"EVALUATION OF POTENTIATED YASHADA BHASMA FOR SAFETY, BIOAVAILABILITY AND SPERMATOGENIC ACTIVITY"

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THE KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI

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For the award of the degree of

Doctor of Philosophy

In Rasashastra and Bhaishajya Kalpana

Under the Faculty of Ayurveda

By

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Geetad .

Dr. GEETA G. GADAD

LIST OF ABBREVIATIONS USED

AAS	:	Atomic Absorption Spectroscopy
Al	:	Aluminium
API	:	Active Pharmaceutical Ingredient
AUC	:	Area Under the Curve
BCA	:	Bicinchoninic Acid (BCA) Assay
BPR	:	Ball-to-Powder Ratio
c	:	Concentration
CMC	:	Carboxymethyl Cellulose
CRS	:	Chronic Restraint Stress
cu.mm	:	cubic millimeter
CVD	:	Chemical Vapor Deposition
DLS	:	Dynamic Light Scattering
DNA	:	Deoxyribonucleic Acid
EDAX	:	Energy Dispersive X-ray Analysis
EDTA	:	Ethylenediaminetetraacetic Acid
EDX	:	Energy Dispersive X-ray Spectroscopy
e.g.	:	For example
EMF	:	Electrical Muffle Furnace
FESEM	:	Field Emission Scanning Electron Microscope
fl	:	Femtoliter
FSH	:	Follicle-Stimulating Hormone
FTIR	:	Fourier Transform Infrared Spectroscopy
gm	:	Grams
gm%	:	Gram percent

gm/L	:	grams per liter
GnRH	:	Gonadotropin-Releasing Hormone
hr	:	Hour
HPR	:	Histopathological report
HRTEM	:	High-Resolution Transmission Electron Microscopy
IAEC	:	Institutional animal Ethics Committee
ICP-AES	:	Inductively Coupled Plasma – Atomic Emission Spectroscopy
ICP-MS	:	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	:	Inductively Coupled Plasma – Optical Emission Spectroscopy
IC50	:	Half Maximal Inhibitory Concentration
JB	:	Jashada Bhasma
Kg	:	Kilogram
L	:	Liter
LD & KD	:	Liver Depression and Kidney Deficiency
LH	:	Luteinizing Hormone
LOD	:	Loss on Drying
MCH	:	Mean Corpuscular Hemoglobin
MCV	:	Mean Corpuscular Volume
MCHC	:	Mean Corpuscular Hemoglobin Concentration
mg	:	Milligram
mgs%	:	Milligrams percent
mIU/ml	:	Milli-international units per milliliter
MI	:	Male Infertility (MI)
mL	:	Milliliter
mV	:	Millivolt

nm	:	Nanometer
ng/ml	:	Nanogram per milliliter
No	:	Number
NP	:	Nanoparticle
NPST	:	Namburi Phased Spot Test
OECD	:	Organization for Economic Co-operation and Development
PCV	:	Packed Cell Volume
PDI	:	Poly Dispersibility Index
pg	:	Picogram
ppb	:	Parts Per Billion
ppm	:	Parts Per Million
Q.S	:	Quantity Sufficient
R^2	:	Correlation coefficient
RBC	:	Red Blood Cell
RDW	:	Red Cell Distribution Width
RPM	:	Revolutions Per Minute
S	:	Sulphur
S.D	:	Standard Deviation
S.E	:	Standard Error
SGOT	:	Serum Glutamic-Oxaloacetic Transaminase
SGPT	:	Serum Glutamic-Pyruvic Transaminase
Si	:	Silicon
Sl. No	:	Serial Number
TA	:	Total Ash
v/v	:	Volume/Volume

WSA	:	Water-Soluble Ash
XRD	:	X-ray Diffraction
XRF	:	X-ray Fluorescence
Zn	:	Zinc
Zn^{2+}	:	Zinc ion (with a +2 charge)
ZnO	:	Zinc Oxide
ZNP	:	Zinc Oxide Nanoparticles
U/L	:	Units per liter
µg/mL	:	Micrograms per milliliter
µm	:	Micrometer
µL	:	Microliter
°C	:	Degree celcius
%	:	Percentage
±	:	Plus-Minus Sign
cm^{-3}	:	Per cubic centimeter
θ	:	Theta
<	:	Less than

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ABSTRACT

Background: Bhasma, traditional Ayurvedic formulation, are renowned for their nanoscale dimensions and superior bioavailability. Yashada bhasma, a zinc-based preparation, has demonstrated therapeutic potential, particularly in enhancing male fertility and spermatogenesis. Zinc's pivotal role in sperm motility, morphology, and spermatogenesis underscores the importance of optimizing Yashada bhasma preparation through modern techniques to enhance its efficacy.

Objectives:

1. To potentiate and characterize Yashada bhasma
2. To evaluate the safety and bioavailability of Potentiated Yashada bhasma
3. To evaluate Potentiated Yashada bhasma for spermatogenic activity

Methodology: The study involved the incorporation of advanced size reduction techniques, such as ball milling, wet grinding, and extended incineration cycles, to achieve nanoparticle uniformity within the classical Bhasmikarana method. Both Classical Yashada bhasma (CYB) and Potentiated Yashada bhasma (PYB) were characterized using instrumental analysis emphasizing mainly particle size with DLS analysis. The novel preparation methodology prompted safety evaluations through an Acute Toxicity Study, which was conducted on female Wistar rats following OECD 420 guidelines. Subsequently, a comparative *in vivo* bioavailability study was performed. Furthermore, the comparative efficacy of CYB and PYB was evaluated over a 30-day period using an adenine-induced fertility suppression model in male Wistar rats, focusing on spermatogenic activity parameters.

Results:

- PYB showed reduced particle size (261.1 nm vs. CYB's 693.6 nm), higher stability (zeta potential: -28.20 mV), and more homogeneous particle distribution. XRD confirmed ZnO as the primary crystalline phase for both.
- PYB demonstrated no mortality at higher dose (2000mg/kg) and minor histopathological changes, with no significant abnormalities in biochemical and hematological parameters.
- PYB showed slightly higher zinc bioavailability, though the difference was statistically insignificant.
- PYB II exhibited superior spermatogenic activity, with significantly improved sperm motility (59.17%), viability (92.33%) and count (83.27 × 10^6/ml) compared to CYB.

Conclusion: Modern processing techniques, including ball milling and wet grinding, significantly enhanced the physicochemical properties of Potentiated Yashada bhasma (PYB), emphasizing particle size reduction and comparatively slightly higher bioavailablility compared to Classical Yashada bhasma (CYB). Acute toxicity studies confirmed PYB's safety even at high doses. PYB II demonstrated significantly improved sperm quality and testicular recovery, outperforming CYB even at reduced doses.

Keywords: Bhasma; Yashada bhasma; Zinc nanoparticles; Bioavailability; Spermatogenesis; Male infertility; Nanomedicine; Toxicity; Ayurveda; Nanoparticle characterization.

1. INTRODUCTION

The concept of Bhasma**,** an ancient Ayurvedic wisdom, has been widely recognized for its efficacy at very low doses and its safety profile due to the unique physical and chemical properties of its stable nanoparticles in contrast to conventional nanoparticles.[1] Nanoparticles of bhasma are organo-metallic or organo-mineral complexes integrated with biological molecules from organic liquid media during the course of pharmaceutical processing. This integration enhances their stability, functionality, absorption, assimilation, bioavailability,[2] biocompatibility, targeted drug delivery and overall effectiveness.[3]

Yashada bhasma (Zinc calyx), specifically, has been extensively studied for its zinc content, which plays a critical role in numerous biological functions, including enzyme activity, cellular function and reproductive health.[4] Yashada bhasma containing zinc oxide nanoparticles are synthesized by series of Ayurvedic pharmaceutical processing,[5] of metallic yashada into submicronic to nano size range zinc oxide. Studies have shown that these nanoparticles exhibit improved therapeutic properties[6] due to their increased surface area and smaller particle size.[7] This emphasizes the classical concept of bhasmikarana, which involves subjecting metals or minerals to a series of purification and incineration processes before they are administered internally. These steps are designed to reduce the particle size, making them easily absorbable, biocompatible, bioassimilable and suitable for usage in the human body. Based on this Yashada bhasma can be further potentiated by additional size reduction methods in existing pharmaceutical processing steps, in presence of herbal extracts by grinding and milling for its improved therapeutic effect.

Thus, study aims to evaluate potentiated Yashada bhasma, which is produced by added intervention in the classical preparation method, by incorporating additional size reduction steps. These modifications are expected to further micronize the particle size, enhancing its bioavailability and therapeutic efficacy. This provides a modern adaptation of traditional Ayurvedic practices. Prior studies have highlighted the potential of size-reduced bhasmas in increasing the pharmacokinetic profiles of active metals and metal oxides.[8]

Though the evidence from previous research highlights the safety of bhasmas when prepared using stringent classical methods, which involve repeated purification and incineration processes to remove impurities and mitigate potential toxic effects.[9] As a novel approach in the preparation of bhasmas, this study necessitated a thorough evaluation of the safety profile of potentiated Yashada bhasma, to confirm its safety for subsequent bioavailability and efficacy studies.

The rationale for this study is rooted in the observation that bhasmas, due to their nano-sized particles, enhance bioavailability and therapeutic potential. Thus, the present work of Yashada bhasma potentiation, followed by comparative bioavailabilty and its potential to enhance spermatogenic activity in vivo, may offer a novel approach to address male reproductive health concerns.

This research is driven by the increasing evidence that metal-based nanomedicines, inspired by the traditional Ayurvedic bhasmas, offer significant potential for advancing nanotechnology applications in Ayurvedic drug pharmacology. Thus, the research seeks to integrate ancient knowledge with modern preparation methods, aiming to bridge the gap between traditional wisdom and contemporary science, thereby contributing to the development of innovative therapeutic interventions.

1.1 BACKGROUND

Bhasma, an important dosage form of Ayurvedic medicine, represents a unique amalgamation of traditional wisdom and advanced material science, characterized by its remarkable efficacy at minimal doses and exceptional safety profile. These properties stem from its nanoscale dimensions and sophisticated preparation processes, which endow it with better bioavailability and therapeutic potential.[9] Among the various bhasmas, Yashada bhasma stands out for its potential role in addressing male reproductive health, particularly its effect on spermatogenesis.[10] Yashada bhasma, prepared from Yashada, is an Ayurvedic preparation traditionally prepared through rigorous methods involving purification (shodhana) and incineration (marana). These processes result in the production of a bio-compatible, organomineral compound containing nanoparticles of zinc oxide, which are believed to enhance its therapeutic properties.

Zinc, an indispensable trace element, is abundantly present in seminal plasma,[11] where it is integral to maintaining sperm integrity and enhancing reproductive functions. Its critical involvement spans several physiological processes, including fortifying cell membrane stability, supporting spermatogenesis by ensuring optimal sperm motility and morphology. It contributes to epithelial integrity in cell membranes, supports the physiological and functional properties of sperm and plays a pivotal role in spermatogenesis.[12] Zinc deficiency has been associated with impaired spermatogenesis, reduced sperm quality and lower fertilization rates.[13] Studies have shown that seminal zinc levels correlate positively with sperm count, motility and morphology, highlighting its critical role in male fertility.[14] Furthermore, zinc influences several physiological processes in the male reproductive system, including the development of gonads, prostate function, and testosterone production.[15] The high

zinc concentration in the prostate gland underscores its importance in enzymatic reactions, sperm motility preservation and regulation of sperm functions.[16,17,18 & 19]

Yashada bhasma's potential for enhancing male reproductive health is supported by its property of bala-veerya vardhana, which translates to enhancing strength and potency. The term "veerya" in Ayurveda is synonymous with the reproductive potency of sperm, aligning with modern findings on zinc's role in improving sperm morphology and count.[20, 21 & 22] This dual perspective provides a strong rationale for exploring Yashada bhasma's spermatogenic efficacy through systematic scientific studies. Additionally, classical methods of bhasma preparation, while highly effective, have been further advanced by integrating modern techniques to achieve more uniform nanoparticle dimensions, thereby expecting enhanced bioavailability and therapeutic effects.

The preparation of Yashada bhasma follows intricate traditional processes that are central to its therapeutic efficacy. Shodhana, the purification step, employs diverse Ayurvedic techniques, including repeated melting and immersion in organic medias are believed to imbue the formulation with bio-compatible properties and eliminate impurities.[23] This meticulous approach underscores the holistic principles of Ayurveda, aiming not only for physical purification but also for an enhancement of the material's therapeutic potency. This detoxification step is followed by marana, which involves grinding, mixing with herbal additives and repeated cycles of incineration under controlled conditions.[24] These steps transform the raw zinc into a bio-compatible, therapeutically potent form. Recent analytical advancements have confirmed that traditional bhasma preparations contain submicronic or nanoparticles, contributing to their high bioavailability and efficacy. However, classical methods may not be seen as limitations, can further excel with classical hints like increased

number of puta increases therapeutic potential as evident in bhasma like abhraka, where 100, 1000 puta are advised.[25 & 26]

In this regard, innovative interventions have been integrated into the classical preparation methods, focusing on enhancing particle size uniformity and optimizing bioavailability. By introducing steps like dry milling in a ball mill and wet grinding, alongside additional cycles of incineration, the process may achieve a more consistent nanoscale structure. These interventional steps need to be substantiated through sophisticated instrumental techniques, ensuring the potentiated Yashada bhasma meets contemporary characterization standards.

Nanotechnology is a cutting-edge field within modern materials science.[27] Contemporary synthesis methods, such as top-down and bottom-up approaches, provide various techniques for achieving nanoscale particle size. While the bottom-up strategy concentrates on assembling atoms or molecules into nanoscale configurations, the top-down approach uses mechanical techniques to convert bulk materials into nanoscale structures.[28] In the fabrication of nanoparticles, both physical techniques such as UV irradiation, sonochemistry and laser ablation, as well as chemical reduction methods involving agents like sodium borohydride and hydrazine, are commonly employed. Biological methods, including the use of microorganisms and plant extracts, provide eco-friendly alternatives that often yield nanoparticles with better-defined sizes and morphologies.[29] However, these modern methods are often associated with high costs, potential toxicity and complexity in separation processes. In contrast, classical methods of bhasma preparation are cost-effective, safe and capable of bulk production, making them an attractive alternative for therapeutic applications.

The additional interventions in the classical preparation of Yashada bhasma necessitated further safety and efficacy evaluations. Yashada bhasma's complex composition, primarily comprising zinc oxide nanoparticles, positions it as a potential therapeutic agent for addressing male infertility.[30] The systematic evaluation of its safety, bioavailability, and spermatogenic efficacy provides a robust scientific basis for its application in addressing male infertility. This study highlights the importance of combining traditional wisdom with modern scientific advancements to explore the full therapeutic potential of Yashada bhasma.

1.2 REVIEW OF LITERATURE

1.2.1 Drug Review

The main drugs like Yashada, Parada, Gandhaka and other drugs which were used for the Shodhana, Jarana and Marana were reviewed.

1. YASHADA

Historical Review:

Yashada had been known since ancient times, but under the name Rasaka Satva. It wasn't until the end of the 14th or the beginning of the 15th century that Shri Adhamalla gave it the name "Yashada" in his commentary on the Dhatu vargikarana by Acharya Sharangadhara and Shri Madanapala, who then added it to Loha varga. Due to its low melting point it is included in Puti loha group along with Naga and Vanga.

Vernacular names: [31]

1. Arabi - Shibaha
2. Bengali - Dasta
3. Burma - Thwot
4. Chinese - Tutenague
5. English - Zinc
6. Gujarathi - Jasada
7. Hindi - Jasta, Dasta
8. Irani - Tutia
9. Kannada - Sattu
10. Latin - *Zincum*
11. Malayalam - Nagam, Tamagaputi

12. Marathi - Jasta
13. Nepali - Dasta
14. Persian - Tutia Rumi
15. Sanskrit - Yashada
16. Tamil - Tutanagam
17. Telugu - Tuttanagam, Kharparam

Synonyms (Paryaya): [32]

Classical texts of Rasashastra mention the following synonyms for Yashada: Yashadam, Kharparaja, Kharparasatva, Jasada, Ranga Sankasham, Rangasadrusha and Ditihetu.

Grahya Lakshanas (Characteristics of Acceptable Yashada): [33]

- Chede samujwalam (Shines bright when cut)
- Snigdham (Unctuous)
- Nirmalam (Clean)
- Mrudulam (Soft)
- Druta dravam (Melts quickly)
- Mahabharam (Very heavy)

Agrahya Lakshanas (Characteristics of Non-Acceptable Yashada): [33]

- Kathina (Hard)
- Kathinadrava (Not easy to melt)
- Ruksha (Dry)
- Ruksha prabha (Dull luster)
- Laghu (Light weight)
- Malina (with visible impurities/dirty appearance)

Ashodhita Yashada Sevana Janya Vyadhi:[34] Prameha, ajirna, vami, bhrama, gulma, kshaya and kushta are the rogas which were found in classical texts of rasashastra.

Ashodhita Yashada Sevana Janya Vikara Shanti: [35]

Bala and Haritaki are to be taken along with sita for three days to overcome the ashuddha Yashada sevana janya vikaras.

Yashada Shodhana:

Samanya Shodhana:

- Dhalana in tila taila, takra, gomutra, kanji and kulattha kwatha for 7 times.[36]
- Dhalana in kanji, takra, kulattha kwatha, gomutra and tilataila for 3 times.[37]
- Dhalana using swarasa of Kadali mula for 7 times.[38]

Vishesha Shodhana: [39]

- Dhalana in Godugdha for 21 times.
- Dhalana in Sudha dugdha for 7 times.
- Dhalana in Churnodaka for 7 times.
- Dhalana in Nirgundi mula swarasa for 7 times.
- Dhalana in Godugdha for 21 times or Triphala kwatha for 21 times or Arka dugdha for 3 times.[40]
- Dhalana in Kanji or Gomutra or Katutumbika for 7 times.[41]
- Dhalana in Ravi dugdha for three times.[42]

Jarana:

Classically Jarana is carried out by apamarga (*Achyranthes aspera* Linn.) panchanga churna or nimba (*Azadirachta indica* Linn.) kashta by continuous stirring of melted yashada with a loha darvi (iron rod).[43]

Marana:

Direct method: Texts have mainly mentioned drugs like Parada, Gandhaka, Haratala etc for the marana of Yashada. Rasataranginikara has mentioned in the first method of Marana that the amalgam of Yashada and Parada should be prepared first. The amalgam is triturated with nimbu swarasa and washed for several times. Lastly Gandhaka is added and triturated properly and then subjected to puta. Final product obtained is Yashada bhasma.[43]

Indirect method: This method is adopted after Jarana process. According to Rasamrutam, fine powder is obtained from Jarana process after filtering through vastra (cloth). Sieved fine powder is triturated with Kumari swarasa, pelletized, dried, enclosed in the sharava samputa and is subjected to Gaja puta. This process is repeated for 7 times to obtain the Yashada bhasma.[44]

Marana of Yashada according to different classical texts:[45]

1. Ayurveda Prakasha: Jarana with apamarga panchanga churna.

2. Rasa Tarangini:

- Marana with parada, gandhaka & nimbu swarasa bhavana – followed by puta.
- Marana with shuddha haratala- puta.
- Jarana with apamarga churna /rigorous trituration with loha darvi.

3. **Rasa jala nidhi**: Jarana with nimba kashta- only for anjana, not for internal use. Marana with kajjali (1/4th of Yashada) nimbu warasa bhavana – gajaputa.
4. **Rasamritam:** Jarana with bhanga churna & kumari swarasa bhavana – 7 gajaputa.
5. **Rasendra purana**: Jarana with nimba kashta, Marana with parada and gandhaka, kumari swarasa & nimbu swarasa bhavana – followed by 1 gajaputa.
6. **Rasachandamshu:** Marana with parada and gandhaka, kumari swarasa & nimbu swarasa bhavana – 1 gajaputa.

Bhavana Dravyas: The classical texts frequently recommend Nimbu Swarasa and Kumari Swarasa as the primary bhavana dravyas for processing Yashada bhasma.

Properties of Yashada bhasma:[46]

Rasa	: Kashaya, Tikta and Katu
Guna	: Sara
Veerya	: Sheeta
Doshagnata	: Kapha pitta hara
Karma	: Balya, Veerya vardhaka and Chakshushya
Vyadhi Prabhava	: Pandu, Prameha, Shwasa, Kasa, Kampavata etc.

Matra:[46]

- ½ to 1 Gunja (bala kalaadyapekshya)
- 2 Gunja

ZINC – MODERN REVIEW: [47 & 48]

Introduction: From the perspective of quantity used in our highly industrialized society, zinc is one of the four most important metals, surpassed by copper, steel and aluminum.

History: The metal Zinc has been used for over 2000 years, notably as brass by the Romans and ancient Middle and Far Eastern civilizations. Smelting knowledge originated in India and China with moderate zinc production in China by the early 1700s. Johann Henckel isolated zinc in 1721, marking its discovery in the Western world. Production began in Great Britain in 1740 and in the U.S. in 1835, achieving commercial success by 1860.

Properties:

- Zinc : Metallic Element
- Atomic Number : 30
- Isotopes (stable) : 5
- Density : 7.14
- Melting point : 419º C
- Boiling Point : 907º C
- Malleable at : 100º C to 150º C

General Characteristics:

- Bluish-gray sheen on a gleaming white metal (spelter).
- Strongly Electropositive.
- Ignites in presence of Moisture.
- Insoluble in Water.

Major Ores of Zinc:

1. **Calamine (Anhydrous Carbonate)**: Contains 52% zinc found in granular or slab form.
2. **Zinc Bloom (Hydrous Carbonate)**: Contains 56% zinc, has a shining appearance and is shapeless in form.
3. **Zinc Blende (Sulfide)**: Contains 66% zinc, white or reddish and typically appears in granular or slab form.
4. **Zincite (Oxide)**: Contains 80% zinc, with a specific gravity of 4 to 4.5 and hardness of 5. It is typically orange-yellow or red in color.

Occurrence: India, China, Canada, U.S.A, Australia, Mexico, Germany, Poland and Belgium are the important nations where the Zinc ores are mined. Bihar, Rajasthan, Chennai, Punjab, Kashmir, Madhya Pradesh and Uttar Pradesh are the source of Zinc ores in India.

Zinc in Human Health: Zinc is a well-studied trace element essential for metabolism, with a total body store of 1.4–2.3 g distributed across tissues. It is primarily absorbed in the small intestine and excreted mainly via feces with minor amounts in urine, sweat, and menstrual blood.[49]

Functions: [50]

It is crucial for the metabolism of nucleic acids and the production of proteins.

1. **Enzymatic:** At least 90 enzymes and 40 metallozymes that perform structural, regulatory or catalytic functions such as carbonic anhydrase, carboxypeptidase, amino peptidase, alkaline phosphatase, etc. need zinc.

2. **Cell functions:** Important for cell function because it plays a role in genetic expression. In lymphocytes, Zinc acts as mitogen, it stabilizes lysosomal plasma and sub cellular membranes and microtubules.

3. **Endocrine function:** Zinc plays a crucial role in insulin binding and degradation at the hepatocyte plasma membrane. Both elevated and reduced zinc concentrations can inhibit the conversion of testosterone to dihydrotestosterone.

4. **Zinc and Male Reproductive Health**[51 & 52]

Physiological Roles: Normal growth and the development of the gonads depend on zinc. It is a key factor in maintaining the proper function of the prostate gland, enhancing testosterone production, and ultimately supporting spermatogenesis.

Zinc in the Testis: In vertebrates, the testis contains high concentrations of zinc, comparable to levels found in the liver and kidneys. Zinc accumulates in germ cells, and its concentration in the testis increases significantly during spermatogenesis. A deficiency in zinc disrupts spermatogenesis, leading to sperm abnormalities.

Zinc and Male Hormonal Regulation: Zinc contributes to male fertility in multiple ways. Insufficient zinc levels negatively affect serum testosterone concentrations, which can impair overall reproductive health.

Zinc and the Prostate: The prostate holds exceptionally high zinc levels, serving as a marker of prostatic function. Zinc regulates sperm function, acts as a co-factor for enzymes, and preserves sperm motility.

Roles of Zinc in Sperm Quality and Function: Zinc is essential for sperm function. The low zinc levels in semen are linked to reduced sperm quality and a decreased chance of successful fertilization.

Sperm Functions: Higher sperm concentration in the ejaculate, greater antioxidant activity, improved sperm motility, and increased viability are all associated with optimal zinc concentrations in seminal plasma.

Sperm Quality: Supplementing with supra-nutritional doses of zinc, along with cobalt (Co) and selenium (Se), has been shown to improve the production of motile sperm with intact membranes. Studies have also highlighted a strong association between seminal zinc levels and sperm count, that zinc is essential for sperm function. Low zinc levels in semen are linked to reduced sperm quality and a decreased chance of successful fertilization

5. Toxicity: [53]

Acute poisoning is characterized by metallic taste, sub-sternal and abdominal pain, vomiting, purging, convulsions, collapse and death. Chronic Zinc poisoning may occur among Zinc smelters. Inhalations of fumes result in severe respiratory irritation and cyanosis called "metal fume fever". Chronic over ingestion causes dyspepsia, colic, diarrhea or constipation, anorexia, peripheral neuritis leading to paralysis. Excessive intake of Zinc may produce evidence of copper deficiency.

Clinical Manifestations of Zinc Deficiency:

Bullous pustular dermatitis, baldness, diarrhea, emotional instability, weight loss, and recurring infections as a result of compromised cell-mediated immunity are all signs of severe zinc deficiency in humans. Additionally, it may result in delayed ulcer healing, neuro-sensory problems, and male hypogonadism.

Severe zinc deficiency can be lethal if undiagnosed and untreated.

Male hypogonadism and growth retardation in teenagers are linked to moderate zinc insufficiency. Rough skin, low appetite, mental sluggishness, delayed wound healing, immunological malfunction mediated by cells, and aberrant neurosensory alterations are additional symptoms.

These findings underscore the critical role of zinc in maintaining various physiological functions and highlight the importance of adequate zinc intake for overall health.

2. PARADA

Historical Review

Parada, regarded as the soul of Rasashastra, is indirectly mentioned in the Atharvaveda and Yajurveda. In classical texts like Charaka Samhita, Sushruta Samhita and Ashtanga Samgraha, Parada is extensively referenced. Charaka recommended its external use, while Sushruta and Vagbhata advocated both internal and external applications. Systematic utilization of Parada for Dehavada and Lohavada began in the 8th century A.D., establishing it as a vital component of Rasashastra.

Synonyms: [54]

1. **Dehavadatmaka**: Rasayana, Amritam and Mrityunashana
2. **Dhatuvadatmaka**: Maharasa, Rasottama and Suta
3. **Vishishta Gunatmaka**: Ananta, Sukshma and Saubhagya
4. **Swarupatmaka**: Galada, Raupyanibhama, Mahateja and Shiva Virya
5. **Devatmaka**: Harabija, Shiva Bijam, Hara Ret and Trilochanam
6. **Gatyatmaka**: Chapala and Khechara

Vernacular Names: [55]

1. **Sanskrit** **:** Parada
2. **Hindi** **:** Parada
3. **Gujarati** **:** Paro
4. **Marathi** **:** Para
5. **Latin** **:** *Hydrargyrum*
6. **English** **:** Mercury and Quick Silver

Grahya–Agrahya Lakshanas: [56]

Mercury with a bright, mid-day sun-like shine and a bluish tinge from within, resembling liquefied silver (Galada roupyanibham), is considered acceptable (Grahya). Mercury that appears smoky, dull, or displays yellowish-white or mixed colors is unsuitable (Agrahya).

Doshas of Parada: Parada may contain natural (Naisargika), chemical (Yougika), or artificial (Aoupadika) impurities due to its association with metallic elements or commercial adulteration.

Samanya Shodhana (General Purification): [57]

The most common method involves rubbing Parada with Sudha Churna for three days, filtering it, and triturating with Lashuna and Saindhava Lavana until it turns black. The mixture is washed with hot water to obtain purified Parada.

Vishesha Shodhana (Special Purification): [58]

Impurities like Visha, Vahni, Mala, Naga, Vanga, Chapalya, and others are removed through Ashtadasha Samskaras to enhance and potentiate Parada.

Pharmacological and Therapeutic Properties: [59]

- **Rasa** **:** Shad Rasa
- **Guna** **:** Snigdha, Sara and Guru
- **Virya** **:** Ushna
- **Vipaka** **:** Madhura
- **Karma** **:** Yogavahi, Rasayana, Balya and Vrushya
- **Dosa Prabhava** **:** Tridoshaghna
- **Vyadhi Prabhava** **:** Krimi, Kustha, Vataroga and Valipalita

MERCURY (Hg) – Modern Review:

English Name: Quick Silver **Latin Name**: *Hydrargyrum*

Ores of Mercury: The primary ore of mercury is Cinnabar (mercury sulfide). Other notable ores include Meta Cinnabar, Calomel (mercury chloride), Stonite, and Worsenite (containing antimony and sulfur).

Physical Properties:

1. Color : Shining silver-white
2. Atomic Number : 80
3. Atomic Weight : 200.61
4. Specific Gravity : 13.595 at 25°C
5. Boiling Point : 357°C
6. Freezing Point : 38.9°C

Absorption, Distribution and Excretion: [60]

Mercury salts are absorbed slowly from the alimentary tract, with soluble salts having systemic effects. Insoluble salts, such as mercury sulfides, are minimally absorbed and largely inert. Mercury is excreted primarily through the kidneys and colon, with smaller amounts via bile, saliva, lungs and skin.

Therapeutic Uses:

Mercury salts are used externally as antiseptics and disinfectants, particularly in surgical and obstetric practices. Ointments like blue ointment and calomel ointment relieve itching and promote absorption in conditions like eczema, psoriasis, and pruritis. Mercury-based preparations are also used in chronic joint diseases and certain eye conditions like conjunctivitis and keratitis.

3. GANDHAKA

Historical and Mythological Background of Gandhaka: [61]

Gandhaka (*Sulfur*) holds a significant place in Rasashastra, classified under the Uparasa group. While not mentioned in the Vedic period, its therapeutic applications are noted from the Samhita era. Detailed descriptions appear in Rasashastra texts. Mythologically, Gandhaka is believed to have originated from volcanic activity, symbolizing the Artava (menstrual essence) of Prakriti. It is said to have emerged during the Samudra Manthana and was named for its pleasing fragrance (Gandha).

Synonyms: [62]

- Appearance-based **:** Navanita, Gandhapashan and Gandhopala
- Fragrance-based **:** Gandhaka, Divyagandha and Putigandha
- Therapeutic Action **:** Kitaghna, Lekhi and Kushtari
- Mythological Origin **:** Gauribija and Balivasa
- Physical Characteristics**:** Shukapiccha, Shukapuccha &Shukapicchasamacchayo

Grahya Lakshana (Qualities of Acceptable Gandhaka): [63]

High-quality Gandhaka resembles the greenish-yellow like tail of a parrot (Shukapiccha), is smooth, hard, unctuous, and translucent like Amalaki fruit. Such Gandhaka, termed Amalasara, is preferred for therapeutic use.

Impurities and Purification

Impurities:

1. **Physical:** Clay and sand (Shilachurna*)*
2. **Chemical**: Arsenic and Lead (Visha Dosha*)*

Impure Sulphur gives rise to kushta, taapa, brama, pitta roga and destroys roopa, veerya, bala and shukra.[64]

Shodhana Methods:

1. An equal part of powdered Gandhaka is added to heated ghee in an iron vessel. The combination is added to milk after the Gandhaka has melted.[65]
2. Cover the mouth of a milk-filled vessel with a thin cloth. Melt Gandhaka with an equal amount of ghee in a ladle over mild heat and pour it into the milk through the cloth for filtration.[66]
3. Heat Sarshapa or Kushmanda tila add powdered Gandhaka in equal proportion and melt over low heat. Pour the molten Gandhaka into milk, cool it, and wash with hot water to complete the purification.[67]
4. **Damaru yantra method:** For four Praharas, four pala of Gandhaka powder are cooked over a moderate fire in a Damaru Yantra. The Yantra is opened once it has cooled naturally, and the Gandhaka gathered in the upper chamber is taken out and put away.[68]
5. **Kurma puta method:** Spread powdered Gandhaka over a cloth-covered vessel containing milk, seal it with an earthen lid and bury it up to the neck in the ground. Ignite cow dung cakes on top of the setup. The heat melts the Gandhaka, which drips into the milk below for collection.[69]

Pharmacological and Therapeutic Properties: [70 & 71]

- Rasa : Madhura, Katu, Tikta & Kashaya
- Guna : Ushna, Sara & Snigdha
- Virya : Ushna
- Vipaka : Katu
- Karma : Deepana, Vishahara, Jantughna & Rasayana
- Doshagnata : Alleviates Kapha Vata & increases Pitta.
- Vyadhi prabhava : Kandu, Visarpa, Krimi, Kustha & Pleeha.

SULPHUR – Modern Review:

History: Originally called "brimstone" (fire stone), sulfur was used for fumigation and medicine by the Aryans, Greeks, Romans, and Indians. Antoine Lavoisier identified it as an element in 1777. In the cosmos, it is the tenth most plentiful element.

Physical Properties:

Latin Name : *Sulphur* (S)

Atomic Number : 16

Atomic Mass : 32.06

Melting Point : 112.8°C

Boiling Point : 444.6°C

Classification : Non-Metal

Crystal Structure : Orthorhombic

Physiological Role:

- Essential for bile acid production aiding fat digestion.
- Maintains healthy skin, hair and nails.
- Deficiency is linked to eczema and poor hair and nail growth.

Therapeutic Uses: [72]

1. **Internal**: Converts to alkali sulphides in the small intestine, acting as a mild laxative (10–12 hours) or purgative in larger doses and stimulates skin and bronchial secretions.
2. **External**: Treats parasitic skin diseases like scabies, ringworm, and pediculosis.

4. ASSOCIATED DRUGS:

1. **Kanji:** For Samanya Shodhana of Yashada.
2. **Takra**: For Samanya Shodhana of Yashada.
3. **Kulattha Kwatha:** For Samanya Shodhana of Yashada.
4. **Gomutra**: For Samanya Shodhana of Yashada.
5. **Tila Taila:** For Samanya Shodhana of Yashada.
6. **Sudha Churna:** For Vishesha Shodhana of Yashada and Samanya Shodhana of Parada.
7. **Godugdha:** Shodhana of Gandhaka and Takra preparation.
8. **Saindhava:** For Samanya Shodhana of Parada.
9. **Lashuna:** For Samanya Shodhana of Parada.
10. **Apamarga:** For Jarana of Yashada.
11. **Kumari:** For Bhavana during Chakrika nirmana.
12. **Nimbuka:** For Bhavana during Chakrika nirmana.

Table No. 01. Ayurveda Pharmacological properties of Associated drugs

Sl. No.	Drug	Rasa	Guna	Virya	Vipaka	Karma
1.	Kanji[73]	Amla	Teekshna, Laghu	Ushna	Amla	Vatakaphahara, Rochana, Pachana, Deepana, Basti Shodhana, Bhedana
2.	Takra[74]	Kashaya, Amla, Madhura.	Laghu	Ushna	Madhura	Pathya, Deepana, Preenana, Vrushya
3.	Kulattha[75]	Kashaya	Laghu, Vidahi, Sara	Ushna	Katu	Vatakapha shamaka, Pittaraktakara, Bhedana, Sangrahi, Swedasangrahaka
4.	Gomutra[76]	Katu, Tikta, Kashaya	Teekshna, Laghu, Kshara	Ushna	Katu	Vatakapha shamaka, Pittaraktakara, lekhana, deepana
5.	Tilataila[77]	Madhura, Tikta, Kashaya	Sookshma, Vyavayi, Teekshna	Ushna	Madhura	Vata-kaphahara, Raktapittakrit, Lekhana, Twachya, Keshya
6.	Godugdha[78]	Madhura	Guru, Snigdha, Picchila, Manda	Sheeta	Madhura	Jeevaneeya, Brumhaneeya, Rasayana, Ojo Vriddhi kara, Vrushya, Balya
7.	Saindhava[79]	Lavana, Madhura	Laghu, Snigdha	Sheeta	Madhura	Deepana, Pachana, Vrushya, Hrudya Tridosha hara
8.	Lashuna[80]	Madhura, Lavana, Katu, Tikta, Kashaya.	Snigdha, Guru, Teekshna, Sara.	Ushna	Katu	Vata-kaphahara, Balya, Brahmhana, Rasayana
9.	Apamarga[81]	Katu Tikta	Teekshna, Laghu, Ruksha	Ushna	Katu	Kapha Vata shamaka, Kapha Pitta shodhaka, Krimigna, Shotha hara
10.	Kumari[82]	Katu, Tikta	Guru Snigdha, Picchila	Sheeta	Katu	Kapha pittahara, Shothahara, Vedana sthapana, Vrunaropaka
11.	Nimbuka[83]	Amla	Laghu Tikshna	Ushna	Amla	Deepana, Rochaka, Anulomana, Pachaka

Table No. 02. Pharmaceutical relevance of Associated drugs

Drug/Drugs	Evidences of Research
1. Tila (*Sesamum Indicum* Linn.) taila 2. Takra 3. Kanji 4. Gomutra 5. Kulattha (*Dolichos biflorus* Linn.*)* kwatha 6. Churnodaka	Evidence from previous research demonstrates that another putiloha: Naga was sequentially melted and quenched three times in all these five media sequentially utilizing media ranging from slightly acidic to strongly basic. This process effectively reduced particle size, increased brittleness and enhanced exposure to the purifying medium. Post-purification analysis using TGA and FTIR confirmed significant alterations in the physicochemical properties of Naga, indicating reduced toxicity and improved therapeutic potential.[84] Another study demonstrates the role of various Shodhana media in metal purification. The sequential use of acidic (e.g., Takra, Kanji) and basic (e.g., Gomutra, Churnodaka) media removes soluble, evaporable, and washable impurities, reduces particle size, and enhances brittleness for improved exposure to the purifying medium. Organic compounds from media like Kanji and Kulattha Kwatha are introduced, contributing to therapeutic efficacy.[85]
7. Sudha churna 8. Saindhava 9. Lashuna (*Allium sativum* Linn.)	Previous research demonstrates that Samanya Shodhana of Parada using Sudha Churna**,** Saindhava and Lashuna effectively removes toxic impurities. After 36 -36 hours of trituration in Sudha**,** followed by Lashuna and Saindhava**,** the color change of the mercury indicated impurity removal. The process also improved its organoleptic characteristics like luster, suggesting enhanced therapeutic potential. These findings show that the media purified mercury, reducing its toxicity and making it more suitable for further pharmaceutical use.[86]
10. Apamarga *(Achyranthes aspera* Linn.)	The study observed that the use of herbal substances like Apamarga Panchanga Churna during Jarana leads to a series of physicochemical changes, including reduced particle size and enhanced surface area. Also revealed the presence of potassium in the elemental analysis of the bhasma, which was primarily attributed to the potassium content in *Achyranthes aspera.* [87]
11.Kumari (*Aloe vera* Linn.*)* 12. Nimbuka (*Citrus Limon* Linn.)	This study highlights interaction between Yashada and herbal juices during the Bhavana process resulting in chemical changes that enhance the bioavailability of zinc compounds. Further, add that the Bhavana process contributes to a reduction in the particle size which is essential for increasing the surface area of the metal, thereby enhancing its absorption and bioavailability in the body.[88]

1.2.2 PHARMACEUTICAL REVIEW

1. Shodhana (Purification):

Metallurgical Perspective: [89 & 90]

Shodhana, a purification and transformation process, fundamentally alters the physical and chemical properties of substances based on their nature. Softer substances like Parada, Gandhaka, and Sudhavarga dravyas undergo gentler treatments, while harder substances such as Abhraka, Loha, Tamra and Yashada require more rigorous methods to break their compact structures. For metals, the primary step involves heating to red-hot or melting, followed by quenching in a liquid medium at room temperature, leading to significant structural transformations.

The Role of Grain Boundaries and Recrystallization[91 & 92]

Quenching introduces structural defects in metals. Grain boundaries, which separate crystals of similar or differing phases, play a pivotal role in determining metal properties. Rapid cooling after heating disrupts the grain structure, creating smaller grains with entrapped liquid. These boundaries serve as sites of increased energy due to dislocations and stress, leading to recrystallization during subsequent heat treatments. By replacing the deformed grains with fresh, strain-free ones, recrystallization lowers internal energy and improves grain structure. Recrystallization also depends on factors such as temperature, cooling rate, and the number of quenching cycles. Rapid cooling generates smaller grains due to limited movement of grain boundaries, enhancing structural deformation. Conversely, slower cooling allows for larger grains as grain boundaries traverse greater distances. The entrapped liquid forms boundary films, altering the properties of the metal, which in Rasashastra is a desired effect.

Application of Collision Theory[93 & 94]

The collision theory explains the molecular interactions during Shodhana. Heating metals increases the kinetic energy of atoms, leading to more frequent and energetic collisions. During quenching, the rapid temperature drop causes abrupt lattice distortions as atoms from the liquid medium occupy interstitial lattice sites. These interactions affect the grain boundary energies and the extent of deformation, contributing to the unique physico-chemical changes sought in Shodhana.

Repeated quenching cycles amplify these effects, as successive thermal shocks create smaller, more stable grains and introduce elemental interactions with the quenching medium. The specified cycles (e.g., seven per liquid) ensure the metal achieves consistent and desirable properties.

Significance in Rasashastra

In metallurgy, the structural changes caused by quenching often weaken metals; however, in Rasashastra, these changes enhance their suitability for medicinal applications. The process ensures the breakdown of the metal's compact structure, creating bio-compatible forms enriched with elemental additions from the liquid medium.

2. Marana (Calcination) for Puti lohas:

In the pharmaceutical process, transforming metals into bioavailable forms involves distinct stages, with Marana being a critical step where metals are converted into their oxidized or incinerated forms (bhasma). Before metals can be transformed into Bhasma, they must first be rendered workable. For Puti-Lohas (low-grade metals), this involves two primary preparatory steps: Jarana and Pishti preparation (amalgamation with Parada).

- **Jarana (Intermediate Step):**

Jarana is an intermediary metallurgical process, where metals are treated to alter their physical and chemical properties, making them more amenable to further transformation. Although the term "Jarana" is not explicitly mentioned in ancient texts, its application has been widely recognized in practice for convenience. The process typically involves reactions such as oxidation, phase transformation, or alloying, depending on the treatment medium and conditions.

From a material science perspective, Jarana can be viewed as a step involving:

1. **Surface Oxidation**: Oxidation disrupts the metal's compact structure, forming oxide layers that are more chemically reactive.
2. **Grain Refinement**: Thermal cycling and controlled mechanical treatments during Jarana reduce grain size, introducing new grain boundaries that enhance reactivity.[95]
3. **Phase Transformation**: Transitioning metals into mixed phases (e.g., metal oxides or alloys) increases the surface area for subsequent reactions, facilitating their integration into the pharmaceutical process.[96]

- **Pishti: Amalgamation with Parada**

In Pishti preparation, Parada (mercury) amalgamates with the metal, creating an alloy or intermetallic compound. Mercury acts as a solvent, diffusing into the grain boundaries and weakening the crystalline lattice.[97] This step aligns with principles of solid-state diffusion and interstitial alloying**,** enhancing the reactivity and bioavailability of the treated metal.[98]

3. Marana (General Method): Marana involves calcination or incineration of the treated metal to produce Bhasma, a bio-compatible and therapeutic form. During Marana:

1. **Thermal Decomposition**: The metal undergoes controlled heating, leading to the breakdown of its compact structure and formation of stable oxides or other compounds.[99]
2. **Structural Reorganization**: High temperatures promote recrystallization and phase transformations, stabilizing the material in its desired form.
3. **Enhanced Reactivity**: The final Bhasma form is characterized by a fine particle size and increased surface energy, crucial for its therapeutic efficacy.[100]

Pharmaceutical and Therapeutic Implications

In some cases, such as Yashada (zinc), the Jarana stage itself may yield a product with sufficient therapeutic efficacy, reducing the need for further processing. This decision depends on the metal's structural and chemical properties after Jarana and its intended medicinal application. If the partially processed form is found to be unsafe or ineffective, the process is extended through Bhavana (levigation with herbal or mineral liquids) and Marana to ensure safety and efficacy.

4. Jarana (General Method):[101]

It refers to the process of incineration, roasting, or occasionally calcination. During this procedure, the molten metal is stirred with an iron ladle or branches of specific herbal materials. Organic substances may be added to the molten metal and stirred to promote incineration, while inorganic substances like lavana (salts) or kshara (alkaline extracts of herbal drugs) may be incorporated to aid in converting the metal into a fine powder.

The resulting material, reduced to an ash-like form, is then heated to a red-hot state. In some cases, it is covered mud saucer and subjected to prolonged heating for 12 to 24 hours. The ash thus produced can be used directly. Classical texts do not prohibit its internal use, though some recommend washing the ash with water and subjecting it to Puta (repeated heating cycles) to enhance its quality and safe administration.

5. Pishti (General method):

In Rasashastra, Bhasma prepared using Mercury (Parada) is considered the best. For metals like Yashada (zinc), an intermediate step involves mixing it with purified Mercury (Shodhana) before proceeding to Marana (the process of calcination). The Mercury is triturated with molten metal, forming an amalgam. Mercury helps to embrittle metals by penetrating their grain boundaries, making it an effective powdering medium. The resulting amalgam, or Pishti, is then washed with acidic liquids like Nimbu Swarasa to remove acid-soluble impurities that could interfere in later stages.

This Pishti, which is in a favorable pharmaceutical form, is typically combined with Gandhaka (Sulphur) before Marana. Gandhaka acts as a binder for Mercury, stabilizing it through the heating process. Sulphur enables the controlled release of Mercury, preventing its loss. The final bhasma may contain minimal Mercury, as Gandhaka facilitates the formation of sulphides or sulphates, compounds that are beneficial for medicinal use.

6. Bhavana (Levigation):

Bhavana is defined in various ways across texts, with differences in the amount of liquid used and the duration of the process. These definitions include:

Amount of Liquid:

- Equal to the solid material, either volumetrically or gravimetrically.
- Sufficient to immerse or triturate the solid into a dough-like consistency.
- For herbal decoctions, the amount of dry herb equals the solid material, with eight parts water added, reduced by boiling to one-eighth. This liquid is used for levigation.
- The liquid is absorbed until the material forms a dough-like consistency, suitable for making Chakrika.

Subjective Signs:

- The liquid is fully absorbed when the material returns to its powdered state.

Duration:

- The liquid is added and left for 24 hours, repeated for seven days and nights. If it evaporates, it should be replenished in the same amount.

7. Chakrika:

Chakrika preparation in Rasashastra is a pelletization process similar to kuchala beeja samana, which resembles modern pharmaceutics, where fine powders are agglomerated into spherical units (0.5 to 1.5 mm). It is a key step in the Marana process, though sometimes powders are used directly. The necessity of Chakrika includes:

1. Heat Testing: Chakrika helps gauge the amount of heat applied during Marana.
2. Ease of Transfer: It facilitates the movement of material from the mortar to the Marana apparatus.
3. Prevents Loss: In traditional Puta, it prevents material from being lost if the samputa topples or if the material isn't properly contained.

Intricacies: Molding & Drying: The dough should be soft enough to form uniform, smooth Chakrikas, typically round and convex and dried in shade.

8. Concept of Puta:

The process of subjecting Rasadravyas (Medicinal substances) to a specific quantum of heat following established principles is known as Puta. This procedure is carried out in a sharava samputa (a sealed earthen saucers). After undergoing preliminary purification (Shodhana) or incineration (Jarana), the materials are combined with plant juices or decoctions, shaped into coin-like pellets, dried and subjected to controlled heating using cow dung cakes as fuel.

The heating process or Puta, involves carefully structured arrangements of cow dung cakes, either on the ground or within specially designed pits. These pits, such as mahaputa, gajaputa, and varaha puta, provide varying degrees of heat intensity tailored to the hardness of the material, enhancing its therapeutic properties progressively.[102]

Effect of Puta on the Drug:

The Puta process induces chemical transformations within the drug, resulting in observable changes such as color alteration and the formation of organo-metallic compounds. These transformations improve the drug's biocompatibility, stability, digestibility, and absorption, making it more effective for therapeutic use.[103]

Assessment of Bhasma: [104]

To ensure the quality of bhasma (calcined preparations), classical texts prescribe specific tests (bhasma pareekshas). These include:

- Varitara**:** Floating on water.
- Rekhapurnata**:** Filling the grooves of a finger.
- Apunarbhava**:** Irreversibility to the original form.
- Nischandrata**:** Absence of metallic luster.
- Niruttha**:** Stability and non-reactivity.

Contemporary Understanding of Bhasmikarana:[105]

The process of Bhasmikarana can be elucidated using Griffith's theory of mechanical force, which underpins pharmaceutical techniques such as shodhana, jarana, bhavana and marana. These methods employ heat and mechanical attrition to systematically reduce particle size, enhancing therapeutic efficacy by fracturing particles at their irregularities. This reduction in particle size improves bioavailability and augments the therapeutic effects.

In addition, the principles of thermodynamics provide a contemporary framework to understand Bhasmikarana.

- **Fourier's Law of Heat Conduction**: Explains the heat transfer within the sharava samputa, ensuring uniform heating of the material.[106]
- **Hess's Law of Thermodynamics**: Highlights the total energy changes during the chemical reactions in the Puta process, emphasizing energy conservation and transformation within the system.[107]

These modern interpretations enrich our understanding of traditional processes, offering insights into the scientific principles underlying the ancient art of bhasma preparation.

REVIEW ON NANOTECHNOLOGY

Nanotechnology is a rapidly advancing field of science that focuses on particles ranging from 1 to 100 nanometers (nm). These extremely small particles possess unique physical, chemical, and biological properties, making them applicable across various industries. Prominent uses of nanotechnology can be observed in the manufacturing of sunscreens, cosmetics, textiles, and sports equipment.

Nanotechnology is essential to the creation of Novel Drug Delivery Systems (NDDS) in the medical arena. These systems utilize nanoparticles to deliver drugs with high precision to targeted sites in the body. The ultra-fine particle size of nanoparticles enhances solubility and permeability, resulting in improved therapeutic efficacy even with minimal drug dosages.[108]

Types of Nanoparticle Synthesis: [109]

Nanoparticle synthesis can be broadly categorized into two main approaches based on the methodology:

1. **Top-Down Approach:** Larger, bulk materials are broken down into smaller, nanoscale particles using the top-down method. This method is widely employed in processes such as:

    - **Mechanical milling**: Grinding bulk materials to achieve nanoscale particles.
    - **Electrospinning**: Creating nanofibers using electrical forces.
    - **Lithography**: Patterning materials on a nanoscale using a mask.
    - **Sputtering**: Depositing thin films on surfaces by bombarding a target material with high-energy particles.
    - **Laser ablation**: Using lasers to vaporize material into nanoparticles.

2. **Bottom-Up Approach:** The bottom-up method concentrates on assembling atoms or molecules to create nanoparticles. Better control over the particles' size, shape, content, and structure is possible with this approach. Techniques used in this approach include:

- Chemical vapor deposition (CVD): Depositing material from a gas phase to form nanoparticles.
- Solvothermal and hydrothermal methods: Synthesizing nanoparticles under high temperature and pressure.
- Sol-gel method: Producing nanomaterials by transitioning from a solution to a gel state.
- Soft and hard templating: Using templates to create nanostructures with defined shapes.
- Reverse micelle method: Forming nanoparticles within nanoscale micelles.

Methods for Nanoparticle Synthesis:[110]

Physical, chemical, or biological techniques can be used to create nanoparticles; each is designed to meet precise specifications for size and shape.

- **Physical and Chemical Methods:** Although these techniques are efficient, they are frequently expensive and present environmental dangers because they involve the use of dangerous materials.

- **Biological (Green) Synthesis:** Green synthesis has become a viable and environmentally responsible substitute. In order to create nanoparticles, it makes use of natural resources like plant extracts. This method is economical, scalable for a range of applications, and less harmful to the environment.

Review on Mechanical Milling for Nanoparticle Synthesis:[111]

Introduction:

Mechanical milling is a prominent physical method in nanoparticle synthesis, belonging to the top-down approach. It involves the mechanical breakdown of bulk materials into nanoscale particles by applying high-energy impact, shear, and friction forces. This method is widely used due to its simplicity, scalability, and applicability to various materials.

Process and Techniques:

Mechanical milling primarily employs high-energy ball mills or planetary ball mills. The process involves grinding materials within a rotating chamber filled with grinding balls and powder. The impact and frictional forces generated cause the material to fracture into smaller particles. Key techniques include:

- **High Energy Ball Milling**
  - Most common technique for producing nanoparticles.
  - Uses rotational and/or vibrational motion to grind materials.
- **Cryogenic Milling**
  - Involves milling at cryogenic temperatures to process heat-sensitive materials.
  - Reduces thermal decomposition and oxidation.
- **Attrition Milling**
  - Combines shear forces with impact to refine materials to nanoscale.

Parameters Influencing Mechanical Milling:

The efficiency and quality of nanoparticle synthesis depend on several parameters:

- **Milling Speed**: High speeds increase impact energy and reduce particle size.
- **Ball-to-Powder Ratio (BPR)**: Affects the collision frequency and energy transfer.
- **Milling Time**: Extended milling leads to finer particles but may induce contamination.
- **Material Properties**: Hardness and brittleness influence the fragmentation process.

Applications: Because of its versatility, mechanical milling can be used on a wide range of materials:

1. **Metals**: Produces ultra-fine metal powders used in catalysts and conductive materials.
2. **Ceramics**: Generates nanoscale ceramic particles for advanced structural applications.
3. **Composites**: Combines nanoparticles with polymer matrices for improved properties.

Advantages:

- Cost-effective and simple.
- Suitable for industrial-scale production.
- Can process a wide range of materials.

Limitations:

- Broad size distribution and irregular particle shapes.
- Potential contamination from milling tools.
- Long processing times for uniform size reduction.

Biomedical Applications

- **Drug Delivery Systems**: Controlled drug delivery systems for cancer and chronic diseases use cryogenic milled nanoparticles, such as those composed of biodegradable polymers.

- **Magnetic Hyperthermia**: Magnetic nanoparticles, like iron oxide, synthesized by physical methods are employed in hyperthermia treatments to target and destroy cancer cells.

Limitations in Biomedical Application:

1. **Size Distribution and Uniformity**: Mechanical milling often results in a broad size distribution, which can hinder predictable behavior in biological systems, where uniformity is crucial for drug delivery and cellular uptake.

2. **Surface Contamination**: Contaminants from milling tools can affect the biocompatibility of nanoparticles, potentially leading to toxicity in biomedical settings.
3. **Irregular Particle Shape**: Nanoparticles from mechanical milling may have irregular shapes, affecting their stability and interaction with cells, tissues, and biomolecules, thus reducing efficacy in applications like drug delivery.
4. **Agglomeration**: Due to high surface energy, nanoparticles can agglomerate, reducing surface area and altering properties like drug release rates and cellular uptake.
5. **Limited Control over Composition**: Mechanical milling may not offer precise control over nanoparticle composition, which is essential for ensuring appropriate biological interaction and minimizing toxicity.

6. **Toxicity Concerns**: High-energy collisions can generate toxic byproducts or form sharp-edged particles that may cause cellular damage or inflammation.
7. **Scalability Issues**: Scaling up mechanical milling for large-scale production while maintaining quality and size control is challenging, especially for clinical applications.
8. **Energy Consumption and Processing Time**: High energy and long processing times are required, increasing production costs and raising concerns about sustainability in large-scale manufacturing for biomedical applications.

Advantages of Classical Bhasmikarana (Nanoparticle preparation):[112 & 113]

1. **Biocompatibility**: Ayurvedic bhasmas are traditionally used for therapeutic purposes in Ayurveda, indicating their inherent biocompatibility. The process of preparing bhasmas involves careful purification and calcination, which can produce nanoparticles that are compatible with biological systems. This makes them potentially safer for biomedical applications compared to some synthetic methods.
2. **Eco-Friendly and Sustainable**: The preparation of bhasmas uses natural sources and avoids harmful chemicals or solvents. This makes the process eco-friendly, sustainable, and a low-cost alternative to modern nanoparticle synthesis methods that often rely on toxic chemicals or high energy inputs.
3. **Controlled Particle Size and Structure**: The bhasmikarana process often results in nanoparticles with controlled size and morphology, which are critical for applications such as drug delivery. Traditional Ayurvedic methods also provide the advantage of natural functionalization with medicinal properties, enhancing their therapeutic potential.

4. **Low Toxicity and Better Safety Profile**: The use of herbal and natural substances in bhasmikarana can reduce the risk of toxicity compared to synthetic nanoparticle synthesis methods. The nanoparticles prepared through this method are considered to be safer for human use, with fewer side effects.
5. **Enhanced Efficacy in Drug Delivery**: Nanoparticles prepared via bhasmikarana have been shown to exhibit enhanced drug delivery properties, including better solubility, targeted action, and improved bioavailability. The method's ability to control size and surface properties enhances the effectiveness of pharmaceuticals.

1.2.3 ANALYTICAL REVIEW

Instrumental Analysis Review:

1. Dynamic Light Scattering (DLS)[114]

A popular technique for determining the size distribution of tiny particles and nanoparticles in suspension or dispersion is dynamic light scattering, or DLS. DLS works by examining the variations in scattered light intensity brought on by the particles' Brownian motion. These variations aid in figuring out the particles' hydrodynamic diameter, which offers important information on their stability, behavior in various media, and effectiveness in industrial and therapeutic settings.

The technique is highly sensitive, capable of measuring particles in the size range of 1 nm to several micrometers. It is non-destructive and can be performed on small sample volumes. DLS is particularly useful in characterizing protein aggregates, polymer nanoparticles, and emulsions, offering quick and reproducible results.

Advantages of DLS:

1. Rapid analysis of particle size.
2. Requires minimal sample preparation.
3. Provides size distribution information for complex samples.

Limitations:

1. Primarily measures the hydrodynamic size, which can be affected by the particle’s surface properties.
2. Not ideal for measuring particles with a broad size distribution or aggregates larger than a few micrometers.

2. XRD- X Ray Diffraction [115]

X-ray Diffraction (XRD) Analysis is a highly effective method for determining the crystallographic structure, phase composition, and other structural characteristics of materials. The method measures the X-ray intensities and angles that are diffracted by a crystalline material. The material's atomic arrangement, crystallite size, and orientation can all be determined by analyzing the diffraction pattern created by X-ray interaction with the crystal lattice.

XRD is widely applied in various fields, including material science, chemistry, geology, and pharmaceuticals, due to its ability to characterize both crystalline and amorphous materials. It is particularly useful for identifying phases in complex mixtures, studying thin films, and determining the purity of substances.

Advantages of XRD:

1. Non-destructive and requires minimal sample preparation.
2. Capable of determining phase composition, crystallite size, and lattice parameters.
3. Provides high precision for phase identification and structural analysis.

Limitations:

1. Cannot detect non-crystalline or amorphous phases in materials.
2. The interpretation of data can be complex, particularly for multi-phase samples

3. SEM analysis [116]

A popular method for high-resolution imaging and material surface analysis is scanning electron microscopy, or SEM. A concentrated electron beam is directed onto a sample, and the secondary electrons released from the surface are detected. This makes it possible to see surface composition, topography, and morphology at nanoscale to micrometer scales.

SEM may produce fine-grained surface pictures of the material, displaying details on the dimensions, form, and texture of any particles or structures.

SEM and Energy Dispersive X-ray Spectroscopy (EDX) can be used together for imaging as well as elemental analysis, which provides information about the chemical makeup of materials. Numerous fields, including materials science, biology and nanotechnology use SEM.

Advantages of SEM:

1. High resolution, allowing for detailed imaging of surface features.
2. Capability for elemental analysis when coupled with EDX.
3. Suitable for a wide variety of sample types and applications.

Limitations:

1. Sample preparation may require coating for non-conductive materials.
2. SEM only provides surface information and may not reveal bulk characteristics.

4. ICP-MS Analysis [117]

An incredibly sensitive technique for identifying and measuring trace elements and isotopes in a variety of samples is Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The method entails ionizing a sample in an inductively coupled plasma (ICP) and determining the mass-to-charge ratio of the resultant ions. With detection limits as low as parts-per-trillion (ppt), ICP-MS is capable of detecting a wide variety of elements, including transition metals, heavy metals, and rare earth elements. Because of its accuracy and sensitivity, ICP-MS is frequently utilized in geology, environmental analysis, pharmaceuticals, food and clinical diagnostics.

Advantages of ICP-MS:

1. High sensitivity and low detection limits for trace element analysis.

2. Capability to analyze complex samples with minimal sample preparation.
3. Can provide isotopic information, useful for tracing sources and understanding processes.

Limitations:

1. The technique requires expensive equipment and skilled operators.
2. Interferences from matrix effects or other ions can complicate analysis, though these can often be mitigated with appropriate methods.

5. ICP-AES Analysis [118]

ICP Optical Emission Spectroscopy (ICP-OES) is another name for this potent method of determining a sample's elemental makeup. It works by introducing a liquid or solid sample into an inductively coupled plasma (ICP), where it is atomized and excited. The excited atoms and ions emit light at characteristic wavelengths, which is then detected and analyzed to quantify the elements present.

ICP-AES is widely used for detecting and quantifying a broad range of elements in complex matrices such as water, soil, metals, and biological samples. It is known for its high sensitivity, rapid analysis, and ability to analyze multiple elements simultaneously.

Advantages of ICP-AES:[119]

1. Capable of multi-element analysis with high sensitivity and precision.
2. Wide dynamic range, allowing for the analysis of both trace and major elements.
3. Relatively simple sample preparation and rapid results.

Limitations:

1. Requires expensive instrumentation and trained personnel.
2. The technique may suffer from matrix interferences, which can affect accuracy, though this can be mitigated through calibration and internal standards.

PREVIOUS RESEARCH WORKS

Recent Research Works on Pharmaceutico - Analytical Study

1. Pareek A. et. al. (Sept. 2020) showed notable changes through the three-step Shodhan, Jarana and Marana procedure. The addition of plant extracts and herbs improved its therapeutic qualities and reduced the particle size to the nanoscale in the manufacture of Yashada bhasma. While the raw zinc metal showed crystallite zinc, X-ray diffraction (XRD) investigation showed a hexagonal ZnO crystalline phase in Yashada bhasma. Dynamic light scattering (DLS) indicated a reduction in particle size from 2063 nm for raw metal to 339.8 nm for Yashada bhasma, which was further confirmed by scanning electron microscopy (SEM), showing a particle size of 324 nm for the Bhasma compared to 1-2 μm for raw zinc. Additionally, zeta potential values confirmed the stability of the nanoparticles, and energy dispersive X-ray analysis (EDAX) highlighted differences in zinc and oxygen concentrations between the Bhasma and raw metal, validating the physicochemical transformations that occur during the preparation process.[120]

2. Sharma H. et al. (Mar. 2022) compared the physicochemical properties of Yashad Bhasma (YB) and Green-synthesized Zinc Oxide Nanoparticles (ZNP) using Aloe vera L. leaf extract. YB was prepared through 11-12 puta cycles with a gradual heating process, reaching a maximum temperature of 850°C. ZNP was synthesized by treating zinc nitrate hexahydrate with Aloe vera leaf juice, followed by 8 hours of heating at 150°C and 4500 RPM, resulting in a yield of 33.33%. Zinc oxide was identified as the predominant phase in both YB and ZNP by SEM-EDAX analysis, with average particle sizes of 20.237 μm and 1.846 μm, respectively. Additionally, trace components including carbon and nitrogen were detected in both samples. Functional groups such nitriles, alkenes, and nitrates were detected by FTIR analysis. Overall, the two synthesis methods yielded similar results analytically.[121]

3. Balakrishna A et al. (Jan 2023) examined the physicochemical changes of Jashada bhasma (JB) samples that were acquired through several marana cycles using a range of analytical methods, including as DLS, FTIR, FESEM, EDAX, XRD, and Raman spectroscopy. XRD study showed that the hexagonal ZnO wurtzite crystals' size decreased from 53.14 to 42.40 nm as a result of successive heat treatments that realigned the zinc and oxygen molecules. The morphological transition from 1.5 μm rods to 31 nm particles was shown by FESEM and SAXS. HRTEM verified that 10 nm nanoparticles were present. FTIR and Raman spectroscopy confirmed the presence of ZnO. Cell viability assays showed no toxicity in human colon Caco-2 cells (IC50 > 1000 μg/mL). ICP-MS analysis indicated a dose-dependent uptake and intracellular accumulation of JB nanoparticles, with a 6% w/w bioavailability of Zn^{2+} ions observed under acidic conditions. The study concluded that the physicochemical transformation of JB nanoparticles through incineration cycles enhances their bioavailability and biocompatibility, supporting their therapeutic potential as described in Ayurveda.[122]

4. Veda S. M. et al. (Feb. 2023) studied the classical preparation of Yashada bhasma, characterizing it using SEM and EDAX analysis. The carbon content was higher in shodhita yashada, while aluminum (Al) was present in shodhita and jarita samples but absent in marita, likely due to chemical transformation during incineration. Silicon (Si) was found in the jarita sample but not in the final product. The Zn content increased from 43% in shodhita to 48% in jarita, reaching 78% in the final marita product. SEM analysis showed a reduction in particle size at each stage, enhancing drug absorbability.[123]

5. Singh H. et al. (Aug. 2023) The study examined the function of particular phytochemicals, including proteins, terpenoids, and phenolic compounds, in the plant-

mediated synthesis of nanoparticles and evaluated their impact on particle stability, size, and characteristics. Additionally, it emphasized the prospective uses of these bio-derived nanoparticles, specifically in the fields of bioremediation, agriculture, disease control, and medicine administration, among other industries.[124]

6. Mahadeorao KV et al. (Oct 2023) carried out a pharmaceutical standardization of different processes in the production of Yashada bhasma, utilizing quality control tests to compare the chemical and physical characteristics of three batches. Following the Marana procedure, XRF analysis verified that Yashada had been converted to zinc oxide (ZnO). Particle size analysis revealed that 10% of particles had a size of 0.722 µm, 50% had 3.45 µm, and 90% were 16.6 µm. The physicochemical tests showed a loss on drying (LOD) of 0.03%, total ash content of 99.92%, acid-insoluble ash (AIA) of 98.86%, and water-soluble ash (WSA) of 2.86%.[125]

1.2.4 EXPERMENTAL STUDY REVIEW

1. Toxicity Study

a) Acute Toxicity Study:

Pareek A. et. al. (Oct 2013) used acute toxicity tests on Wistar rats to show that traditionally made Yashada bhasma (zinc ash) is harmless. No aberrant behavior, clinical symptoms, or death were noted when zinc ash was given orally at doses of 30 mg/kg and 300 mg/kg. In comparison to controls, there were no appreciable variations or pathological alterations in body weight increase, relative organ weights, or histological analysis of the main organs (liver, kidney, spleen, and heart). These findings indicate that Yashada bhasma is safe even at doses 10-100 times the optimal therapeutic dose (3 mg/kg).

b) Sub-Acute Toxicity Study

Pareek A. et. al. (Oct 2013) demonstrated the safety of zinc ash in a sub-acute toxicity study. There were no discernible changes in body weight, relative organ weights, SGOT, SGPT, serum creatinine, or urea levels in Wistar rats given dosages of 30 mg/kg and 300 mg/kg for 28 days. This suggests that there was no hepatotoxicity, cardiotoxicity, or nephrotoxicity. Histopathological examination revealed no major organ damage, confirming the safety of prolonged high-dose administration of zinc ash.[126]

c) Sub-Acute Toxicity Study

A sub-acute toxicity study on Swiss albino mice compared the safety profiles of Yashada bhasma and Yashada Pushpa Bhasma. Mice were treated with low (15.6 mg/kg) and high doses (78 mg/kg) for 28 days, and assessments included body weight, hematological and biochemical parameters, and tissue parameters.

Yashada bhasma: Showed no appreciable negative impacts on blood parameters, liver and kidney function tests, body weight, or organ weight. Histopathology revealed no structural abnormalities, confirming its safety even at higher doses.

Yashada Pushpa Bhasma: At higher doses, it caused a mild reduction in hemoglobin and RBC counts, elevated SGOT, SGPT, serum creatinine, and blood urea levels, indicating potential liver and kidney toxicity. Histopathology showed hepatocellular necrosis and proximal tubular damage, highlighting dose-dependent toxicity.[127]

2. Bio Availability study

a. In Vitro Bioavailabilty study:

Balkrishna A. et. al. (Jan 2023) assessed the JB cycle 17 nanoparticles' in vitro bioavailability in Caco-2 cells. For a full day, the cells were exposed to different doses of nanoparticles (1–300 μg/mL). The Pierce BCA test was used to determine the protein content, and ICP-MS was used to assess the total zinc concentration after normalizing it to protein levels. No cell viability loss was observed up to 1000 μg/mL (IC50 > 1000 μg/mL), confirming biocompatibility. A dose-dependent increase in intracellular Zn at 100 and 300 μg/mL indicated active internalization via endocytosis. These results suggest that JB cycle 17 nanoparticles can be absorbed in the gastrointestinal tract without cytotoxicity, supporting their bioavailability.

b. In Vivo Bioavailability Study:

Pareek A. et. al. (Oct 2013) conducted pharmacokinetic study on adult Wistar rats investigated the oral absorption of zinc ash. Rats were given either zinc ash (300 mg/kg) or 0.5% CMC solution orally after being split into control and treated groups (6 rats each). Atomic Absorption Spectroscopy (AAS) was used to measure the zinc levels in blood samples that were taken at different intervals. Serum zinc concentrations in the treatment group were significantly higher (~3.5 mg/L) than in the control group (~1 mg/L), peaking at 2 hours and rapidly declining to baseline levels within 4 hours. These results suggest that while zinc ash is absorbed through the intestine, a significant portion is likely excreted in feces. The transient rise in serum zinc confirms oral absorption, but the overall intestinal uptake appears limited.

3. Spermatogenic Activity Study

Review on Male Infertility Rat Model Development[128]

Male infertility rat models are commonly used in reproductive research to simulate human male infertility conditions. These models help investigate the underlying causes, mechanisms, and potential treatments for male infertility by employing various methods as below.

1. **Chemical Induction:** Chemical agents are used to induce male infertility in rats by disrupting hormonal regulation, spermatogenesis, or causing direct damage to reproductive organs. Common chemicals like adenine, cyclophosphamide, busulfan, or vincristine are used to induce infertility. These agents can cause testicular atrophy, hormonal imbalance, and reduced sperm count.

2. **Surgical Models:** Surgical approaches can involve vasectomy, epididymal ligation, or orchidectomy (removal of one or both testicles) to investigate infertility caused by mechanical blockages or hormonal disruption.

3. **Endocrine Disruption**: The use of exogenous hormones or the administration of hormone inhibitors can create infertility by disrupting the normal hormonal environment necessary for spermatogenesis. For example, administration of GnRH antagonists or aromatase inhibitors to reduce estrogen levels or disrupt FSH and LH levels.

4. **Genetic Models:** Genetic engineering techniques, such as knockout mice or gene editing, can create infertility by knocking out genes involved in spermatogenesis, such as those affecting sperm motility, acrosome formation, or the blood-testis barrier.

5. **Heat-Induced Infertility:** Heat exposure is another method to induce infertility, either by environmental temperature changes or using scrotal heat treatment. High temperatures impair sperm production by causing damage to Sertoli cells and germ cells.

6. **Diabetes-Induced Male Infertility:** Diabetic male rats, induced by streptozotocin or other diabetes-inducing chemicals, are a model for studying infertility due to metabolic disorders. Diabetes impacts sperm motility, DNA integrity, and hormonal balance.

Chemical Method: Adenine induced male infertility rat model:[129]

Adenine is a purine nucleobase that is a fundamental component of DNA and RNA, playing a key role in cellular processes such as energy transfer and cell signaling. In the context of adenine-induced infertility, high doses of adenine are administered to rats, where it accumulates in the testes and disrupts spermatogenesis. This leads to testicular damage, including degeneration of seminiferous tubules, inflammation, and a reduction in sperm count, making it an effective model to study testicular toxicity and infertility mechanisms. The model is also useful for exploring oxidative stress and potential therapeutic interventions for male infertility.

PREVIOUS RESEARCH WORKS:

a. Adenine induced male infertility rat model

Chen J. et al (2014) used adenine treatment (300 mg/kg/day) for **7,** 14, and 21 days to induce male infertility in rats. All durations effectively induced infertility, with the 21-day treatment causing the most severe effects, including significant changes in SMAD expression, hormonal imbalances (lower testosterone, higher LH and FSH),

and testicular damage. The 14-day and 7-day treatments showed less pronounced effects, but also contributed to infertility symptoms. The 21-day duration is considered the most effective for establishing a severe infertility model.[130]

Shen Y. et al. (2024) In this study, by exposing rats to chronic restraint stress (CRS) and adenine therapy, a rat model of male infertility (MI) with liver depression and kidney deficiency (LD & KD) was created. Significant MI symptoms, such as decreased responsiveness, altered mating behaviors, and testicular and epididymal atrophy, were displayed by the animal. Sperm abnormalities rose while sperm viability and count declined. Dysregulated reproductive hormones and elevated proinflammatory cytokines were observed, along with enhanced oxidative stress in reproductive tissues. Histopathological analysis revealed damaged seminiferous tubules.[131]

b. Adenine induced male infertility and zinc supplement

Yu ZZ. et al. (2014) investigated the impact of micronutrients on adenine-induced male infertility in rats. Four groups were used: adenine-only (inducing infertility), zinc supplementation (which improved sperm count, motility, testosterone levels, and antioxidant activity), other micronutrient supplementation (which also restored testicular function), and a control group. Zinc showed the most significant therapeutic effect, suggesting its potential as a treatment for male infertility caused by chemical agents like adenine.[132]

c. Impact of spermatogenesis and zinc deficiency

The molecular underpinnings of the impact of zinc shortage on spermatogenesis were investigated by **Omu AE. et al.** In rats, the researchers found that zinc shortage impacted spermatogenesis by causing decreased testosterone synthesis, elevated

oxidative stress, and apoptosis. Supplementing with zinc was associated with a reversal of these adverse effects, suggesting zinc's crucial role in male reproduction.[133]

d. **Yashada bhasma in Oligospermia**

Rajani B. et. al. evaluated the effect of Yashada bhasma (zinc calyx) as an internal medication for oligospermia in 42 patients, divided into two groups. The results showed a significant improvement in sperm count in the Yashada bhasma group**,** with 92.3% improvement**,** compared to the other group (Group B), which showed only a 0.27% improvement**.** This study highlights the therapeutic potential of zinc supplementation, in the form of Yashada bhasma, in treating oligospermia and improving sperm quality.[134]

1.3 JUSTIFICTAION

Zinc, a vital trace element, plays a critical role in numerous physiological processes, particularly in male reproductive health. As a significant component of seminal plasma, zinc influences various sperm parameters, including motility, morphology and overall semen quality.[135] The median concentration of zinc in seminal fluid, approximately 123 mg/L, underscores its importance in ensuring optimal reproductive function.[136] Zinc's deficiency has been directly associated with reduced sperm quality, impaired spermatogenesis and diminished fertility, highlighting its crucial role in maintaining male reproductive health. Yashada bhasma, an Ayurvedic preparation primarily composed of zinc oxide nanoparticles, offers a traditional yet scientifically intriguing approach to addressing male infertility and other zinc-related deficiencies.

The classical preparation of Yashada bhasma involves well-defined processes such as shodhana (purification) and marana (incineration), which are aimed at detoxifying the metal and transforming it into a bio-compatible and therapeutically potent form. For low-melting-point metals like zinc, an additional step called jarana (roasting) is employed between these processes to facilitate easier incineration and transformation into bhasma. These traditional methodologies have demonstrated safety and efficacy over centuries. However, advancements in material science and nanotechnology provide an opportunity to further potentiate these formulations through modern interventions.

One promising area of enhancement lies in the reduction of particle size. Classical bhasma preparation methods, while effective, can benefit from additional steps such as dry milling and wet grinding to achieve a finer, more uniform nanoscale

particle size. Reducing particle size has been consistently shown to increase solubility, thereby improving the bioavailability of active compounds. Modern research corroborates this phenomenon; for instance, Ishihara et. al. reported that micronized zinc oxide exhibited significantly higher bioavailability compared to its bulk counterpart.[137] Similarly, nanoparticle formulations of poorly water-soluble iron compounds have demonstrated bioavailability comparable to soluble salts.[138] Such findings suggest that further size reduction in Yashada bhasma could amplify its bioavailability and therapeutic potential.

Despite its well-documented benefits, there remains a notable gap in scientific research on the potentiation of Yashada bhasma through advanced size reduction techniques. While traditional preparation methods have been validated for safety and efficacy, their combination with modern size reduction techniques, such as ball milling, wet grinding, and subsequent characterization in the nano range, has yet to be explored. This innovative approach holds the promise of enhancing the bioactivity of Yashada bhasma, making it more effective for therapeutic applications, particularly in the domain of male reproductive health.

The planned study, “Evaluation of Potentiated Yashada bhasma for Safety, Bioavailability, and Spermatogenic Activity”, aims to address this gap by systematically investigating the impact of additional size reduction techniques on the bioavailability and spermatogenic efficacy of Yashada bhasma. This study integrates the classical principles of Ayurveda with contemporary scientific advancements, offering a unique opportunity to validate and refine traditional formulations.

2. AIM, OBJECTIVES AND HYPOTHESIS

Aim:

- To emphasize concept of potentiation of Yashada bhasma, its safety and spermatogenic activity.

Objectives:

- To Potentiate and Characterize Yashada bhasma
- To Screen Safety and Bioavailability of Potentiated Yashada bhasma
- To Evaluate Potentiated Yashada bhasma for Spermatogenic activity

Research Hypothesis (H_1):

- There is increased Spermatogenic effect of Potentiated Yashada bhasma over Classical Yashada bhasma.

3. MATERIALS AND METHODS

The study was conducted in two main phases: Pharmaceutical and Experimental. The pharmaceutical study encompasses following points:

- Procurement of genuine raw material and associated drugs for Shodhana, Jarana, Marana and authentified by the subject experts.
- Proper methods of processing like Samanya shodhana, Vishesha shodhan and Jarana.
- Marana procedure to obtain Classical Yashada bhasma (CYB).
- Marana procedure with additional size reduction methods to obtain Potentiated Yashada bhasma (PYB).
- Quality analysis of both the finished products.

3.1 PHARMACEUTICAL PART

Materials and Methods:

Materials include:

i. Major raw drugs.
ii. Associated drugs.
iii. Equipment's and associated equipment's.

i. Major Raw Drugs:

The raw material Yashada (raw zinc) has been provided by Dhootapapeshwar Pvt. Ltd (Batch no. 1PRML00278/Jun/2021). Parada and Gandhaka were procured from GMP certified K. L. E. Ayurveda Pharmacy, Khasbag, Belgaum.

ii. Associated Drugs:

- Kanji (sour gruel), Takra (butter milk), Kulattha Kwatha (decoction of *Dolichus biflorus* Linn.), Gomutra (cow's urine) and Tila taila (oil of *Sesamum indicum* Linn.*)* were used for samanya shodhana of Yashada. Gomutra was freshly collected from the local cow shed. Tila taila was procured from the K. L. E. Society's Ayurveda Pharmacy, Belgaum and other drugs were prepared freshly.

- Churnodaka (lime water) was used for vishesha shodhana of Yashada and it was prepared freshly in the department.

- Apamarga panchanga (*Achyranthes aspera* whole plant) churna was used for the Jarana of Yashada. Apamarga was procured from the K. L. E. Society's Ayurveda Pharmacy, Belgaum

- Fresh Godugdha (cow's milk), procured from the local cow shed was used for Gandhaka (*S*) Shodhana.

- Sudha Churna (lime stone), Rasona Kalka (paste of *Allium sativum* Linn.) and Saindhava lavana (rock salt) were used for samanya shodhana of Parada (*Hg*). Sudha and saindhava were procured from K. L. E. Society's Ayurveda Pharmacy, Belgaum. Rasona purchased from the market and got authenticated from the subject experts.

- Ghrita kumari swarasa (fresh juice of *Aloe vera* Tourm.) and Nimbu swarasa (fresh juice of *Citrus limon* Linn.) were used for Bhavana during Marana. Kumari and Nimbuka were procured from the market.

iii. Equipment's:

- Pithara yantra was used for shodhana of Yashada. A cement block with hole at the centre was used to cover the mouth of steel vessel (2.5 lit capacity).
- Khalva Yantra for parada, gandhaka shodhana and for bhavana.
- Ball mill was used for dry milling of Jarita yashada and PYB kajjali preparation and wet grinder was used for bhavana and chakrika nirmana in PYB preparation.
- Horizontal Electric Muffle furnace was used for Bhasmikarana.

Associated equipment's: Stainless steel vessels, iron ladle, earthen crucibles, gas stove, knife, juice extractor, measuring cylinder etc.

A. Schematic presentation of Classical Yashada bhasma preparation

Ashodhita Yashada (Ref: R. T: 19/95)

↓

Samanya shodhana (Ref: R. T: 15/ 4-6)
(Dhalana in Kanji, Takra, Kulattha kwatha, Gomutra and Tila Taila)

↓

Vishesha shodhana (Ref: R. T: 19/98,99)
(Dhalana in Churnodaka)

↓

Jaarana (Ref: R. T: 19/112-115)
(Roasting with Apamarga panchanga churna)

↓

Kajjali (Ref: Rasachandamshu Pu. Kh: Verse 575 & 576)
(Mardana with Shuddha Parada and Shuddha Gandhaka)

↓

Bhavana (Kumari swarasa and Nimbu swarasa)

↓

Chakrika Nirmana

↓

Sharava Samputa

↓

Gajaputa (in EMF)

B. Schematic presentation of Potentiated Yashada bhasma preparation

Ashodhita Yashada (Ref: R. T: 19/95)

↓

Samanya Shodhana (Ref: R. T: 15/ 4-6)

(Dhalana in Kanji, Takra, Kulattha kwatha, Gomutra and Tila Taila)

↓

Vishesha shodhana (Ref: R. T: 19/98,99)

(Dhalana in Churnodaka)

↓

Jaarana (Ref: R. T: 19/112-115)

(Roasting with Apamarga panchanga churna)

↓

Dry Milling in Ball mill for 24 hours

(1st additional size reduction)

↓

Kajjali (Ref: R.Ch. Pu. Kh: Verse 575 & 576)

(Trituration with Shuddha Parada and Shuddha Gandhaka in ball mill for 70 hours

(2nd distinct size reduction)

↓

Bhavana with Kumari swarasa and Nimbu swarasa in wet grinder for 24hours before each puta

(3rd distinct and additional size reduction)

↓

Chakrika Nirmana

↓

Sharava Samputa

↓

Gajaputa (in EMF)

(4th additional size reduction by additional 2 puta even after attainment of Bhasma siddha laxanas by the end of 2nd puta)

Practical No. 01

Name	:	Samanya Shodhana of Parada
Aim	:	To do shodhana of Parada for Yashada marana
Date of Commencement	:	19/09/2022
Date of Completion	:	23/09/2022
Reference	:	R. T: 5/27-29.
Materials	:	1. Parada – 300 gm 2. Sudha churna – 300 gm 3. Sudha mardita Parada – 250 gm 4. Saindhava Lavana – 125 gm 5. Lashuna – 250 gm
Instruments & Equipment's:		Khalva yantra, steel vessels, gas stove etc.

Procedure:

With Sudha Churna

Initially 300 gm each of Parada and sieved sudha churna were triturated in a khalva yantra.

During Mardana:

- Initially, Sudha churna was light and was spattering out.
- After 5 hours: Parada formed small globules, Sudha churna became heavier.
- After 9 hours: Parada formed elongated structures and mixed with Sudha.
- After 17 hours: Sudha churna turned to grey color.
- After 24 hours: Complete color transformation of Sudha to gray color and homogeneous mixing was observed

Observations: Parada became minuscule, with some part collected at the center under a thick sudha layer.

Separation of Parada: Parada globules were collected in stages:

- 200gm initially with a spoon.
- 30 gm after mixing with hot water.
- 20 gm after soaking Sudha with hot water overnight and drying.

Total Parada recovered: 250 gm (50 gm loss due to handling and incomplete separation).

With Saindhava and Lashuna: 125 gm of coarse saindhava lavana and 250 gm dehusked lashuna triturated to form kalka and to this parada was added and mardana performed:

During mardana

- After 2 hours: Kalka turned blackish; Parada became almost invisible.
- Post 30 mins gap: Parada globules united with increased shine.

Observations: Parada regained enhanced shine and formed larger globules.

Separation of Parada:

- 200 gm initially as a large globule.
- 30 gm after treating kalka with hot water and repeated washing.

Total Parada recovered: 230 gm (20 gm loss likely due to Jala gati).

Precautions:

- Careful handling during mardana to prevent Parada spillage.
- Avoiding Jala gati during separation of Parada from Sudha churna and kalka.

Kalpa siddhi pareeksha: Bright shiny parada was obtained at the end.

Practical No. 02

Name	-	Gandhaka Shodhana
Aim	-	To do shodhana of Gandhaka for Yashada marana
Date of Commencement	-	24/09/2022
Date of completion	-	24/09/2022
Reference	-	R. T: 8/7-12
Drugs used	-	1. Gandhaka – 750 gm 2. Godugdha – 3 Liters
Method	-	Dhalana

Instruments & Equipment's: Weighing machine, khalva yantra, iron laddle, stainless steel vessel, steel sieve, cloth, pH meter, thermometer etc.

Procedure:

- 50 gm of authenticated Gandhaka was coarsely powdered using a clean khalva yantra.
- 3 liters of fresh Godugdha (cow's milk) with an initial pH of 5.3 was taken in a clean stainless-steel vessel. The vessel's mouth was covered with a steel sieve and a cotton cloth.
- The powdered Gandhaka was heated in a loha darvi over a mild flame.
- After 15 minutes, at 118°C, the Gandhaka melted completely and was poured through the sieve and cloth into the milk.
- Granular Shuddha Gandhaka was collected, washed with hot water, and dried.
- The procedure was repeated twice more.

Observations:

- Gandhaka emitted a peculiar smell during pounding.
- Gandhaka began melting at 118°C after 15 minutes of heating.
- Godugdha color changed from white (shweta) to yellow (haridra varna).
- The purified Gandhaka formed small masses and granules.

Precautions:

- Avoid burning Gandhaka by maintaining a mild flame.

Kalpa siddhi Pareeksha: Greenish yellow coloured, shiny gandhaka was obtained.

Organoletic Characters:

Color	**:** Greenish yellow
Odour	: Dravyagata
Taste	: Katu
Appearance	: Solid

Results:

- **Initial weight of Gandhaka**: 750 gm
- **Final weight of Gandhaka**: 706 gm
- **Weight loss**: 44 gm (due to impurities, washing loss, and absorption in milk fat).
- **pH of milk after shodhana**: Reduced from 5.3 to 5.1.

Practical No. 03

Name : Yashada Samanya Shodhana

Aim : To do samanya shodhana of Yashada

Date of Commencement : 25/09/2022

Date of Completion : 01/10/2022

Reference : R. T: 15/ 4-6

Materials:

Table No. 03: Ingredients and quantity used

Sl. No.	Media	Quantity of Media (ml)	Quantity of Yashada (gm)
1.	Kanji	2000	1170
2.	Takra	2400	1053
3.	Kulattha kwatha	1950	954
4.	Gomutra	2100	847
5.	Tila taila	2100	798

Note: Takra[139] and Kulattha kwatha were prepared as per classics.

Method : Dhalana

Equipments : Iron ladle, gas stove, steel vessels, pithara yantra, etc.

Procedure:

1. Yashada pieces were melted in an iron ladle over intense heat (teevragni) using a gas stove.
2. The molten Yashada was poured into the respective medium (kanji, takra, kulattha kwatha, gomutra, or tila taila) using a Pithara yantra.
3. After cooling, the Yashada was removed, washed with hot water, and dried using a cotton cloth.

4. This process (dhalana) was repeated three times for each medium, using fresh media each time.

Precautions:

- The molten Yashada was poured slowly and carefully to avoid splashing or spillage.
- Heating was stopped immediately after the Yashada completely melted.
- Care was taken to use the appropriate quantity of liquid media to prevent spilling during Dhalana.

Observations:

- **Kanji:** Black coating appeared on the surface of molten Yashada during the first dhalana. The pH decreased after dhalana, and the liquid showed bubbling and heating effects.
- **Takra:** A yellow tinge was observed on Yashada after dhalana. Takra's colour became greyish and lost opacity after each dhalana.
- **Kulattha Kwatha:** Black fumes and a reddish-brown tinge appeared during the process. The kwatha became more transparent after dhalana.
- **Gomutra:** Frothing and black fumes were observed. The Yashada became brittle, and gomutra turned black after each dhalana.
- **Tila Taila:** The taila caught fire briefly when molten Yashada was added. Its consistency became watery, and the colour changed to brownish.

Common Observations

- The weight of Yashada reduced progressively with each dhalana, indicating the removal of impurities and loss.

- The physical properties of the media (colour, pH and consistency) changed significantly after each dhalana, demonstrating their role in purification.

Kalpa siddhi pareeksha: Silvery white shiny Yashada was obtained at the end.

Organoleptic Characters:

Colour **:** Silver white

Odour **:** Nirgandha

Taste **:** Kashaya & katu

Appearance **:** Solid

Table No. 04: Observations and Results

Media	Melting Started (°C)	Completely Melted (°C)	pH (Before)	pH (After)	Initial Weight of Yashada (gm)	Final Weight of Yashada (gm)	Weight Loss (gm)
Kanji	633	719	4.13	3.71	1170	1053	117
Takra	640	700	3.57	4.13	1053	954	99
Kulattha Kwatha	600	720	5.73	6.18	954	847	107
Gomutra	600	650	7.17	7.30	847	798	49
Tila Taila	600	680	5.84	6.05	798	741	57

Practical No. 04

Name : Vishesha Shodhana of Yashada in Churnodaka

Aim : To do vishesha shodhana of Yashada

Date of Commencement : 03/10/2022

Date of Completion : 03/10/2022

Reference : R. T: 19/ 98, 99

Materials : 1. Samanya shodhita Yashada – 741 gm

2. Churnodaka – 7.5 litres

Instruments &Equipments : Gas stove, iron ladle, steel vessels and pithara yantra.

Procedure:

- The white solid mass of yashada was melted and poured into 700 ml of churnodaka using a pithara yantra. After cooling, the Yashada was removed, washed with hot water, and the churnodaka was replaced for each subsequent dhalana.
- This process was repeated seven times.

Precautions:

- Care was taken while dhalana to avoid spillage.
- After each dhalana, it was washed with hot water.

Key Observations:

- **1st Dhalana:** Melting occurred at 470°C and churnodaka was boiling when molten yashada was added and brittleness slightly increased.
- **2nd Dhalana:** Melting started at 450°C without fire catching. Churnodaka's color remained unchanged, and the Yashada became lustrous and brittle.
- **3rd Dhalana:** Produced hissing sounds and resembled "corn flakes" during heating.

- **4th Dhalana:** Took longer time to melt, with a brownish tinge forming on the surface.
- **5th Dhalana:** Produced hissing sounds and a golden-brown tinge.
- **6th Dhalana:** Blackish tinge appeared after immersion in Churnodaka, forming a brittle, irregular mass.
- **7th Dhalana:** Melted quickly, forming a large, irregular brittle mass.

Kalpa siddhi pareeksha: Silvery bright shiny yashada was obtained at the end.

Organoleptic Characters:

- **Colour** **:** Silver white
- **Odour** **:** Nirgandha
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Solid

Observations and Results:

Table No.05: Observations during Vishesha Shodhana of Yashada

Dhalana	Churnodaka (ml)	Melting Started (°C)	Completely Melted (°C)	pH Before	pH After	Initial Weight (gm)	Final Weight (gm)
1st	700	540	470	11.34	11.02	741	711
2nd	700	450	460	11.34	11.00	711	684
3rd	700	480	510	11.34	11.01	684	681
4th	700	500	510	11.34	11.01	681	670
5th	700	510	520	11.34	11.01	670	667
6th	700	500	550	11.34	11.01	667	663
7th	700	510	560	11.34	11.01	663	660

Practical No. 05

Name	:	Yashada jarana
Aim	:	To do jarana of Yashada
Date of Commencement	:	04/10/2022
Date of Completion	:	04/10/2022
Reference	:	R. T: 19/ 112-115
Materials	:	1. Shuddha Yashada – 660 gm 2. Apamarga Panchanga churna – 165 gm
Equipments	:	Iron pan, gas burner, iron pestle etc.

Procedure:

- A clean iron pan was heated red-hot to remove any residues, then cooled (Swangasheeta) and used.
- The gas burner was placed near a ventilated window for proper airflow.
- Shodhita Yashada was placed in the pan and melted completely.
- Using an iron pestle, vigorous mardana (triturating) was performed after adding 10–15 grams of Apamarga Panchanga Churna.
- A further 10 to 15 grams was added once the churna had burned entirely, and the mardana proceeded.
- During mardana, offensive grey vapors developed that necessitated wearing a mask. Yashada turned into ash or powder after 70 mins of nonstop mardana.
- After gathering the powder in the middle of the pan and covering it with an earthen sharava, the pan was heated to a red-hot temperature for an hour.
- After that, the pan was left to cool overnight after the fire was put out.

- The sharava was taken out the following morning, and the grey powder (Jaarita Yashada) was gathered and weighed.

Precautions:

- A face mask was worn to avoid inhaling fumes.
- Mardana was performed continuously and with pressure.

Kalpa siddhi pareeksha: Grey colour yashada powder was obtained at the end.

Organoleptic Characters:

- **Colour** **:** Grey
- **Odour** **:** Dravyagata
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Powder

Observations:

- Offensive grey fumes evolved during mardana.
- A total of 165 gm of Apamarga Panchanga Churna (1/4th of Yashada) was used.
- Continuous vigorous mardana for 70 minutes was required.
- The resulting Jaarita Yashada was a fine blackish powder with tiny metallic particles.
- Final weight of Jaarita Yashada: 600 gm (weight loss: 60 gm).

Practical No. 06

Name	:	Yashada Marana (CYB)
Aim	:	To do marana of Yashada
Date of Commencement	:	27/11/2023
Date of Completion	:	07/12/2023
Reference	:	Rasachandamshu: Pu.Kh. Verse 575 & 576
Materials	:	1. Jarita Yashada: 200 gm 2. Shuddha Parada**:** 50 gm 3. Shuddha Gandhaka: 50 gm 4. Kumari Swarasa**:** 150 ml 5. Nimbu Swarasa**:** 150 ml
Instruments & Equipments:		Khalva yantra, spoon, muffle furnace, earthen sharavas, cotton cloth and multani mitti.

Procedure:

1. Mardana of Jarita Yashada with Parada

- Jarita Yashada (200 gm) was triturated with Shuddha Parada (50 gm) in a Khalva Yantra in two batches over 15 hours.
- Initially, rough particles of Yashada resisted the flow of mardana. After nine hours, Parada was completely amalgamated, forming a fine, grey, heavy powder.

2. Mardana with Gandhaka

- The prepared mixture was triturated with Shuddha Gandhaka (50 gm) in two batches over 15 hours (7 hours on Day 1 and 8 hours on Day 2).
- The final product was a fine, dark grey-black powder without any visible metallic particles.

3. Mardana with Kumari Swarasa

- The Kajjali mixture was triturated with Kumari Swarasa (150 ml) in three batches, adding 50 ml at two-hour intervals.
- After 8 hours, the paste was black, soft, and dried.

4. Mardana with Nimbu Swarasa and Chakrika Formation

- The Kajjali mixture was triturated with Nimbu Swarasa (150 ml) in two stages, forming a black, thick paste after 6 hours.
- Chakrikas (67 in total) were prepared, weighing 330 gm, and dried in shade.

5. Sharava Samputa Preparation

- Chakrikas were placed in earthen sharavas, arranged into two samputas, sealed with Multani Mitti-coated cotton cloth (seven layers), and dried layer-wise over 48 hours.

6. Marana (Incineration)

- Samputas were incinerated in a muffle furnace with a peak temperature of 950°C, maintained for 3 hours.
- After cooling, the chakrikas (330 gm) reduced to 150 gm, resulting in an off-white to dull yellow powder.

Precautions:

- Proper ventilation and face masks were used during mardana to avoid exposure to fumes and powder spillage was minimized during trituration.
- Swarasa was added in controlled quantities as per Rasatarangini guidelines.
- Uniform size and proper drying of chakrikas ensured consistency.

Kalpa siddhi pareeksha: Light yellow colour, fine bhasma was obtained at the end.

Organoleptic Characters:

- **Colour** **:** Light yellow
- **Odour** **:** Nirgandha
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Powder

Observations:

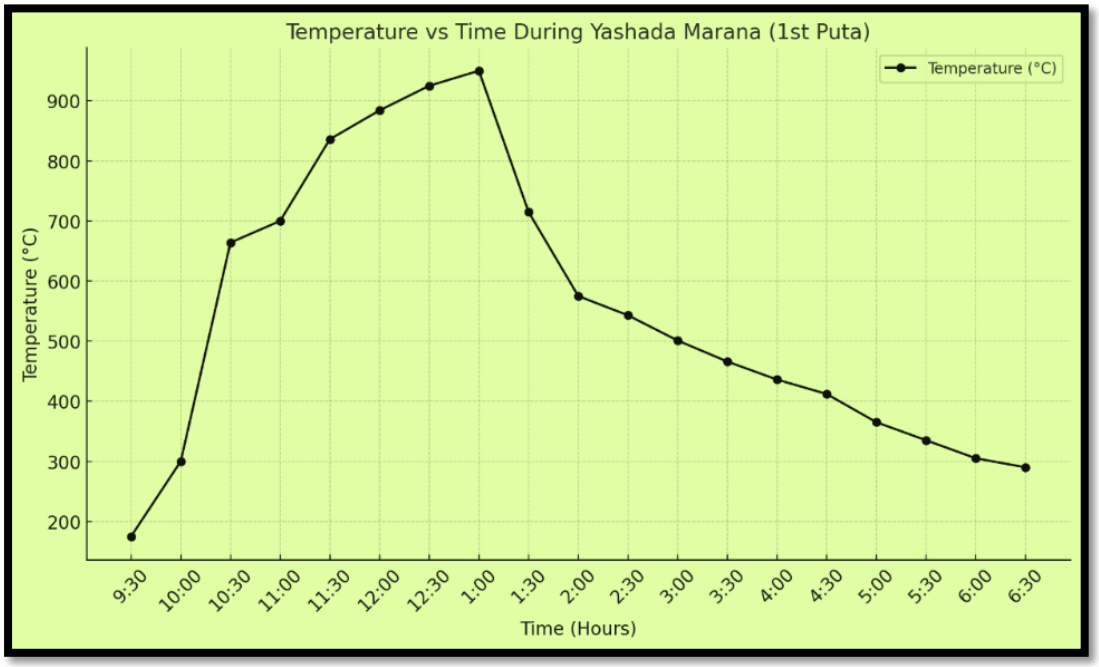
- The Yashada powder transformed into fine bhasma with no visible metallic particles.
- Rekhapurnata, Nishchandrata, Nirdhuma, and Niswadu tests were passed, but the Varitara test was partially passed, as trace particles settled down.
- A second puta was planned for further refinement.

Table No. 06: Temperature pattern during Yashada marana (1st Gajaputa)

Time (hr)	Temperature (°C)
9:30 AM	175
10:00 AM	300
10:30 AM	664
11:00 AM	700
11:30 AM	836
12:00 PM	884
12:30 PM	925
1:00 PM	950
1:30 PM	715
2:00 PM	575
2:30 PM	543

3:00 PM	501
3:30 PM	466
4:00 PM	436
4:30 PM	412
5:00 PM	365
5:30 PM	335
6:00 PM	305
6:30 PM	290

Graph No. 1: Temperature graph during CYB 1st Gajaputa



Practical No. 07

Name	:	Yashada marana second puta (CYB 2nd Puta)
Aim	:	To do Marana of Yashada
Date of commencement	:	07/12/2023
Date of completion	:	10/12/2023
Reference	:	R. Ch: Purva khanda Verse 575 & 576
Materials	:	1. Bhasma after 1st puta – 150 gm 2. Parada – 37.5 gm, Gandhaka – 37.5 gm 3. Kumari swarasa – 100 ml 4. Nimbu swarasa – 70 ml
Instuments &Equipments	:	Muffle furnace, khalva yantra, spoon, earthen sharavas, cotton cloth and multani mitti.

Procedure:

- The procedure remains same as done above.
- Yashada bhasma after 1st puta and shuddha parada were taken and triturated in a clean khalva yantra for five hours until all the parada was mixed homogenously with bhasma.
- Then shuddha gandhaka was added to the above mixture and triturated for three hours.
- Later bhavana with kumari swarasa was given for three hours and nimbu swarasa for three hours.
- When the mixture turned to semisolid state, chakrikas were prepared and dried under shade.
- Sandhibandhana was done as before.

Observations:

- The colour of chakrikas was greyish.
- Weight of chakrikas was 270 gm.
- Total 38 chakrikas were prepared.
- Room temperature was 19 ^{o}C and humidity was 65%.
- Temperature initially was 35° C.
- Maximum peak temperature recorded was 950°C.
- Maximum temperature range was 700 to 950°C which was maintained for 3 hours.
- After swangasheeta, sharava were opened and the chakrikas were obtained which were light yellow in colour.
- Weight of these chakrikas was 167 gm.
- There were no shining particles in these chakrikas.
- After pounding in khalva we got very fine bhasma which was very soft to touch.
- Final weight of bhasma after powdering was 160 gm.
- All the bhasma parikshas including Varitara, unama, nirdhuma, apunarbhava, niruttha, niswadu and nishchandrata were passed by this bhasma.

Kalpa siddhi pareeksha: Light yellow yashada bhasma was obtained at the end.

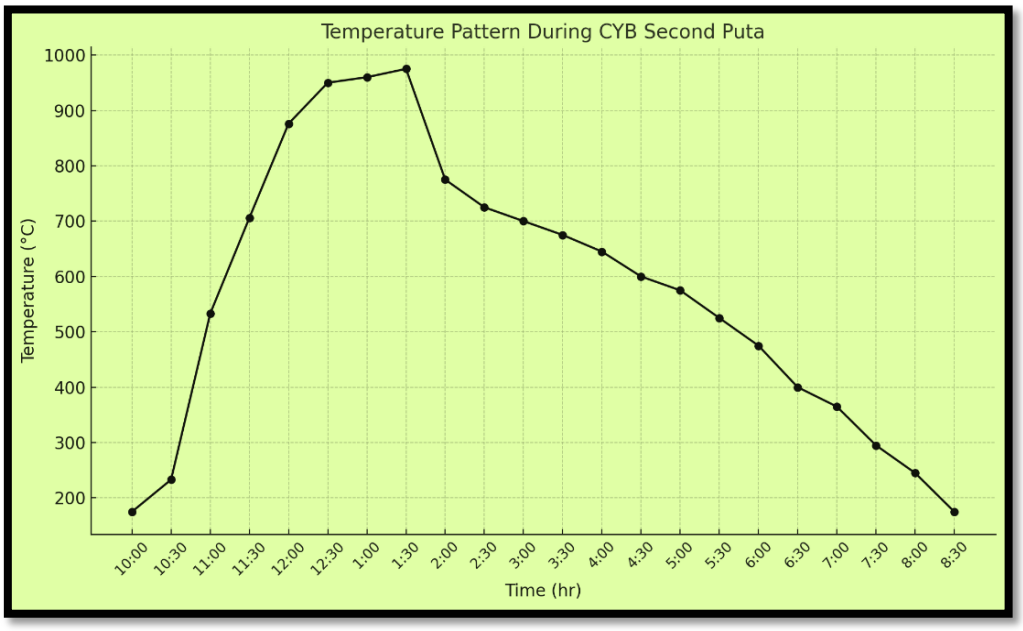
Organoleptic Characters:

- **Colour** **:** Light yellow
- **Odour** **:** Nirgandha
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Shlakshna churna

Table No. 07: Temperature pattern during CYB second puta

Time (hr)	Temperature (^{o}C)
10.00 AM	175
10.30 AM	233
11.00 AM	533
11.30 AM	706
12.00 PM	876
12.30 PM	950
1.00 PM	960
1.30 PM	975
2.00 PM	775
2.30 PM	725
3.00 PM	700
3.30 PM	675
4.00 PM	645
4.30 PM	600
5.00 PM	575
5.30 PM	525
6.00 PM	475
6.30 PM	400
7.00 PM	365
7.30 PM	295
8.00 PM	245
8.30 PM	175

Graph No. 02: Temperature graph during CYB 2nd puta



Sample 2 Potentiated Yashada bhasma (PYB) Preparation

Practical No. 08

Name	:	Ball milling of Jarita Yashada (Additional size reduction by Dry milling)
Aim	:	To dry mill the jarita yashada for additional size reduction.
Date of commencement	:	04/01/2024
Date of completion	:	09/01/2024
Materials	:	Jarita Yashada – 450 gm
Instrument & Equipments	:	Ball mill, weighing machine, spoon & plate. Ball Mill Specifications • (Science Industries Pvt. Ltd., 5 kg capacity) • RPM – 136/min • Ball no. and sizes (1:2:2:3) (Big size 6, Medium size -12, Small size 12 & Very small size – 18)

Procedure:

- 500 gms of jarita yashada was taken and added to jar of the ball mill.
- Jar was closed with additional rubber gasket followed by plate.
- Milling was done for 24 hours with intermittent scraping of the material from the walls of the jar and closing plate.

Precautions:

- Continuous milling should be avoided as it led to increased temperature of the jar and intermittent scraping should be done to keep material in the track.

Observations and results:

- After milling for two hours continuously the jar was getting hot.
- When the jar was opened and scraped the sticked material to the walls it was in small flakes form and colour more darker and powder was very fine.

Kalpa siddhi pareeksha: Greyish black colour fine powder was obtained at the end.

Organoleptic Characters:

- **Colour** **:** Greyish black
- **Odour** **:** Dravyagata gandha
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Shlakshna churna

Practical No. 09

Name	:	Potentiated Yashada bhasma kajjali (PYB K) preparation (Additional size reduction by Dry milling)
Aim	:	To prepare Potentiated Yashada bhasma kajjali by dry milling.
Date of commencement	:	09/01/2024
Date of completion	:	31/01/2024
Materials	:	1. Ball milled jarita yashada – 200 gm 2. Shuddha Parada – 50 gm 3. Shuddha Gandhaka – 50 gm
Instruments & Equipments	:	Ball mill, khalva yantra, spoon and plate

Procedure:

- Ball milled jarita yashada was taken in a clean khalva yantra and mardana was done to make it fine powder. Out of this 200 gm was taken for practical.
- After one hour of mardana shuddha parada and shuddha gandhaka were mixed in it and mardana was continued.
- After one hour the mardana, the mixture was transferred to ball mill.
- Milling was done intermittently for 70 hrs.

Precautions:

- As the milling continued, the weight of the powder reduced and dusting was seen. So, care was taken for proper closure of the jar.
- Intermittent milling was done to avoid heating up of the motor leading to flaking of the material.

- Intermittent scraping of the material done every day to keep the material in track.

Observations and results:

- After first hour of mardana on day 1, the small flake like aggregations reduced and it became fine powder.
- When shuddha parada and gandhaka were added and mardhana was done the mixture was not homogenous still small globules of mercury were seen before transferring ball mill.
- After 08 hours milling of day 1, the parada shiny particles were almost invisible and the whole mixture was turned into grayish black colored fine powder with dusting on opening the jar.
- At every 08 hours a small sample was taken from the jar for noting physical appearance.
- At the end of milling the powder was dark grey to almost black colour with no shining particles of Jarita yashada or parada visible to naked eyes.

Kalpa siddhi pareeksha: Blackish grey colour fine powder without any shiny particles was obtained at the end.

Organoleptic Characters:

- **Colour** **:** Blackish grey
- **Odour** **:** Dravyagata gandha
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Atyanta **s**hlakshna churna

Practical No. 10

Name	:	Wet grinding with Kumari Swarasa and Nimbu Swarasa (Additional time of wet grinding)
Aim	**:**	To do bhavana of Potentiated Yashada bhasma kajjali.
Date of commencement	:	05/02/2024
Date of completion	:	10/02/2024
Materials	:	1. Kumari Swarasa – 800 ml 2. Nimbu swarasa – 250 ml 3. Potentiated Yashada bhasma kajjali- 350 gms
Instruments & Equipments	:	Wet grinder (Capacity – 2 Kg), measuring jar, spatula & spoon.

Procedure:

- To the clean wet grinder, PYB kajjali was added and 200 ml of kumari swarasa was added initially to it and roughly mixed with spatula before starting the grinder.
- Then grinder was started and intermittent grinding was done for 15 hours with timely addition of kumari sawara to facilitate proper grinding.
- After 15 hours of grinding to the same mixture nimbu swarasa of 150 ml added initially and grinding was done for 9 hours with intermittent addition of nimbu swarasa.

Precautions:

- The quantity of liquid media taken was not according to the reference in Rasatarangini which states that the liquid should be taken only so much so as to wet the powder, because grinding was fixed for 24 hours.

- Liquid media were added in between to facilitate proper grinding.
- The size of the chakrikas was maintained uniformly and they were dried in shade.

Observations and results:

- Total hours of grinding done were 24 hrs.
- The grinding was very easy in the beginning due to sufficient quantity of liquid media and was difficult when it depleted which can be appreciated by consistency of material and sound heard during grinding.
- The paste formed after adding of swarasa was black coloured.
- As in this procedure bhavana was fixed for 24 hours subhavita laxanas were not considered for termination after onetime addition of liquid media.
- At the end of 24 hrs of wet grinding the consistency was thick semisolid paste with soft texture.
- At the end of grinding subhavita laxana were observed and chakrikas were prepared.
- The weight of wet chakrika was 345gms and after drying weight was 323 gms.

Kalpa siddhi pareeksha: Grey colour chakrika were obtained at the end.

Organoleptic Characters:

- **Colour** **:** Grey
- **Odour** **:** Dravyagata gandha
- **Taste** **:** Kashaya & katu
- **Appearance** **:** Solid round pellets

Practical No. 11

Name : Potentiated Yashada bhasma 1st Puta sharava samputikarana

Aim : To do samputikarana of sharava for bhasmikarana.

Date of commencement : 11/02/2024

Date of completion : 14/02/2024

Instruments & Equipments : Weighing machine, earthen sharavas, cotton cloth and multani mitti.

Procedure:

- Four equal sized big earthen sharavas with circumference 15.5 cm and 2.5 cm depth at the center, weighing around 165-166 gm were purchased from the local market and were kept immersed in water for 02 hours.
- After drying of sharavas, the chakrikas were arranged and kept inside one of the sharavas and covered by another.
- In each sharava 33 and 34 chakrikas were kept and sandhibandhana was done.
- Cotton cloth of 4 angula width smeared with multani mitti was used for sandhibandhana. Total seven layers of sandhibandhana was done.

Table No. 08: Seven layers of Sandhibandhana

Date	Time (hr)	Layer
11/02/2024	10.00 AM	First
11/02/2024	04.00 PM	Second
12/02/2024	10.00 AM	Third
12/02/2024	04.00 PM	Fourth
13/02/2024	10.00 AM	Fifth
13/02/2023	04.00 PM	Sixth
14/02/2024	10.00 AM	Seventh

Practical No. 12

Name : Marana (PYB 1st Puta)

Aim : To do marana of Yashada

Date of commencement : 15/02/2024

Date of completion : 16/02/2024

Reference : Rasa chandamshu Pu. Kh: Verse 575 & 576

Instruments & Equipments : Muffle furnace & samputita sharavas

Procedure:

- Samputita sharavas were kept inside the muffle furnace and it was closed.
- Temperature was set to 950 ^{o}C.
- Furnace was made on.
- Every half hour once the temperature was recorded.

Observations and results:

Initial weight of chakrikas - 323 gm

Weight of chakrikas after incineration - 196 gm

Loss - 127 gm

Colour of the chakrikas after incineration - Off white to dull yellow

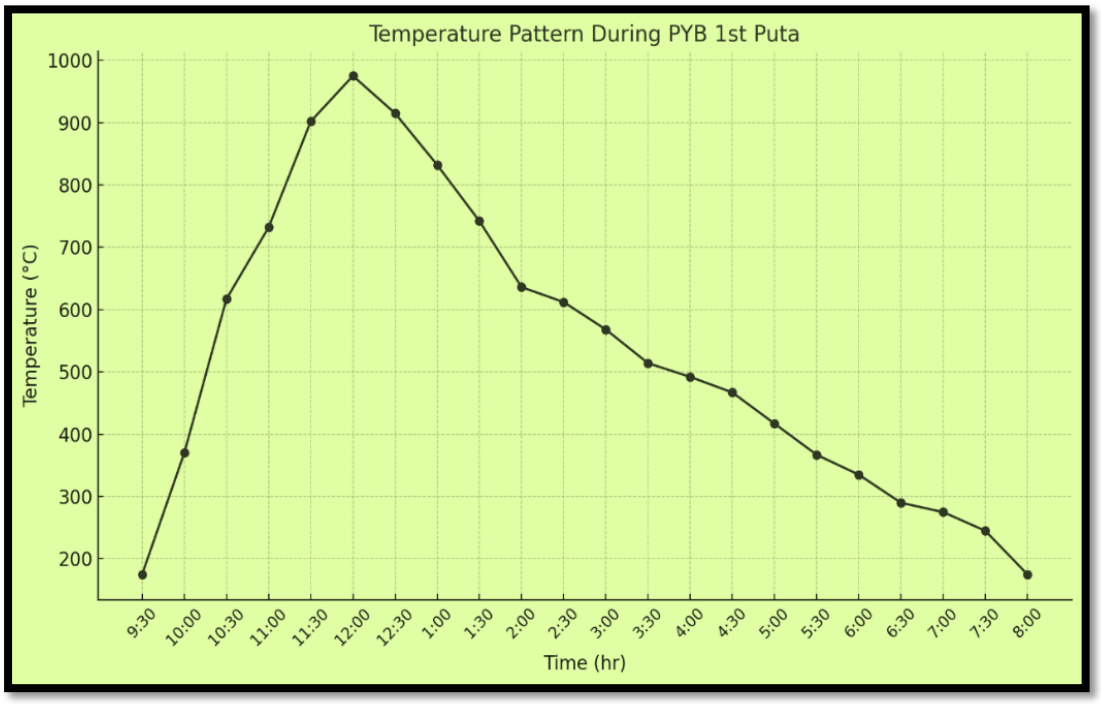
- There were no shining particles present on the surface of chakrikas.
- They were pound well in a khalva yantra and made into powder and subjected to bhasma parikshas like rekhapurnata, varitara, unama and nirdhuma. Among these parikshas rekhapurnata, nishchandrata, niswadu and nirdhuma were passed by the prepared bhasma but varitara was partially passed.
- As some trace particles were settling down in varitara pariksha, second puta was planned.

- A 10gm sample was taken out for analysis and remaining 186 gms was taken forward to 2nd puta.
- Room temperature was 24° C and humidity was 66%.
- Temperature furnace was initially was 35° C.
- Max peak temperature recorded was 950° C.
- Max Temp range was 700 to 950° C which was maintained for 3 hours.

Table No. 09: Temperature pattern during PYB 1st puta

Time (hr)	Temperature (° C)
9.30 AM	175
10.00 AM	370
10.30 AM	617
11.00 AM	732
11.30 AM	902
12.00 PM	975
12.30 PM	915
1.00 PM	832
1.30 PM	742
2.00 PM	636
2.30 PM	612
3.00 PM	568
3.30 PM	514
4.00 PM	492
4.30 PM	467
5.00 PM	417
5.30 PM	367
6.00 PM	335
6.30 PM	290
7.00 PM	275
7.30 PM	245
8.00 PM	175

Graph No. 03: Temperature graph during PYB 1st puta



Note: The temperature gradient in the muffle furnace is fixed. It gradually rises to nearly 100℃ every 15 minutes. It is consistent with the next 3 putas.

Practical No. 13

Name : Marana of yashada (PYB) in successive puta (2nd, 3rd and 4th Puta)

Aim : To do marana of yashada.

Date of commencement : 17/02/2024

Date of completion : 07/03/2024

Reference : Rasa chandamshu Pu. Kh: Verse 575 &576

Instruments & Equipments : Weighing machine, wetgrinder, muffle furnace, spoon, plate, measuring jar, sharavas, cloth and mud etc.

Table No. 10. Ingredients and their quantity in each puta

Sl. No.	Puta	Ingredients	Quantity
1.	2nd Puta	1st Puta Bhasma	140 gm
		Shuddha Parada	35 gm
		Shuddha Gandhaka	35 gm
2.	3rd Puta	2nd Puta Bhasma	60 gm
		Shuddha Parada	15 gm
		Shuddha Gandhaka	15 gm
3.	4th Puta	3rd Puta Bhasma	37 gm
		Shuddha Parada	9.3 gm
		Shuddha Gandhaka	9.3 gm

***Note:** Quantity of bhasma obtained at each puta was not fully taken for next puta as per the quantity of Sh. Parada.

Procedure:

- The obtained quantity of Yashada bhasma from the previous puta was transferred to the Khalwayantra, followed by wet grinder where grinding was conducted with fresh Kumari swarasa for 15 hours and Nimbu swarasa 9 hours.

- When the mixture reached a paste like, semisolid consistency, small chakrikas were formed and dried on the steel plate.
- All dried pellets were weighed before being placed inside the sharava and sealed with another.
- Sandhibhandhana of the sharavas was repeated as with the previous puta.
- The sharava samputa was placed in the electric muffle furnace and subjected to marana (calcination), reaching a maximum temperature of 1000°C maintained for 30 mins before cooling naturally.
- After cooling, the sharava samputa was removed, and the chakrikas were collected.
- This procedure was repeated two more times, with each puta using fresh kumariswarasa and nimbu swarasa for bhavana, until the appearance of Bhasma siddhi lakshanas was achieved.
- The collected Yashada bhasma was powdered and weighed and assessed for Bhasma siddhi lakshanas at each puta.

Precautions:

- Fresh Swarasa was used for each bhavana.
- During grinding, ensure any material sticking to the sides of khalwa yantra is regularly scraped off using a spatula.
- Chakrikas were thoroughly dried in the shade before incineration.

Observations:

- Swarasa quantity was gradually reduced after 3rd and 4th puta.
- Trituration time ranged from 24 hours before each puta.
- Detailed observations of each puta are displayed in Tables No. 11 and 12.

Table No. 11. Bhavana dravya quantity and weight of chakrika at each puta

Sl. No.	Date	Date		Quantity of Bhavana dravya		Weight of Chakrika	
		Commencement	Completion	Kumari	Nimbu	Before	After
1.	2nd Puta	19/12/2024	21/02/2024	600 ml	200 ml	208 gm	116 gm
2.	3rd Puta	24/02/2024	28/02/2024	500 ml	170 ml	87 gm	43 gm
3.	4th Puta	01/03/2024	06/03/2024	200 ml	80 ml	53 gm	25 gm

Table No. 12. Observations during each incineration

Sl. No	Puta	Observations & Results
1.	**2nd** Puta	• All the chakrikas completely baked and turned into yellow colour. • The chakrikas were soft to touch and were breaking with slight difficulty. • After powedring of chakrikas, the Bhasma was smooth in texture. • The colour of the Bhasma faded when compared to the 1st Puta • Varitara was passed completely and Rekhapurnata was appreciated
2.	**3rd** Puta	• All the chakrikas were completely baked and turned into yellow colour. • The chakrikas softer than the 2nd Puta and can be easily broken. • The texture of the chakrikas were very soft like butter while touching • The colour of the Bhasma still more faded when compared to the 2nd Puta • Except niruttha and apunarbhava rest all tests were passed
3.	**4th** Puta	• All the chakrikas completely baked and turned into yellow colour. • The chakrikas softer than the 3rd Puta. • The texture of the chakrikas were softer like butter while touching when compared to 3rd Puta. • The colour of the Bhasma still more faded when compared to the 3rd Puta • Apunarbhava & Niruttha were also passed.

3.2 ANALYTICAL PART

1. Raw drug yashada, provided by GMP certified Shri Dhootapapeshwar Pvt. Ltd (batch no. 1PRML00278/Jun/2021) with certificate of analysis having purity of 99.62% w/w., Parada and Gandhaka procured from GMP certified KLE Ayurveda Pharmacy, were authentified as per grahya laxanas at KAHER's Shri B. M. K. Ayurveda Mahavidyalaya's, Central Research Facility's (CRF) AYUSH approved Drug Testing Laboratory (ASU-DTL) and as per geological parameters at G. S. Science College, Belagavi by the experts.
2. All the raw drugs (major and associated) and final Classical and Potentiated bhasma were tested for physico chemical parameters (LOD, Ash values, Qualitative analysis of major inorganic elements & NPST) as per API guidelines,[140] PSAF[141] and Manual NPST[142] at KAHER's SBMK AYUSH approved Drug Testing Laboratory (ASU-DTL).
3. The instrumental analysis of intermittent and final bhasma samples were done in recognized analytical centers.

- ICPAES analysis was done at Sophisticated analytical Instrument Facility (SAIF), IIT Bombay.
- DLS – Particle size, Zeta potential using Malvern Zetasizer and FTIR analysis were done at KLE college of Pharmacy, Nehru Nagar, Belagavi.
- ICPMS analysis was done at Nanowatts Technologies Ltd. Dollars Colony, Bangalore, 560094. info@nanowattstech.com
- XRD was done at Nanowatts Bangalore (CYB) and Sophisticated analytical Instrument Facility (SAIF), IIT Dharwad (PYB).
- SEM analysis were done Nanowatts Bangalore (CYB) and Sophisticated analytical Instrument Facility (SAIF), IIT Bombay (PYB).

3.3 EXPERIMENTAL STUDY

IN-VIVO STUDY 01 – ACUTE TOXICITY STUDY

Reference: OECD 420 Guidelines

Objective:

To assess the acute oral toxicity of Potentiated Yashada bhasma (zinc calyx) in female Wistar rats, following OECD 420 guidelines.[143]

Materials and Methods

1.1 Experimental Animals:

- **Source:** Procured from a licensed breeder at Pune, Maharashtra.
- **Approval:** IAEC Approval No. BMK/IAEC/Res. No.-211/2021-11.
- **Study site:** Animal House Facility of KAHER's SBMKAM Belagavi.
- **Species and Strain:** Female Wistar rats.
- **Weight:** 150–250 gm (8–12 weeks of age).
- **Housing:** Rats were kept in polypropylene cages with a 12:12 light-dark cycle, a temperature of 22 ± 3°C, and a relative humidity of 50–60%.

  Prior to the experiment, the animals were acclimatized for five days.
- **Water and diet:** Throughout the experiment, all rats had unlimited access to standard foods and water ad libitum.

1.2. Study Design: The study was conducted as per the principles of OECD 420 guidelines. A Group of five animals were dosed using fixed dose of 2000 mg/kg, with an initial dose based on a sighting study.

- Total number of animals: 18 sanctioned.
- Number of animals used for the study: 05 animals, as there was no mortality seen in 1st five groups of animals at highest fixed dose selected (2000mg/kg)

based on previous works, further groups of animals were not used in this study.

- Duration of the study: 14 days
- Route of Drug administration: Oral
- **Vehicle**: Carboxy methyl cellulose (CMC) and distilled water to form a suspension (0.5 %, 1mL/100gm).
- Dose: PYB - 2000 mg/kg
- **Dose Rationale**: The 2000 mg/kg dose was chosen based on the previous work and considering the nontoxic drug nature**.** No human-to-animal dose conversion was required as the study focused directly on evaluating the acute toxicity of drug in rats.
- **Dose calculation**: 2000mg/kg directly in animals as per body weight

1.3. Preparation and Identification

Animals were randomly selected, marked for identification (Head, Tail, Right Arm, Left Arm and Unmarked), and housed individually 5 days prior to dosing.

1.4. Sighting Study

- **Dose:** Potentiated Yashada bhasma at 2000 mg/kg (344 mg for a 172 gm animal) suspended in 2 mL of 0.5 % CMC solution.
- **Fasting Conditions:** 12-hour fasting before dosing (food withheld, water provided) and 3-hour fasting after dosing.
- **Observations:** At 2, 4, 6, 8, 12, 24, and 48 hours, they were observed for mortality and toxicity indicators (skin, fur, eyes, mucous membranes, somatomotor activity, behavior, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma).

- The identical dosage (2000 mg/kg) was used for the main study since there were no deaths or indications of toxicity over the 48-hour timeframe.

1.5. Main Study

- **Subjects:** Four female Wistar rats (excluding the sighting study animal).
- **Fasting Conditions:** 12-hour fasting before dosing.
- **Dose:** Potentiated Yashada bhasma at 2000 mg/kg body weight.

Table No. 13 Details of Animals and Dose Administered

Sl. No.	Animal	Weight (gm)	Dose Administered (mg)
1	Tail (A)	172	344
2	Head (B)	166	332
3	Left Arm (C)	165	330
4	Right Arm (D)	155	310
5	Unmarked (E)	153	306

1.6. Observations:

- No signs of mortality and toxicity were observed throughout the study.
- Stool color changed to light brown for 2 days post-dosing.
- Food and water intake were normal as observed pre dosing of animals.
- Changes in body weight and major organ weight are mentioned in observation and result section.

1.7. Observational Parameters:

- **Signs of Toxicity:** Monitored at 2, 4, 6, 8, 12, 24, and 48 hours for mortality and other clinical signs signs of morbidity.

- **Daily Observations:** Clinical signs and mortality observed for 14 days.
- **Scoring:** Signs of toxicity were recorded on a scale of 0 to 5 as below

Table. No. 14 Signs of Toxicity Scoring

Score	Description	Observable effects
0	No observable effects	The test subject appears normal, with no signs of distress or toxicity.
1	Minimal effects	Mild and transient signs, such as slight lethargy or changes in grooming, but the subject recovers quickly.
2	Mild effects	More pronounced signs like reduced activity or slight weight loss, with gradual recovery.
3	Moderate effects	Clear signs of toxicity, including significant lethargy, weight loss, or mild tremors, but not life-threatening.
4	Severe effects	Severe signs of toxicity such as intense tremors, convulsions, significant weight loss, or extreme distress, potentially life-threatening.
5	Death	The test subject does not survive due to the exposure.

1.8. Sacrifice and Analysis:

- On Day 15, all the animals were anesthetized and blood was collected from retro orbital vein in labeled EDTA and Plain bulbs for hematological and biochemical analysis respectively.

- Same day, animals were euthanized, using diethyl ether inhalation in accordance with ethical guidelines and major organs were weighed and collected for histopathological examination.

- Blood samples and tissue samples were sent to Jeevan Diagnostic Laboratory, Belagavi for hematological, biochemical analysis and histopathological examination

IN-VIVO STUDY 02 – BIOAVAILABILITY STUDY [144]

2.1 Study Objective:

- To compare the bioavailability of CYB and PYB by analyzing zinc levels in blood by AAS at specific time intervals.

2.2. Experimental Animals

- **Source:** Procured from a licensed breeder at Pune, Maharashtra.
- **Species and Strain: Male** & Female Wistar rats.
- **Weight:** 150–250 gm (8–12 weeks of age).
- **Housing:** Rats were kept in polypropylene cages with a 12:12 light-dark cycle, a temperature of 22 ± 3°C, and a relative humidity of 50–60%.
- Prior to the experiment, the animals were acclimatized for five days.
- **Water and diet:**
- Throughout the experiment, all rats had ad libitum access to standard foods and water.

2.3. Ethical Approval

- The study was approved by the IAEC of KAHER's Shri BMK Ayurveda Mahavidylaya with IAEC Approval No. BMK/IAEC/Res. No.-211/2021-11.

2.4 Test Drugs and Dosing

- **Test Drugs:** CYB and PYB
- **Dose Rationale:** The 300 mg/kg dose was chosen based on the previous work.[126] No human-to-animal dose conversion was required as the study focused directly on evaluating the bioavailability of the drugs in rats.
- **Dose calculation**: 300mg/kg directly in animals as per body weight
- **Route of administration**: Oral with 2 mL 0.5 % CMC solution

2.5. Randomization and Grouping

Animals were randomly divided into two groups of 05 male and 05 females in each of the group and housed in four different cages (Male and Female Separately).

Table No. 15 Details of Animal Groups and Interventions

Group	Intervention	No. of Animals
1.	Classical Yashada bhasma (CYB)	10 (05 male and 05 Female)
2.	Potentiated Yashada bhasma (PYB)	10 (05 male and 05 Female)

2.6 Dosing Procedure: Freshly prepared solutions of the test drugs (CYB and PYB) were administered via oral gavage at a dose of 300 mg/kg body weight.

Table No. 16 Weight of the individual animals and dose

Sl. No.	Group I CYB	Weight in gms	Dose mg/kg	Group II PYB	Weight in gms	Dose mg/kg
1.	*M A	315	94	M A	300	90
2.	M B	247	74.1	M B	236	70.8
3.	M C	270	81	M C	271	81.3
4.	M D	256	76.8	M D	259	77.7
5.	M E	292	87.6	M E	276	82.8
6.	*F A	239	71.7	F A	238	71.4
7.	F B	222	66.6	F B	226	67.8
8.	F C	212	63.6	F C	213	63.9
9.	F D	221	66.3	F D	238	71.4
10.	F E	211	63.3	F E	217	65.1
Note: * M – Male, *F - Female						

2.7 Sample Collection and Analysis

a. Blood collection:

- **Time Points**: 0, 0.5, 1, 2, 4, 8, and 24 hours post-dose.

Table No. 17. Details of animals and time points of blood collection

Sample/Time	Group I CYB				Group II PYB			
0 hr	M A	M B	F A	F B	M A	M B	F A	F B
½ hr	M C	M D	F C	F D	M C	M D	F C	F D
1 hr	M E	F E	M A	F A	M E	F E	M A	F A
2 hr	M B	M C	F B	F C	M B	M C	F B	F C
4 hr	M D	M E	F D	F E	M D	M E	F D	F E
8 hr	M A	M B	F A	F B	M A	M B	F A	F B
24 hr	M C	M D	F C	F D	M C	M D	F C	F D
Note: * M – Male, *F – Female								

- **Collection Method**: Blood samples of 1mL were drawn via the retro-orbital plexus under ether anesthesia.
- **Anti-coagulant Used**: EDTA bulbs were used for blood sample collection.
- **Animal sacrifice**: After collection of 24 hour blood sample, all the animals were euthanized using diethyl ether.

b. Sample Preparation for AAS: Acid Digestion

- All blood samples were subjected to acid digestion using a wet digestion method.
- A mixture of concentrated HNO_3 and H_2O_2 (2:1 v/v) was used.
- The digestion process involved heating on a hot plate at 80°C for 1–2 hours until a clear liquid was obtained.
- The digested samples were filtered, and the volume was adjusted to 100 mL from this 25 mL sample is stored containers -20 ^{o}C until analysis.

2.8 Analytical Method: AAS Analysis: Was carried out at Dr. Prabhakar Kore Basic Science Research Center (BSRC), Nehru Nagar, Belagavi.

- Zinc levels were quantified using an AAS instrument (Model AA-7000F, ROM Version - 1.01, SHIMADZU Corporation).
- **Calibration curve preparation and linearity range**: The calibration curve for standard zinc was constructed using standard solutions at concentrations of 2.5, 5.0, and 7.5 c. A linear relationship was observed between the analyte concentration and the instrumental response, with a correlation coefficient (R^2) of 0.9991. The linearity of the method was confirmed in the range of 2.5– 7.5 ppm, demonstrating its suitability for quantitative analysis.
- **Sensitivity and limit of detection**: 0.1-100 ppm

2.9 Pharmacokinetic Analysis:

- Pharmacokinetic parameters were calculated

  C_{max} : Maximum plasma concentration.

  T_{max} : Time at which C_{max} occurs.

  AUC $_{0-\infty}$: Area under the plasma concentration-time curve from time 0 to infinity.

  AUC $_{0-t}$: Area under the curve from time 0 to the last measurable concentration.

2.10. Statistical Analysis:

- Parametric independent T test was used to evaluate comparative bioavailability of CYB and PYB.

IN-VIVO STUDY 03 – SPERMATOGENIC ACTIVITY

3.1. Objective: Evaluation of comparative Spermatogenic Activity of CYB and PYB in Male Wistar Rats in Adenine-Induced Fertility Suppression Model.

3.2. Ethical Approval: The study was approved by the IAEC of KAHER's Shri BMK Ayurveda Mahavidylaya with IAEC Approval No. BMK/IAEC/Res. No.-211/2021-11.

3.3. Chemicals and Drugs:

Test Substances/Drugs: CYB and PYB

Chemical/Inducing Agent:

- **Adenine**: Is procured from Otto Chemie Pvt. Ltd. An ISO, GMP Certified Company with CAS number: 73-24-5 and Purity – 99%.
- **Rationale**: Adenine is a known chemical to suppress fertility in male wistar rats as evident from previous research works.[130, 131 & 132]

Vehicle: Carboxy methyl cellulose (CMC) and distilled water to form a suspension (0.5 %, 1mL/100gm).

3.4. Experimental Animals:

- **Source:** Procured from a licensed breeder at Pune, Maharashtra.
- **Study site:** Animal House Facility of KAHER's SBMKAM Belagavi.
- **Species and Strain: Male** Wistar rats.
- **Weight:** 150–250 gm (8–12 weeks of age).
- The rats were kept in polypropylene cages with a 12:12 light-dark cycle, a temperature of 22 ± 3°C, and a relative humidity of 50–60%.
- Prior to the experiment, the animals were acclimatized for five days.
- Throughout the trial, all rats were provided with unlimited access to standard food and water.

3.5. Experimental Protocol: Two groups of thirty-six male Wistar rats, ages eight to twelve weeks, were randomly assigned: For 30 days

Normal Control Group (n = 6) was administered normal saline intragastrically every day.

Infertility Model Group (n = 30) was given adenine (300 mg/kg) intragastrically every day for 30 days at a volume of 0.5 mL/100 g body weight.

Every week, the rats were weighed and provided complete access to food and water. Physical symptoms were tracked every day for the duration of the investigation. To verify the development of the infertile model, blood samples were taken at the end of 30 days to measure the serum levels of FSH, LH, and testosterone.

The 30 infertile model rats were randomly assigned to five groups (n = 6 each) once model inducement was confirmed. Normal control group remained the same.

Table No. 18 Group Allocation and Interventions – Spermatogenic activity

Group	N	Intervention	Dose (mg)	Duration	Route
CYB I	6	CYB	250 mg	30 days	Oral
CYB II	6	CYB	125 mg	30 days	Oral
PYB I	6	PYB	250 mg	30 days	Oral
PYB II	6	PYB	125 mg	30 days	Oral
Model Control	6	Normal saline	-	30 days	Oral
Normal Control	6	Normal saline	-	30 days	Oral

3.6. Dose Calculation: Animal dose of each interventional group was calculated by dose conversion table of Paget and Barnes.[145]

3.7. Dosing Procedure: All the four interventional group animals were given test drugs CYB and PYB orally at maximum therapeutic dose (250mg) and half of it (125mg) respectively with 0.5% CMC suspension 1 mL/100gm with feeding needle No. 15. Model control and Normal control group animals were given normal saline daily for 30 days.

3.8. Animal Care & Observational parameters:

- All rats were housed in standard conditions in separate cages as per different groups, with free access to food and water throughout the study period.
- The rat's general activity, eating, drinking and urination were monitored daily.
- The rats were weighed weekly for four weeks to monitor any changes in body weight and health status.

3.9. General Observations:

a. During adenine induced infertility suppression: Starting from day 5, the rats exhibited signs of apathy, reduced movement, dull eyes, dry and unkempt fur, as well as a notable increase in water consumption and urination. Additionally, their body weight began to gradually decrease after day 7. In contrast, the rats in the normal control group appeared to be in good health, demonstrating quick reactions, bright eyes, strong appetites, smooth and healthy fur, normal water intake and urination, and a steady increase in body weight.

b. During Intervention with CYB & PYB: After one week of intervention, a slight improvement was observed, as evidenced by a notable increase in food intake, reduced urination, enhanced body movements and improved skin fur appearance. These improvements gradually progressed in all four interventional groups including model control group. In contrast, the rats in the normal control group remained similar as earlier to be healthy.

3.10. Experimental Parameters:

i. **Biochemical Assays:**

- On 30th day, 03 animals randomly from each group were anesthetized by diethyl ether and blood samples of 5 mL were collected from the retro orbital

vein in labeled plain bulbs. Blood samples were sent to Jeevan Diagnostic laboratory, Belagavi for biochemical parameters (Serum FSH, LH and testosterone).

- The rats were euthanized using diethyl ether inhalation in accordance with ethical guidelines.

ii. **Organ weight and Coefficients**:

- The same side's testicles and epididymis were removed after dissection, cleaned with cold saline, dried using filter paper, and weighed.
- The formula Testis coefficient = (Testis weight/Body weight) ×100

iii. Sperm Parameters:

Sperm Suspension Preparation:

- Each rat's epididymis was weighed and then chopped into pieces in a saline solution at 37°C in a sterile petri dish.
- To create the final sperm suspension, the 1 mL combination was further diluted with 9 mL saline at 37°C. It was then transferred right away to a sterile container for analysis of the sperm parameters listed below at Srishti Fertility Centre & Seminology Laboratory, KLE Ayurveda Hospital, Belagavi.

a. Sperm Motility:

- The motility of 200 sperm from a 20 µL sperm suspension was observed under a microscope.
- Categorize each into one of the following motility grades:
- **Progressive Motility:** Spermatozoa moving actively, either linearly or in large circles, regardless of speed.

- **Non-Progressive Motility and Circular:** Spermatozoa exhibiting movement but lacking forward progression, such as swimming in small circles or displaying minimal displacement despite flagellar activity.
- **Immotile:** Spermatozoa that show no movement.
- Sperm with 50% or more forward movement and 25% or more fast forward movement were considered to exhibit normal activity.

b. **Sperm Viability:**

- About 10-20 µL of the sperm suspension was put onto a clean microscope slide.
- The sperm suspension was spread over the surface of the slide using another slide to create a thin smear.
- The slide was stained with 1 drop of eosin and 1 drop of nigrosin onto the sperm smear. Gently mixed the stain and sperm on the slide, ensuring the smear is evenly stained.
- Later the slide was dried at room temperature for a few minutes and examined under the light microscope by placing a coverslip over the sample.
- Live sperms appear white or light-colored (they do not take up the eosin stain).
- Dead sperm appear red or pink, as they absorb the eosin dye.
- At a high magnification of 400 X, the number of live and dead sperm was now counted.
- The percentage of live (uncolored) sperm to total sperm counted was used to determine the sperm viability.

c. Sperm Counts:

- A CBC board was filled with 20 µL of the sperm solution, which was then let to settle for 30 seconds.

- Using the calculation, which reads:
- **Sperm count/L = R×50×dilution factor×10^6**, where R is the count acquired using the red blood cell counting method, the Sperm count was determined.

d. Sperm Morphology:

- The sperm suspension was used to create a smear, which was then dried, fixed with methanol, and stained with methylene blue and basic fuchsin on a hot plate.
- The malformation rate was determined by analyzing the morphology of 100 sperm per rat under the light microscope 40 X objective lens.
- Hook shaped heads with thin neck, mid piece and a long tail and no abnormalities (double head, flattened head, headless, bent neck, bent tail & tailless) in the head, neck, middle or tail regions were considered normal.

iv. Histopathological Analysis:

- One testis from each rat was dissected and cleaned of extraneous tissue and immediately transferred to 10% neutral formalin solution in separate labelled containers. These are sent to Jeevan Diagnostic Laboratory, Belgaum for histopathological study.

3.11. Data Analysis:

- The mean ± standard deviation (SD) was used to express the data.
- To examine differences between the treatment groups, a one-way ANOVA was employed, followed by Tukey's post hoc test. The threshold for statistical significance was $p < 0.05$.

4. OBSERVATIONS AND RESULTS

Results are mentioned in two parts

4.1 Pharmaceutical study analytical results

1. Geological Authentication, Physico-chemical and phytochemical Analysis of raw drugs of Yashada bhasma.
2. Organoleptic characters and Bhasma pariksha results
3. Physico chemical analysis and NPST of CYB & PYB.
4. Instrumental Characterization of both the samples using ICPAES, DLS-Particle size, Zeta potential, XRD, SEM and ICPMS.

4.2 Experimental study results

1. Observations and results of Acute Oral Toxicity Study
2. Observations and statistical results of Bioavailability Study
3. Observations and statistical results of Spermatogenic Activity Study.

4.1 Pharmaceutico Analytical Study Results

Table No. 19 Geological Authentication of Major Drugs

Sl. No.	Geological Parameter	Parada	Gandhaka	Yashada
1.	Colour	Silver / Silver white	Lemon yellow	Silvery white
2.	Streak	None	Pale yellow	Light grey
3.	Hardness	None	1.5-2	2
4.	Fracture	None	Uneven, bladed, sectile	Hackly
5.	Cleavage	None	None, Striations are seen	Perfect
6.	Luster	Metallic	Resinous/greasy	Metallic
7.	Specific gravity	13.6	2.095	6.67
8.	Density	Heavy, 13.6g.cm^{-3}	1.9	Heavy, 7.11 g.cm^{-3}
9.	Form & Habitat	Can take any form, droplets are circular and convex	Irregular, granular/encrusting	Massive
10.	Name	Mercury (element)	Sulphur (native)	Zinc (metal)
11.	Occurrence	Cinnabar ore	In natural form near volcanic vents	Sphalerite ore
12.	Diagnostic properties	Silver white liquid metal	Yellow color, low hardness, low specific gravity and low melting temperature, extremely flammable, burns with blue light and insoluble in water	Silver white metal, heavy & tough and cannot cut easily

Table No 20. Macroscopic characters of Raw drugs.

Sl.No.	Drug	Form/Part	Colour	Taste	Odour
1.	Saindhava lavana	Irregular hard masses	Whitish	Salty	Not specific
2.	Sudha	Fine Powder	White	Slightly chalky	Odourless
3.	Lashuna	Bulbs	Whitish	Acrid	Pungent disagreeable
4.	Kulattha	Seeds	Greyish to reddish brown	Astringent	Not distinct
5.	Apamarga	Whole plant	Yellowish brown	Pungent, Astrigent	Not distinct
6.	Kumari	Pulp	White	Bitter	Characteristic
7.	Nimbu	Juice	Pale yellow	Sour	Characteristic

Table No. 21. Physicochemical Analysis of the Raw drugs

Sl.No.	Drug	Foreign matter	Ash Value in %	Acid Insoluble value in %	Water soluble extract in %	Alcohol soluble extract in %	pH
1.	Saindhava lavana	Nil	-	-	-	-	5.96
2.	Sudha	Nil	59.088	11.165	9.612	0.319	11.34
3.	Lashuna	Nil	2.283	0.245	15.522	3.940	-
4.	Kulattha	Nil	3.626	0.4831	6.463	4.150	7
5.	Apamarga	Nil	5.637	0.340	13.419	3.519	7.6
6.	Kumari	Nil	-	-	-	-	5.29
7.	Nimbu	Nil	-	-	-	-	3.4
Note: According to the Indian Ayurvedic Pharmacopeia, all of the values fall within the range.							

Table No. 22 Qualitative Phytochemical screening of Raw Drugs.

Sl. No.	Drug Name/Test Name	Lashuna		Kulattha		Apamarga		Kumari	Nimbu
		WE	AE	WE	AE	WE	AE	Swarasa	Swarasa
1.	Carbohydrate	-	+	+	+	+	+	+	+
2.	Monosaccharaides	-	-	-	+	-	-	-	-
3.	Reducing Sugar	+	+	-	-	+	+	-	+
4.	Pentose sugar	-	-	-	-	-	-	-	-
5.	Hexose Sugar	-	-	-	-	-	-	-	-
6.	Proteins	-	-	+	-	-	-	+	+
7.	Amino acid	+	-	+	-	-	-	-	-
8.	Steroids	+	+	-	+	+	-	+	+
9.	Cardiac Glycosides	+	+	-	+	-	+	-	+
10.	Saponins	+	+	+	-	+	+	+	-
11.	Alkaloids	-	-	-	-	-	-	+	+
12.	Flavonoids	-	-	-	-	+	+	+	+
13.	Tannins	-	-	-	-	-	+	-	+
Note: WE- Water Extract and AE- Alcoholic extract, + Present. – Absent.									

Table No. 23 Inorganic analysis of Raw drugs

Drug /Element	Sudha	Saindhava	Lashuna	Kulattha	Apamarga
Iron	-	-	-	+	-
Sodium	+	+	-	+	+
Calcium	+	-	-	-	-
Potassium	-	+	-	-	-
Magnesium	+	-	-	-	-
Chlorides	-	-	-	-	+
Sulphates	+	-	+	-	-
Phosphates	-	-	-	-	-
Carbonates	+	-	-	-	-
Nitrates	-	-	-	-	-
Note: + Present. – Absent.					

Table No. 24 Organoleptic characters and bhasma pareeksha of both Bhasma

Parameters	CYB		PYB			
	1st Puta	2nd Puta	1st Puta	2nd Puta	3rd Puta	4th Puta
Colour	Off white	Pale yellow	Off white	Light yellow	Light yellow	Pale yellow
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless
Touch	Soft	Softer	Soft	Softer	Very soft	Very soft
Appearance	Powder	Fine powder	Powder	Fine powder	Amorphous	Amorphous
Rekhapurnatva	Passed	Passed	Passed	Passed	Passed	Passed
Varitara	Partially Passed	Passed	Partially Passed	Passed	Passed	Passed
Unama	Partially Passed	Passed	Partially Passed	Passed	Passed	Passed
Nishchandrata	Passed	Passed	Passed	Passed	Passed	Passed
Niruttha	Not done	Passed	Not done	Not done	Not done	Passed
Apunarbhava	Not done	Passed	Not done	Not done	Not done	Passed

Table No. 25 Physical parameter Analysis of Bhasma samples

Sl.No.	Physical parameters	CYB	PYB
1.	Total ash	96.268%	98.705.2%
2.	Acid insoluble ash	52.338 %	46.115%
3.	Loss on drying	0.09 %	0.298%

Table no. 26. Qualitative major inorganic element analysis of Bhasma samples

Sl. No.	Inorganic elements	CYB	PYB
1.	Calcium	+	+
2.	Magnesium	+	+
3.	Sodium	+	+
4.	Chloride	+	+
5.	Potassium	-	-
6.	Manganese	-	-
7.	Carbonate	-	-
8.	Nitrate	-	-
9.	Sulphate	+	+
10.	Iron	-	-
Note: + Present. – Absent.			

Table No. 27 NPST – Phase wise observations of both the samples

Phases	Observations
Phase 1 reaction 0-5 mins	Wet central spot spread outside with immediate formation of bright white glittering surface over the spot
Phase 2 reaction After 20 mins	The reddish outer ring around the white spot was seen. The white spot was very bright at this stage.
Phase 3 reaction 24-48 hrs	The brightness of white spot was maintained at this stage also. There was a clear yellowish periphery around the central spot

Instrumental Analysis:

1. ICPAES Analysis

Table No. 28 Quantitative Zinc Analysis by ICPAES of at various stages

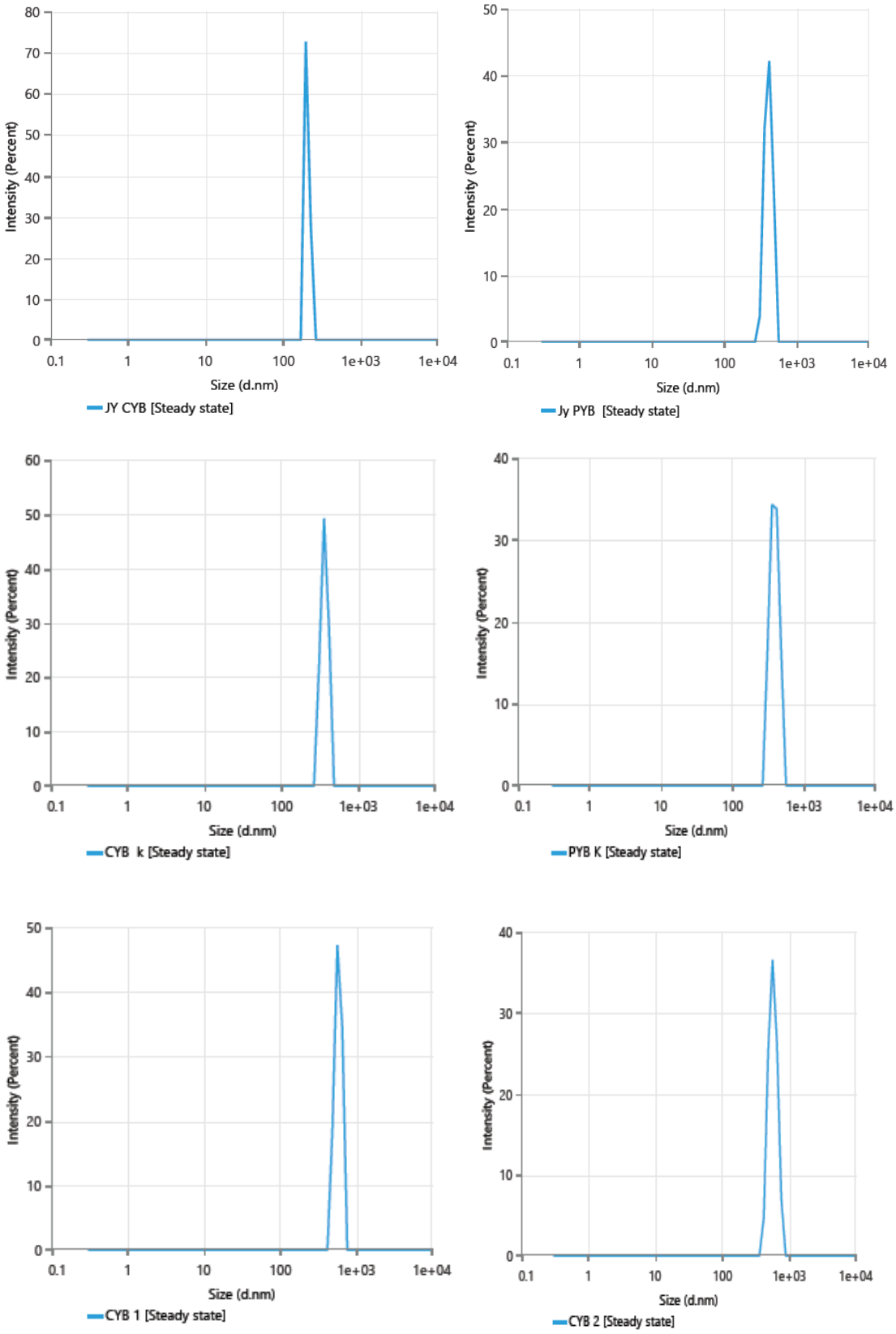
Sl. No.	Yashada samples	% of zinc
1.	Before shodhana	99.88 %
2.	After shodhana	92.92 %
3.	Jarita yashada	82.21 %
4.	Classical yashada bhasma	77.13 %
5.	Potentiated yashada bhasma	70.29 %

2. DLS – Particle size Analysis

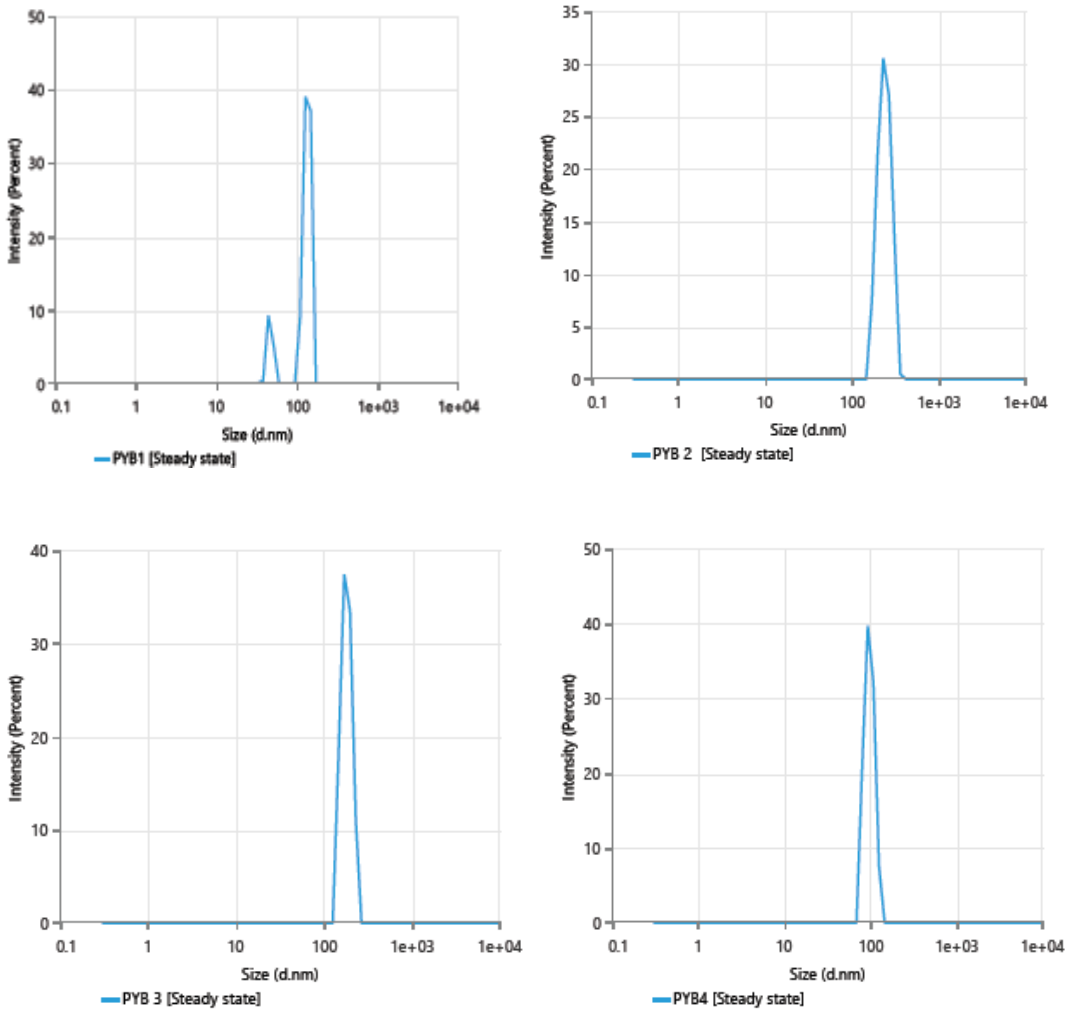
Table No. 29 DLS Particle size & Zeta Potentials at various stages

Sl. No	Sample	Particle Size	Zeta potential (mV)	PDI
1.	Jarita yashada CYB	1598 nm	-23.69	1
2.	Jarita yashada PYB	551.2 nm	-15.40	1
3.	CYB Kajjali	1214 nm	-22.92	1
4.	PYB Kajjali	470 nm	-13.19	1
5.	CYB 1	1328 nm	-28.24	0.72
6.	CYB 2	693.6 nm	-28.88	1
7.	PYB 1	603.7 nm	-25.27	0.84
8.	PYB 2	385.2 nm	-22.92	0.56
9.	PYB 3	344.6 nm	-22.96	0.59
10	PYB 4	261.1 nm	-28.20	0.41
Note: CYB 1 – 1st Puta, CYB 2- 2nd Puta, PYB 1- 1st Puta, PYB 2- 2nd Puta, PYB 3- 3rd Puta, PYB 4 – 4th Puta				

Graph No. 04 DLS Particle size at various stages



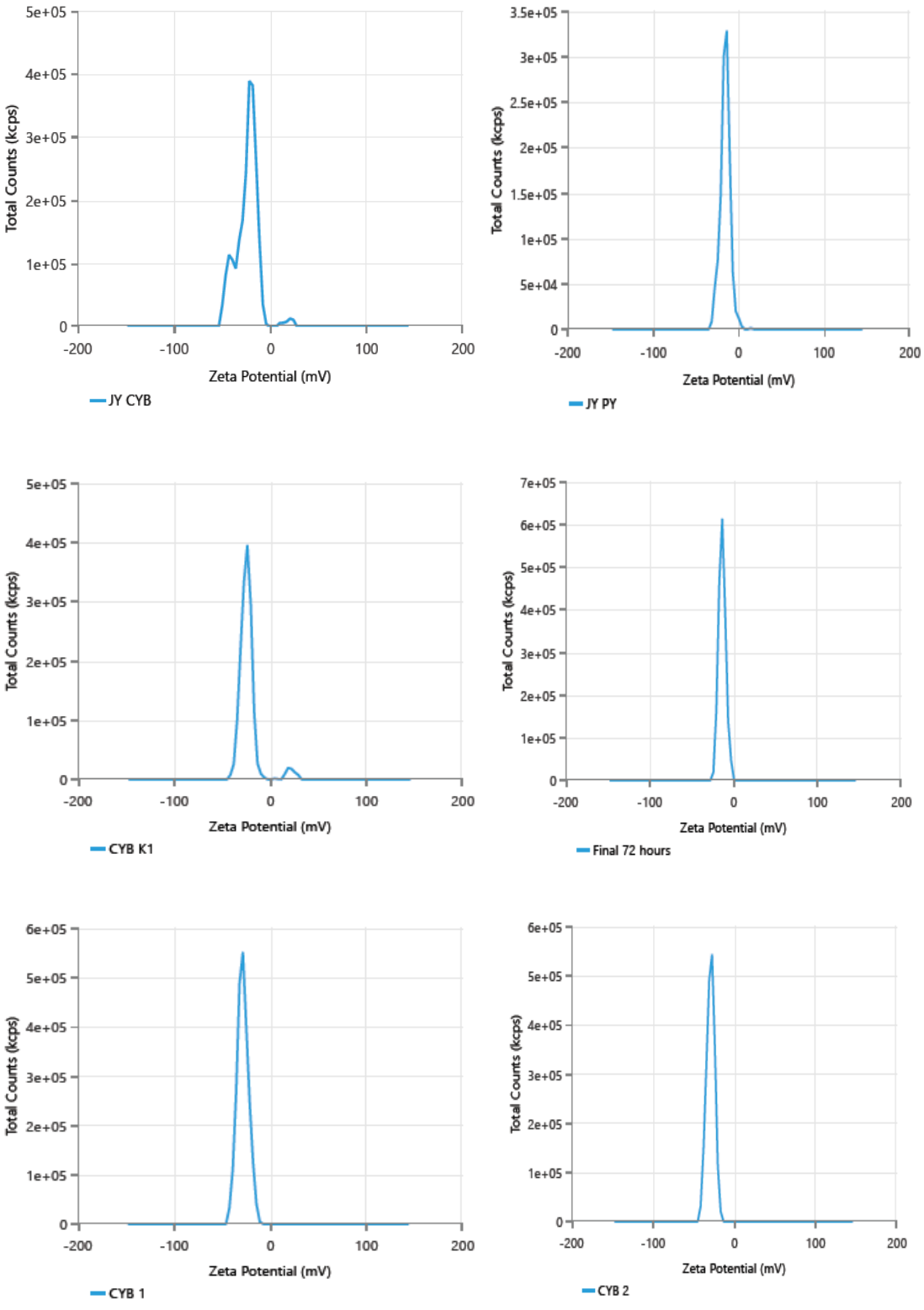
Graph No. 04 DLS Particle size at various stages



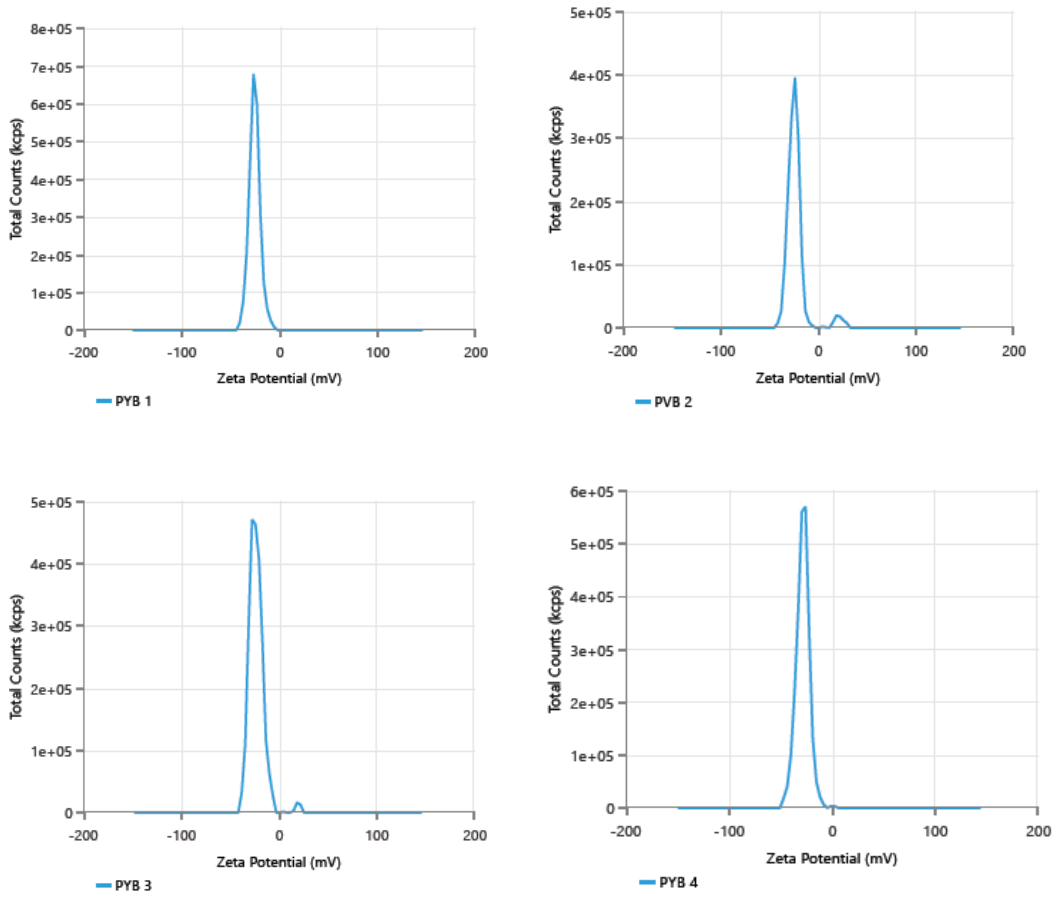
Particle size analysis at various stages of CYB and PYB preparation, using the dynamic light scattering (DLS) are presented in Table No. 29 and Graph No. 4. The findings indicate a stepwise reduction in particle size throughout the preparation process for both CYB and PYB. Notably, the size reduction in the intermittent steps of PYB preparation was more pronounced compared to CYB. By the end of the process, the particle size of PYB was reduced to approximately two and a half times smaller than that of CYB. Additionally, the polydispersity index (PDI) of PYB was 0.412, suggesting a uniform particle size distribution and greater homogeneity. In contrast, CYB had a PDI of 1, indicating slightly greater heterogeneity.

3. DLS Zeta Potential Analysis:

Graph No. 05 DLS Zeta Potential at various stages



Graph No. 05 DLS Zeta Potential at various stages

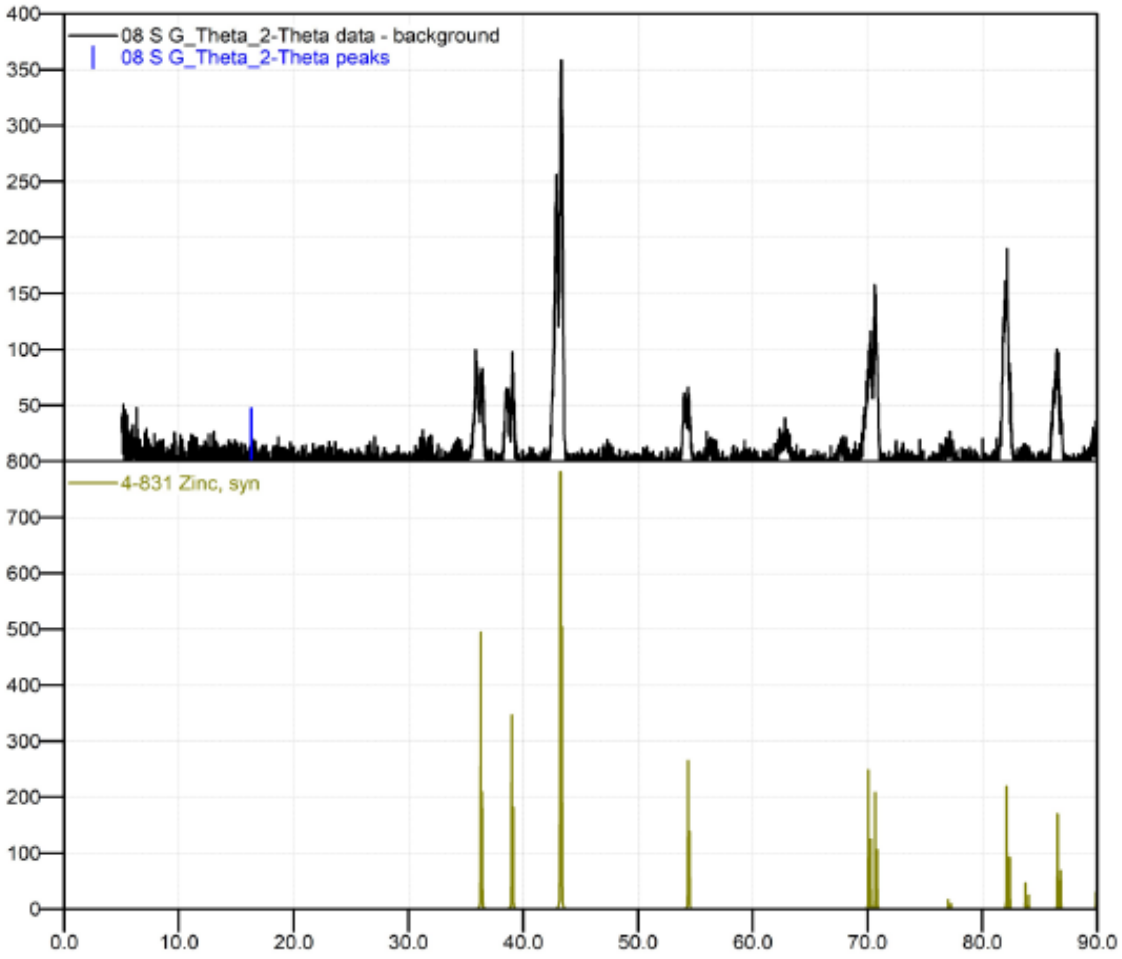


Zeta potential analysis of various stages during the preparation of CYB and PYB was performed using the dynamic light scattering (DLS) method, with the results summarized in Table No. 29 and illustrated in Graph No. 5. All samples exhibited negative zeta potential values ranging from -13 mV to -28 mV, indicating a consistent negative charge across all stages. These negative zeta potentials signify good colloidal stability, as they facilitate strong electrostatic repulsion between particles, thereby preventing aggregation in suspension.

The difference in zeta potential between the final samples of CYB and PYB was minimal, suggesting that both are likely to remain well-dispersed in solution. This stability is critical for maintaining the integrity and performance of the formulations during storage and application.

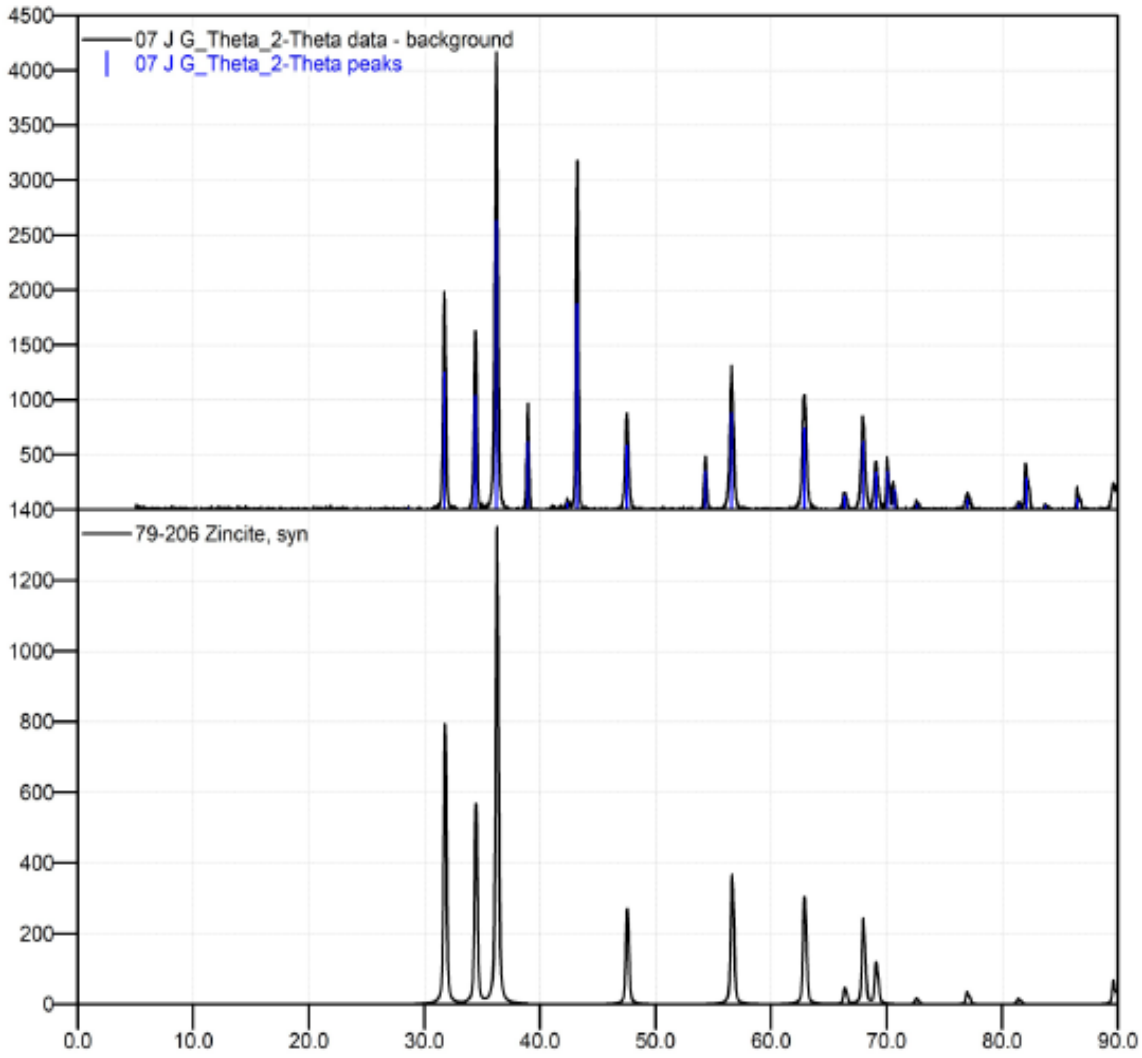
4. XRD analysis at various stages

a. Graph No. 06 Shodhita yashada



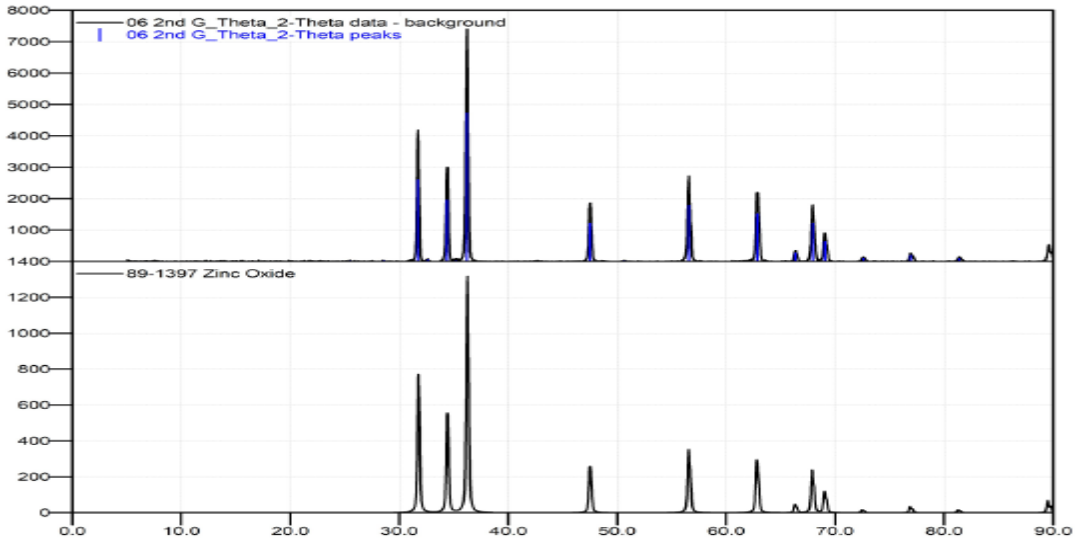
The XRD peaks of the shodhita sample (black line) align well with the reference pattern for zinc (yellow line), particularly at 2θ values corresponding to prominent peaks, such as approximately 36°, 43°, and 54°**,** confirming the presence of zinc in the sample. The sharp and well-defined peaks indicate a high degree of crystallinity. Additionally, there is no significant evidence of additional or unmatched peaks, suggesting that the sample is predominantly composed of the zinc phase with minimal impurities or secondary phases. Overall, the sample demonstrates excellent crystallinity and structural integrity, as it closely matches the reference pattern for zinc.

b. Graph No. 07 Jarita yashada

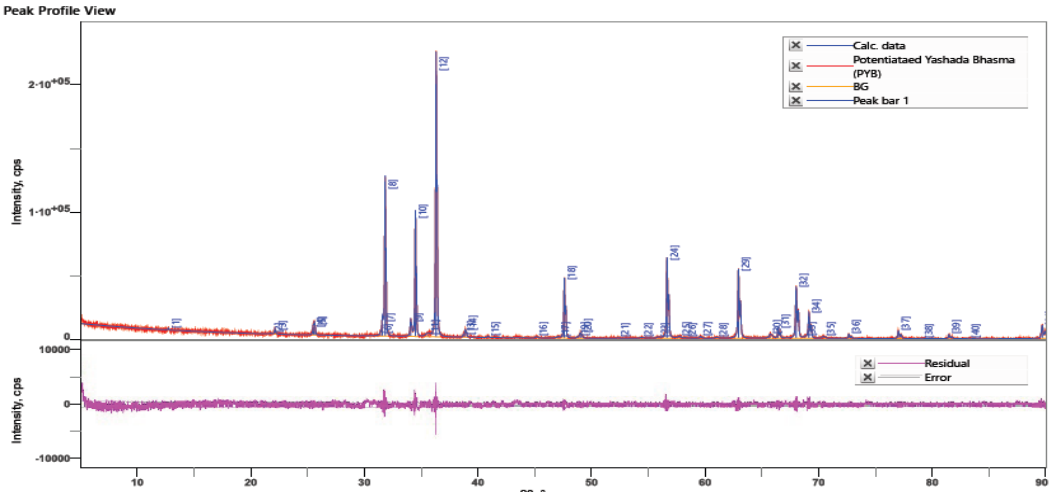


The XRD analysis of the jarita sample (upper blue line) aligns well with the reference pattern for zincite (lower black line, labeled "79-206 Zincite, syn"), confirming the presence of zinc oxide (ZnO) as its crystalline phase. Prominent peaks at 2θ values of 31.8°, 34.4°, and 36.3° are characteristic of zincite and match the reference pattern, indicating a high degree of crystallinity with a well-ordered structure. The absence of additional or unmatched peaks suggest the sample is predominantly composed of ZnO with minimal impurities. Overall, the analysis demonstrates excellent crystallinity and purity of the zinc oxide phase.

c. Graph No. 08 CYB after 2nd Puta



d. Graph No. 09 PYB after 4th Puta



The XRD analysis of CYB (Graph 8, upper blue line) and (Graph 9, PYB, red line) both confirm zinc oxide (ZnO) as the primary crystalline phase, as they align well with the reference pattern for ZnO. For both samples, the prominent 2θ peaks at 31.8°, 34.4°, and 36.3° match the ZnO reference, verifying their ZnO composition. The sharp, intense peaks in both cases indicate high crystallinity with a well-ordered structure. In both cases, the absence of unmatched peaks suggests that both CYB and PYB are predominantly composed of ZnO with minimal impurities.

5. SEM analysis at various stages

Figure No. 01 Jarita Yashada

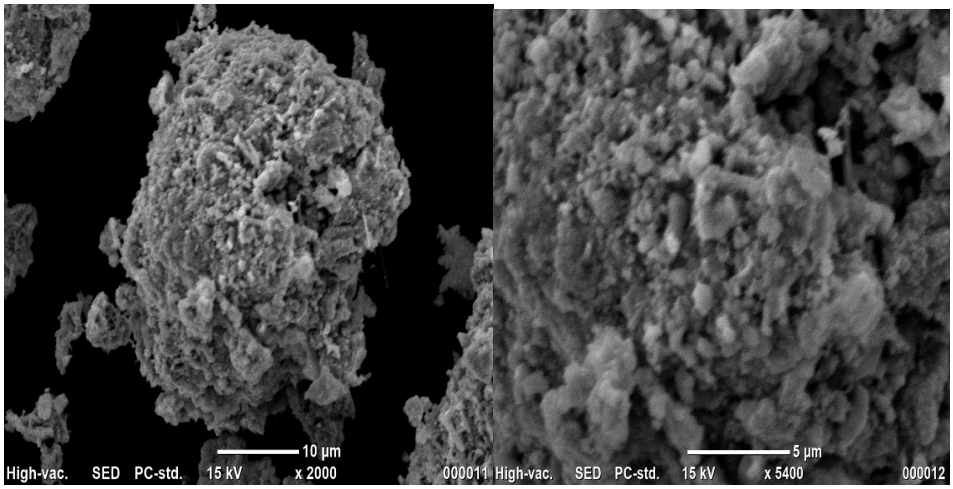


Figure No. 02 CYB after 1st Puta

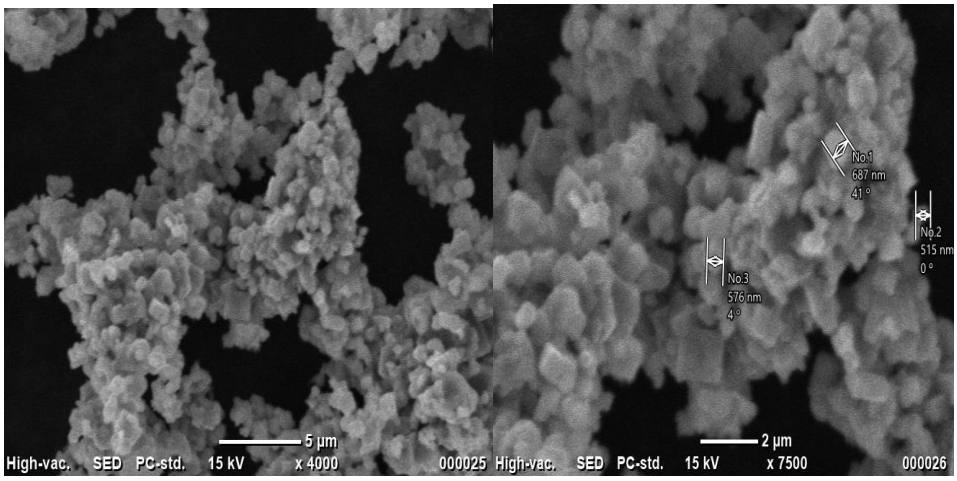


Figure No. 03 CYB after 2nd Puta

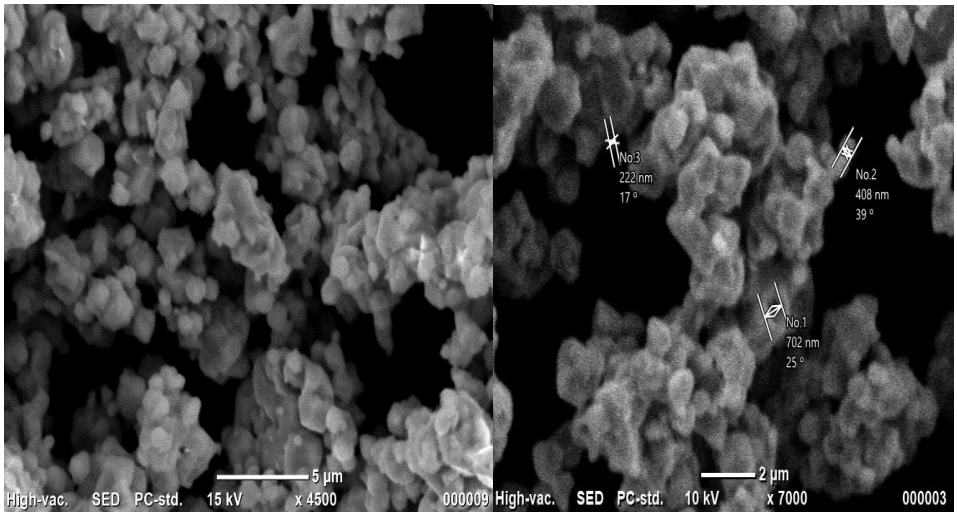


Figure No. 04 PYB after 1st Puta

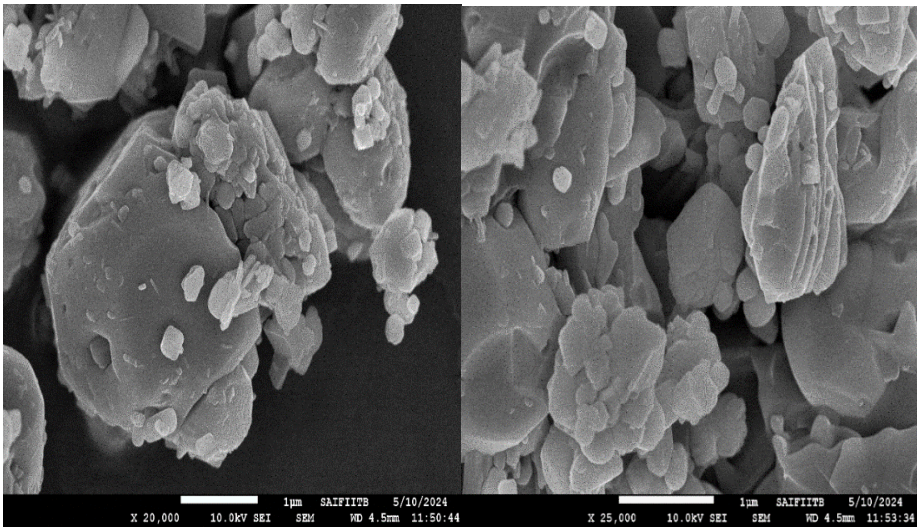
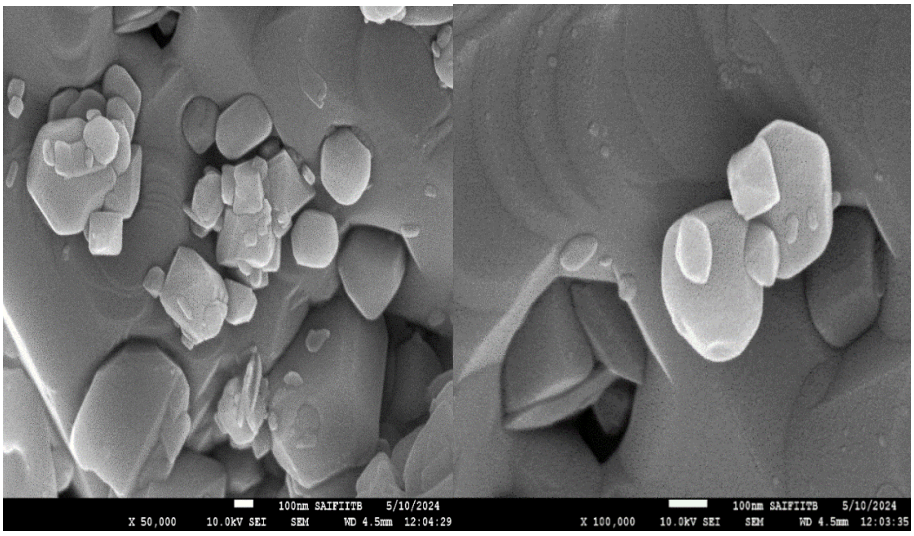


Figure No. 05 PYB after 4th Puta



SEM analysis showed initially porous, agglomerated in jarita yashada but later, the particles in all bhasma samples predominantly exhibited rectangular, spherical and hexagonal shapes. In the final PYB sample, the hexagonal wuzurite structure is more clearly visible compared to CYB after 2nd Puta. The particles in the CYB sample are heterogeneous and agglomerated, with sizes ranging from 200 to 702 nm, while in the PYB sample, the particles are homogeneous and well-dispersed, with sizes ranging from 20 to 263 nm.

6. ICPMS analysis

Table No. 30 ICPMS Results at various stages of Samples

Sl. No	Samples	Zn	Cd	Hg	Pb	Na	Mg	Ca	Fe	Cu
	In ppb									
1.	Raw yashada	2520	1.9	2.3	521	2508	2781	4639	483	4363
2.	Shodhita yashada	15820	0.9	-0.1	9.0	15.2	10.2	20.1	14	0.6
3.	Jarita yashada	15820	0.3	0.1	2.8	40.7	28.9	72.7	107.5	1.2
4.	CYB	15820	0.3	0.4	2.0	51.3	44.7	106.3	150.8	1.6
5.	PYB	15820	00	-0.3	0.1	96.2	96	196.6	133.1	2.7

The results of ICPMS shows that, the processing of Yashada significantly reduced harmful elements like cadmium, mercury, lead, and copper, particularly after Shodhana, with complete removal of cadmium and mercury and near-total elimination of lead and copper by the final PYB sample. Meanwhile, vital elements such as zinc, sodium, magnesium, calcium, iron and copper show increased concentration or retention in the later stages (Jarana, CYB and PYB). This indicates that while undesirable elements are effectively removed, some beneficial or neutral elements are concentrated in the final product. Comparatively, the PYB sample has significantly lower toxic element levels and higher concentrations of some beneficial elements like sodium, calcium, magnesium and copper.

4.2 EXPERIMENTAL STUDY RESULTS

I. Acute Oral Toxicity Study

a. Observations

1. Body Weight of animals:

Table No. 31 Weight Changes during the study

Sl. No.	Animals	Weight of the animals in gms			
		Day 1	Day 7	Day 14	Day 15
1.	A	172	176	184	185
2.	B	166	175	190	192
3.	C	165	172	180	185
4.	D	155	167	182	186
5.	E	153	156	169	173

The weight of all animals gradually increased throughout the study, with steady gains from Day 1 to Day 14, indicating consistent growth.

Table No. 32 Weight of major organs of all the animals

Organs	A	B	C	D	E
Ovaries, Uterus and Tubes	0.79 gm	1.16 gm	0.97 gm	0.75 gm	0.72 gm
Kidney	1.36 gm	1.18 gm	1.38 gm	1.24 gm	1.44 gm
Spleen	0.77 gm	0.59 gm	0.61 gm	0.59 gm	0.91 gm
Pancreas	0.69 gm	0.51 gm	0.48 gm	0.58 gm	0.46 gm
Heart	0.71 gm	1.00 gm	0.67 gm	0.89 gm	0.82 gm
Liver	7.19 gm	7.53 gm	8.18 gm	8.24 gm	7.64 gm
Brain	1.51 gm	1.47 gm	1.60 gm	1.52 gm	1.18 gm

There was not much notable change in the weight of major organs across the animals, with most organ weights remaining relatively consistent.

b. Results

1. Haematological parameters:

Table No. 33 Effect of PYB on Haematological parameters

Parameter	A	B	C	D	E
Hemoglobin (gm%)	14.3	13.4	14.3	14.3	12.9
Total WBC (10^3 cells/cu.mm)	15.22	27.60	11.23	08.03	19.77
Neutrophils (%)	20	84	15	26	15
Lymphocytes (%)	71	04	76	68	74
Eosinophils (%)	01	02	00	01	01
Monocytes (%)	08	00	09	05	10
MCH (pg)	18	18.5	17.6	18	17.4
MCV (fl)	53.3	52.6	51.2	52.1	50.9
MCHC (gm/l)	33.8	35.1	34.3	34.5	34.1
RBC (million cells/cu.mm)	7.93	7.28	8.16	7.97	7.43
Platelets (10^5 cells/cu.mm)	5.24	6.01	4.84	5.08	4.39
PCV (%)	42.3	38.3	41.8	41.5	37.8
RDW (fl)	50.9	44.8	48.7	47.4	41.1

The hematological parameters show mostly normal values. Hemoglobin levels ranged from 12.9 gm% to 14.3 gm%, WBC counts from 8.03 to 27.60 x 10^3 cells/cu.mm, and RBC counts from 7.28 to 8.16 million cells/cu.mm, all within normal limits. Neutrophils and lymphocytes showed typical distributions, though the percentage of neutrophils in one animal (B) was elevated. MCH, MCV, and MCHC values were normal, and platelets ranged from 4.39 to 6.01 x 10^5 cells/cu.mm, with PCV values between 37.8% and 42.3%. RDW values were also within the usual range. Overall, there were no significant abnormalities, indicating no major hematological changes due to acute PYB toxicity.

2. Biochemical parameters:

Table No. 34 Effect of PYB on biochemical parameters

Parameter	A	B	C	D	E
Total bilirubin (mgs%)	0.4	0.4	0.5	0.4	0.5
Direct bilirubin (mgs%)	0.1	0.1	0.1	0.1	0.1
SGOT (U/L)	44	29	46	25	34
SGPT (U/L)	37	41	34	42	43
Alkaline phosphatase (U/L)	140	66	55	32	110
Total protein (gm%)	8.6	8.1	8.5	8.0	7.5
Albumin (gm%)	4.6	4.0	4.5	4.1	3.7
Urea (mgs%)	29	42	36	30	35
Creatinine (mgs%)	0.4	0.5	0.4	0.6	0.5

The biochemical parameters were within normal ranges, with total and direct bilirubin (0.4%–0.5%), liver enzymes (SGOT: 25–46 U/L, SGPT: 34–43 U/L, alkaline phosphatase: 32–140 U/L), total protein (7.5%–8.6%), and albumin (3.7%–4.6%) levels indicating no significant liver damage. Urea (29–42 mg%) and creatinine (0.4–0.6 mg%) levels were within normal renal function limits.

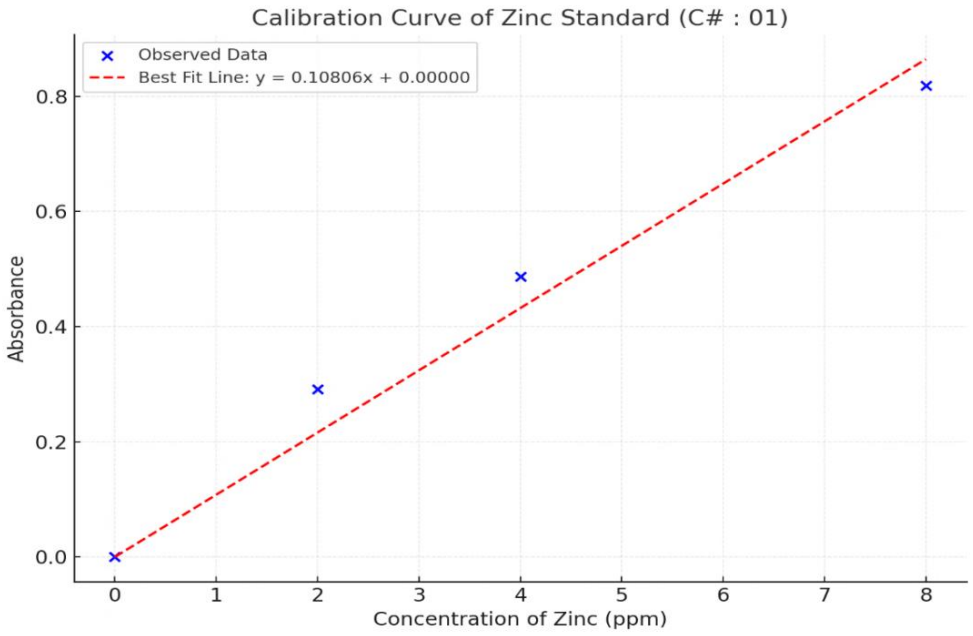
3. **Histopathological Results:** Histopathological examination revealed various changes in major organs. Mild congestion was observed in the ovaries, brain, heart, kidney, and spleen. The brain exhibited mild edema, inflammation, and mild neuronal eosinophilia. The heart showed normal features, while the kidneys displayed mild tubular desquamation. The liver showed signs of mild inflammation, ballooning degeneration, spotty necrosis (1 animal out of 5), and mild cholestasis. Additionally, the spleen displayed mild to moderate congestion along with lymphoid hyperplasia. The photo plates of tissue HPR are attached as annexure.

II Bioavilability Study Results:

Table No. 35 Calibration Data for Zinc Standard

Sl. No	Concentration (ppm)	Absorbance
1.	0	0.0001
2.	2	0.2915
3.	4	0.4869
4.	8	0.8183

Graph No. 10 Calibration Curve of Zinc Standard



The absorbance values were recorded at various concentrations of zinc (0, 2, 4, and 8 ppm) using a spectrophotometer. The linear regression equation y = 0.10806x + 0.0000 (correlation coefficient r = 0.9991) was obtained, indicating a nearly perfect linear relationship. This calibration curve served as the basis for quantifying zinc levels in the test samples analyzed in the bioavailability study.

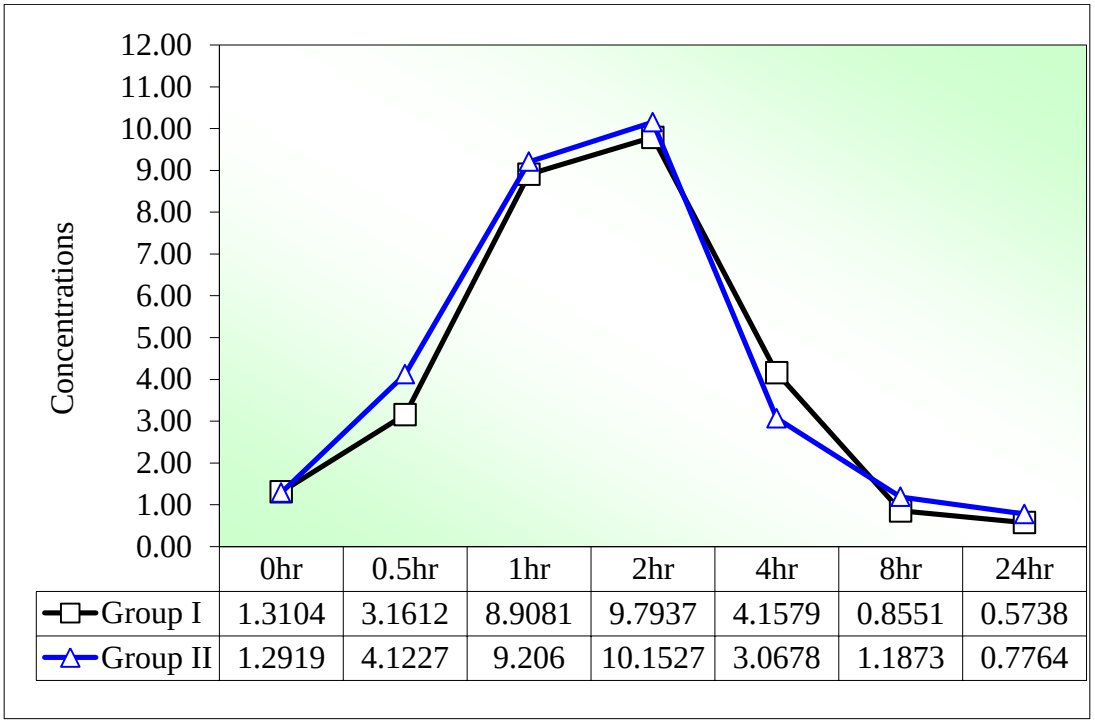
Table No. 36 AAS results at different time intervals of Test samples

Sample/Time	Group I CYB				Group II PYB			
	In ppm							
0 hr	1.256	1.243	1.39	1.353	1.326	1.13	1.43	1.281
½ hr	3.174	2.815	3.287	3.369	3.518	3.835	4.977	4.161
1 hr	9.405	8.942	7.638	9.647	9.811	10.522	8.323	8.168
2 hr	9.37	9.932	9.094	10.779	11.179	9.554	10.063	9.814
4 hr	3.872	3.821	4.319	4.619	2.912	3.557	2.768	3.034
8 hr	0.915	0.882	0.684	0.94	1.257	1.102	1.3	1.091
24 hr	0.553	0.608	0.536	0.598	0.801	0.797	0.745	0.763

Table No. 37 Mean AAS concentrations of both the samples

Sl. No	Time (Hr)	Mean Conc of CYB (ppm)	Mean Conc of PYB (ppm)
1.	0	1.3104	1.2919
2.	0.5	3.1612	4.1227
3.	1	8.9081	9.206
4.	2	9.7937	10.1527
5.	4	4.1579	3.0678
6.	8	0.8551	1.1873
7.	24	0.5738	0.7764

Graph No. 11 Mean concentrations Curve: CYB and PYB



	0hr	0.5hr	1hr	2hr	4hr	8hr	24hr
Group I	1.3104	3.1612	8.9081	9.7937	4.1579	0.8551	0.5738
Group II	1.2919	4.1227	9.206	10.1527	3.0678	1.1873	0.7764

Table No 38 Mean Pharmaco Kinetic Parameters:

Samples/PK parameter	**C_{max}**	**T_{max}**	**AUC_t**	**AUC_∞**
CYB	9.7937ppm	2.0hr	48.89 ppm*hr	71.91ppm*hr
PYB	10.1527ppm	2.0hr	51.81ppm*hr	81.05ppm*hr

Note: C_{max} – The maximum concentration observed, **T_{max} -** The time at which **C_{max}** occurs.

AUC_t – The area under the concentration-time curve from t = 0 to the last measured time point by trapezoidal rule

For two points (t1, C1) and (t2, C2), **AUC** = (C1+C2) / 2 × (t2 − t1)

AUC_∞ - The area under the curve extrapolated to infinity, calculated as:

AUC_∞ = **AUC_t** + C_{last}/ K_{el}

where K_{el} is the elimination rate constant derived from the terminal phase of the concentration-time data.

Statistical analysis of AAS data:

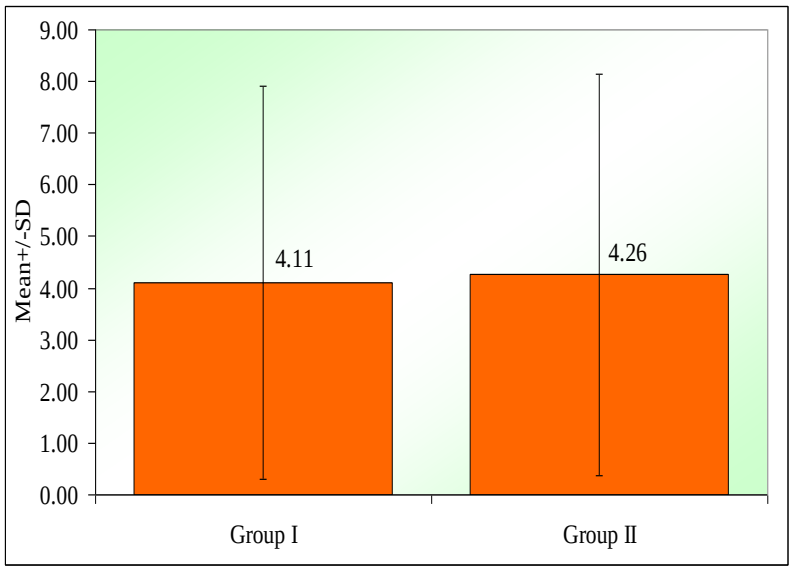
Table No. 39: Normality of mean concentration scores in Group I and Group II

Groups	Kolmogorov -Smirnov	df	p-value	Shapiro-Wilk	df	p-value
Group I	0.2090	7	0.2000	0.8460	7	0.1130
Group II	0.2280	7	0.2000	0.8260	7	0.0730
Note that, the mean concentration scores in Group I and Group II follow normal distribution. Therefore, the parametric tests were applied.						

Table No. 40: Comparison of Group I and Group II with mean concentration scores by independent t test

Groups	Mean	SD	SE	t-value	P-value
Group I	4.11	3.81	1.44	-0.0725	0.9434
Group II	4.26	3.89	1.47		

Graph No. 12: Comparison of Group I and Group II with mean concentration scores



The statistical comparison of zinc bioavailability between Group I (CYB) and Group II (PYB) indicated no significant difference, as the p-value was 0.9434 ($p > 0.05$). However, a slight increase was observed in the mean zinc concentration of Group II (4.26±3.89 ppm) compared to Group I (4.11±3.81 ppm).

III Spermatogenic Activity Study

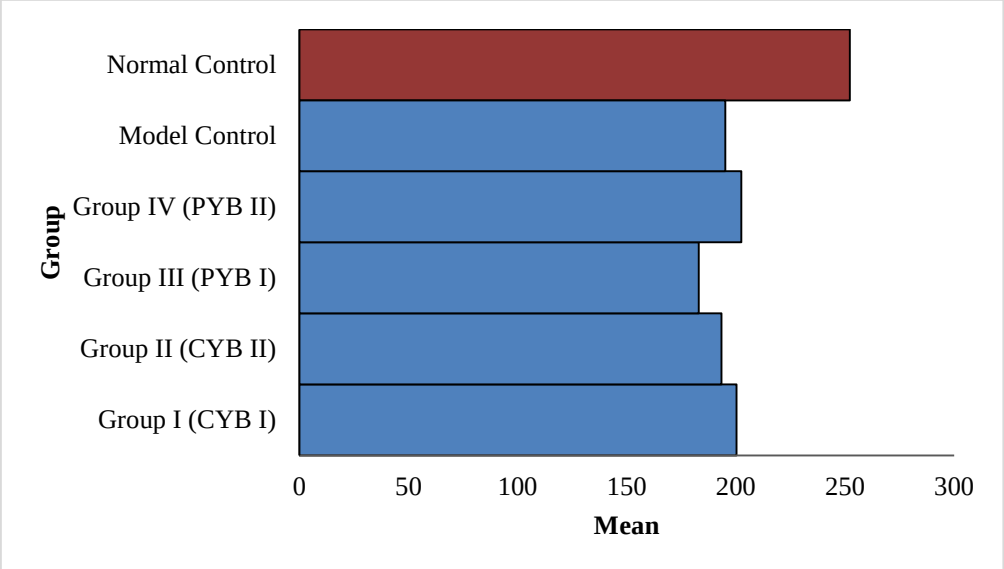
a) Body weight:

Table No. 41 Comparison of weight change in rats with reference to normal control group

Groups		Pre-treatment Mean±SD	Post-treatment Mean±SD
Infertility groups	Group I (CYB I)	222.33 ±17.78	200.33 ±18.13*
	Group II (CYB II)	225.67 ±18.28	193.33 ±23.93*
	Group III (PYB I)	215.00 ±15.27	183.00 ±31.09*
	Group IV (PYB II)	213.00 ±15.35	202.50 ±18.87 *
	Model Control	207.67 ±11.86	195.17 ±21.12*
Normal Control group		203.33 ±15.20	252.17 ±17.93

*<0.05 significance is obtained by comparing post-test to normal control post-test values by independent t test

Graph No. 13 Comparison of weight change in rats with reference to normal control group



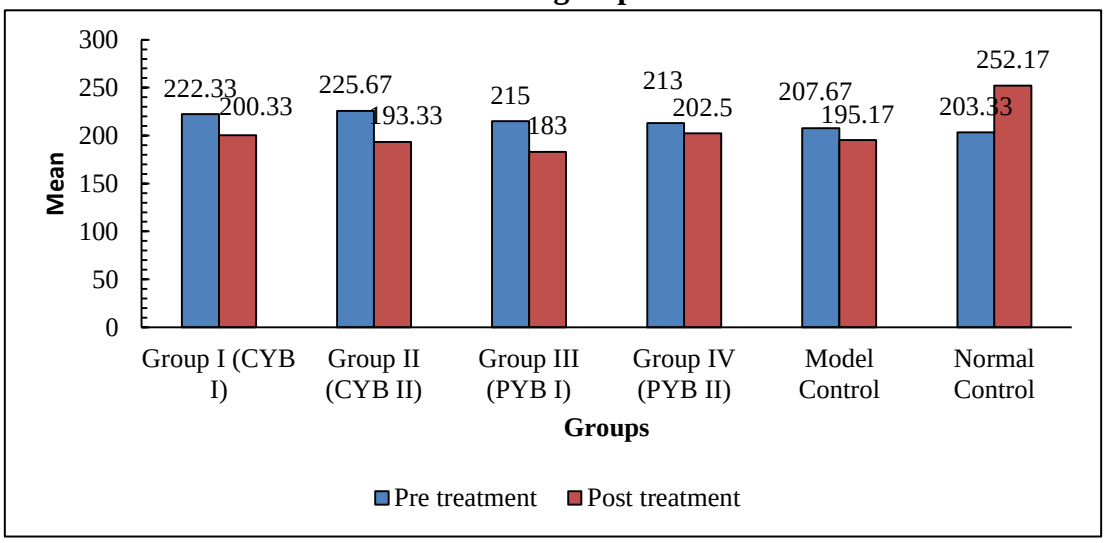
The Normal Control group showed a significant weight increase (203.33 ±15.20 to 252.17 ±17.93), indicating normal physiology. All infertility groups (including Model Control) showed a significant weight decrease ($p < 0.05$), confirming successful adenine-induced infertility compared to the Normal Control.

Table No. 42 Comparison of Pre-treatment and Post-treatment weight change in rats within the groups during infertility model development

Groups		n	Pre-treatment Mean±SD	Post-treatment Mean±SD	Sig.
Infertility groups	Group I (CYB I)	6	222.33 ±17.78	200.33 ±18.13	0.072
	Group II (CYB II)	6	225.67 ±18.28	193.33 ±23.93	0.031*
	Group III (PYB I)	6	215.00 ±15.27	183.00 ±31.09	0.065
	Group IV (PYB II)	6	213.00 ±15.35	202.50 ±18.87	0.351
	Model Control	6	207.67 ±11.86	195.17 ±21.12	0.173
Normal Control Group		6	203.33 ±15.20	252.17 ±17.93	0.01*

*<0.05 significance is obtained by paired t test between pre-treatment and post treatment

Graph No. 14: Comparison of Pre-treatment and Post-treatment weight change within groups



Here, Normal Control group showed a significant increase in weight (p = 0.01), indicating normal physiology. In contrast, the infertility model groups demonstrated varying degrees of weight reduction, with Group II (CYB II) showing the most significant decrease (p = 0.031*), confirming successful induction of infertility due to adenine toxicity.

Table No. 43: Normality of Before intervention and After intervention Body weight in six groups

Times	Groups	Kolmogorov -Smirnov	Df	P-value	Shapiro -Wilk	df	P-value
Before intervention	Group I	0.1390	6	0.2000	0.9880	6	0.9850
	Group II	0.1940	6	0.2000	0.9020	6	0.3850
	Group III	0.2980	6	0.1030	0.8670	6	0.2150
	Group IV	0.3920	6	0.1040	0.7260	6	0.0610
	Model control	0.1470	6	0.2000	0.9760	6	0.9280
	Normal control	0.2900	6	0.1250	0.8520	6	0.1620
After intervention	Group I	0.1780	6	0.2000	0.9370	6	0.6350
	Group II	0.1630	6	0.2000	0.9200	6	0.5070
	Group III	0.2500	6	0.2000	0.9440	6	0.6930
	Group IV	0.2210	6	0.2000	0.9160	6	0.4760
	Model control	0.2220	6	0.2000	0.9150	6	0.4710
	Normal control	0.1700	6	0.2000	0.9590	6	0.8100
Difference	Group I	0.1740	6	0.2000	0.9260	6	0.5460
	Group II	0.1520	6	0.2000	0.9800	6	0.9540
	Group III	0.1760	6	0.2000	0.9540	6	0.7750
	Group IV	0.1970	6	0.2000	0.9330	6	0.6040
	Model control	0.2120	6	0.2000	0.9220	6	0.5210
	Normal control	0.4290	6	0.1010	0.6250	6	0.1010

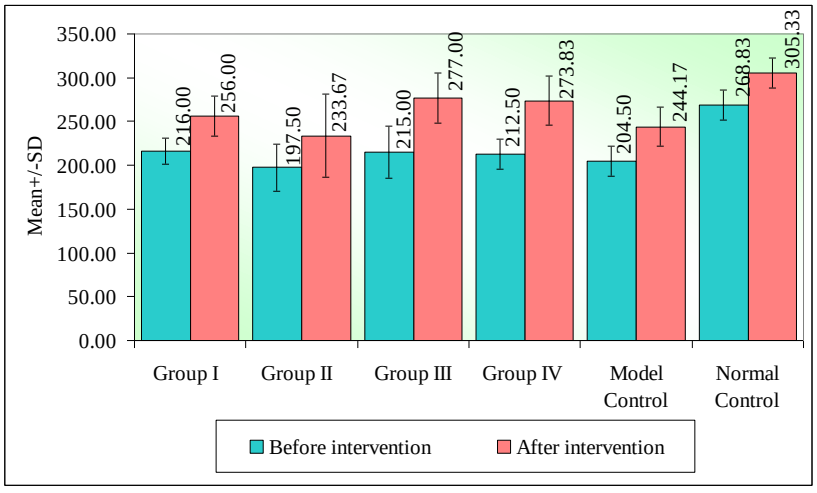
Note that, the before and after intervention Body weight scores in six groups follow normal distribution. Therefore, the parametric tests were applied.

Table No. 44: Comparison of before and after intervention body weight scores within groups by dependent t test (Paired t test)

Groups	Times	Mean	SD	Mean Diff.	% of change	t-value	p-value	Effect size
Group I	Pre	216.00	14.67					
	Post	256.00	22.67	-40.00	-18.52	-6.1094	0.0017*	0.8820
Group II	Pre	197.50	26.55					
	Post	233.67	47.48	-36.17	-18.31	-3.9084	0.0113*	0.7530
Group III	Pre	215.00	29.76					
	Post	277.00	28.39	-62.00	-28.84	-16.9794	0.0001*	0.9830
Group IV	Pre	212.50	17.03					
	Post	273.83	27.98	-61.33	-28.86	-11.2145	0.0001*	0.9620
Model Control	Pre	204.50	17.39					
	Post	244.17	21.83	-39.67	-19.40	-8.2631	0.0004*	0.9320
Normal Control	Pre	268.83	16.89					
	Post	305.33	17.41	-36.50	-13.58	-14.4467	0.0001*	0.9770

*p<0.05

Graph No. 15 Comparison of before and after intervention body weight scores within groups



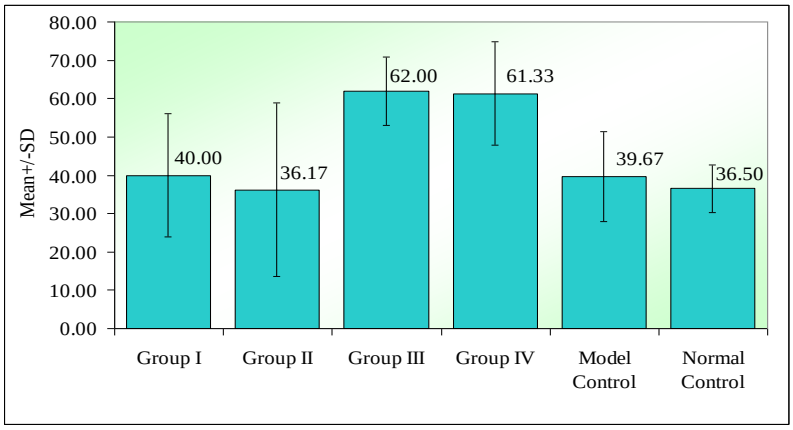
All groups, including Normal Control**,** showed statistically significant weight changes ($p < 0.05$), with Group III and Group IV showing the largest percentage of change (around -28.84% and -28.86% respectively). The effect sizes were large for all groups, with Group III (0.9830) and Group IV (0.9620) exhibiting the strongest effects.

Table No. 45: Comparison between the six groups with changes in Body weight scores from before intervention to after intervention by one-way ANOVA

Groups	Changes from Before intervention to After intervention		
	Mean	**SD**	**SE**
Group I	40.00	16.04	6.55
Group II	36.17	22.67	9.25
Group III	62.00	8.94	3.65
Group IV	61.33	13.40	5.47
Model Control	39.67	11.76	4.80
Normal Control	36.50	6.19	2.53
F-value	4.4986		
P-value	0.0035*		

*p<0.05

Graph No. 16: Comparison between the six groups with changes in Body weight scores



The changes in body weight from before to after the intervention were significantly different across the groups, with an F-value of 4.4986 and p-value of 0.0035***.** Group III and Group IV exhibited the largest changes in weight (62.00 and 61.33), indicating a strong intervention effect, while Model Control and Normal Control, which did not undergo intervention, showed more modest weight changes (39.67 and 36.50), reflecting natural variation.

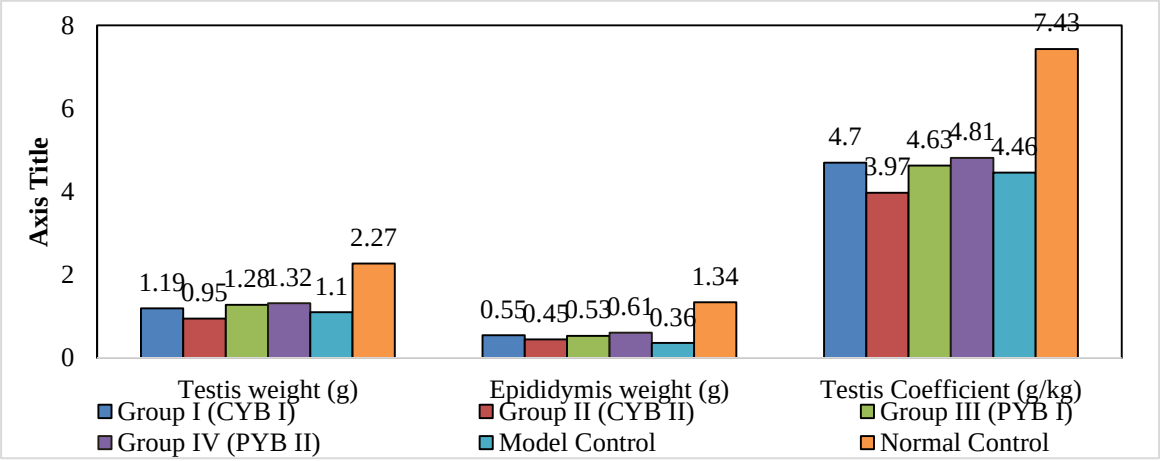
b) Tissue Parameters:

Table No. 46 Comparison of Rat testis weights, epididymis weights, and testis coefficient of varied groups with reference to normal control group

Groups/Tissues	Testis weight (g) Mean±SD	Epididymis weight (g) Mean±SD	Testis Coefficient (g/kg) Mean±SD
Group I (CYB I)	1.19 ±0.13 **	0.55 ±0.11 **	4.70 ±0.84 **
Group II (CYB II)	0.95 ±0.46 **	0.45 ±0.21 **	3.97 ±1.53 **
Group III (PYB I)	1.28 ±0.17 **	0.53 ±0.09 **	4.63 ±0.49 **
Group IV (PYB II)	1.32 ±0.19 **	0.61 ±0.08 **	4.81 ±0.59 **
Model Control	1.10 ±0.22 **	0.36 ±0.13 **	4.46 ±0.59 **
Normal Control	2.27 ±0.25	1.34 ±0.29	7.43 ±0.79

**<0.05 & <0.001 in comparison to normal control group by ANOVA

Graph No. 17 Comparison of tissue weights of varied groups



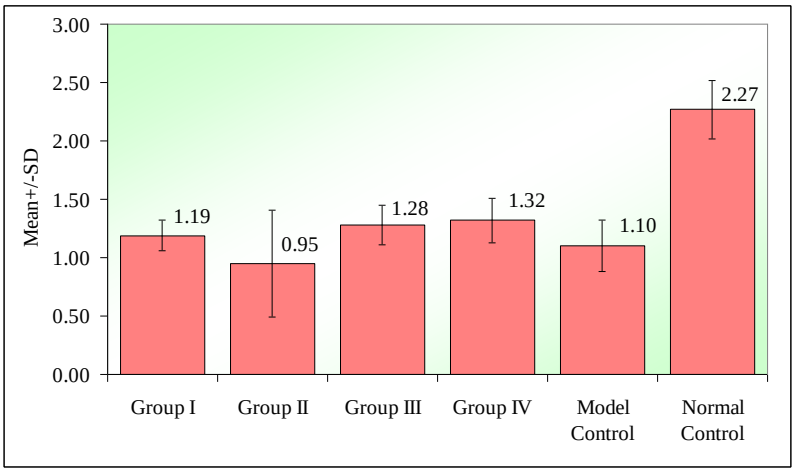
The experimental groups (CYB I, CYB II, PYB I, PYB II, and Model Control) showed significantly reduced testis and epididymis weights, as well as testis coefficients, compared to the Normal Control group, which had the highest values across all parameters ($p < 0.01$). However, the Model Control group exhibited the most pronounced adverse effects, with the lowest epididymis weight (0.36 ± 0.13 g), testis weight (1.10 ± 0.22 g), and testis coefficient (4.46 ± 0.59 g/kg). Treatment groups (CYB I, PYB I, and PYB II) showed some mitigation of these effects compared to the Model Control group.

Table No. 47 Comparison of Rat testis weights between the groups

Groups	Mean±SD	F	Significance
Group I (CYB I)	1.19 ±0.13	19.437	<0.05*
Group II (CYB II)	0.95 ±0.46		
Group III (PYB I)	1.28 ±0.17		
Group IV (PYB II)	1.32 ±0.19		
Model Control	1.10 ±0.22		
Normal Control	2.27 ±0.25		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 18 Comparison of six groups with Testis weight (g) scores



The ANOVA test ($F = 19.437$, $p < 0.05$) revealed significant differences in rat testis weights among the groups. The Normal Control group had the highest testis weight (2.27 ± 0.25 g), significantly greater than all other groups. The Model Control group (1.10 ± 0.22 g) showed reduced testis weight compared to the Normal Control group, indicating adverse effects adenine.

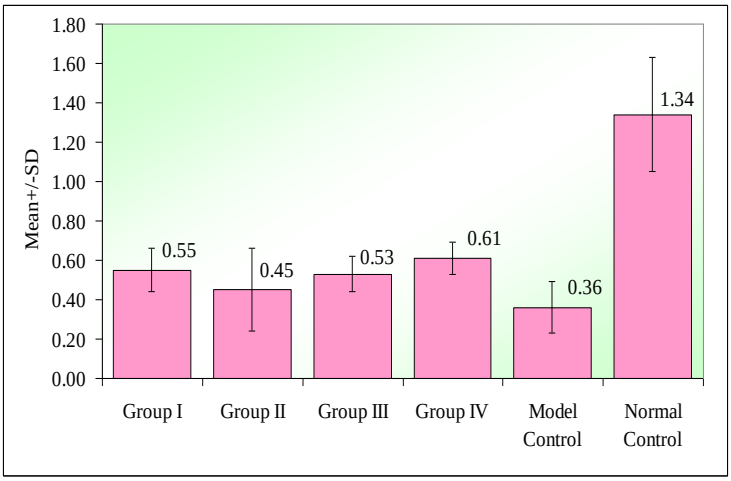
Among the treatments, PYB II (1.32 ± 0.19 g) and PYB I (1.28 ± 0.17 g) demonstrated a partial restorative effect, with higher testis weights compared to the Model Control group. CYB I (1.19 ± 0.13 g) showed slight improvement, while CYB II (0.95 ± 0.46 g) was the least effective.

Table No. 48 Comparison of Rat epididymis weights between the groups

Groups	Mean±SD	F	Significance
Group I (CYB I)	1.19 ±0.13	19.437	<0.05*
Group II (CYB II)	0.95 ±0.46		
Group III (PYB I)	1.28 ±0.17		
Group IV (PYB II)	1.32 ±0.19		
Model Control	1.10 ±0.22		
Normal Control	2.27 ±0.25		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 19 Comparison of six groups with Epididymis weight (gm) scores



The ANOVA test ($F = 19.437$, $p < 0.05$) showed significant differences in rat epididymis weights among the groups. The Normal Control group (2.27 ± 0.25 g) had the highest weight, indicating optimal reproductive health, while the Model Control group (1.10 ± 0.22 g) had reduced weight due to adenine.

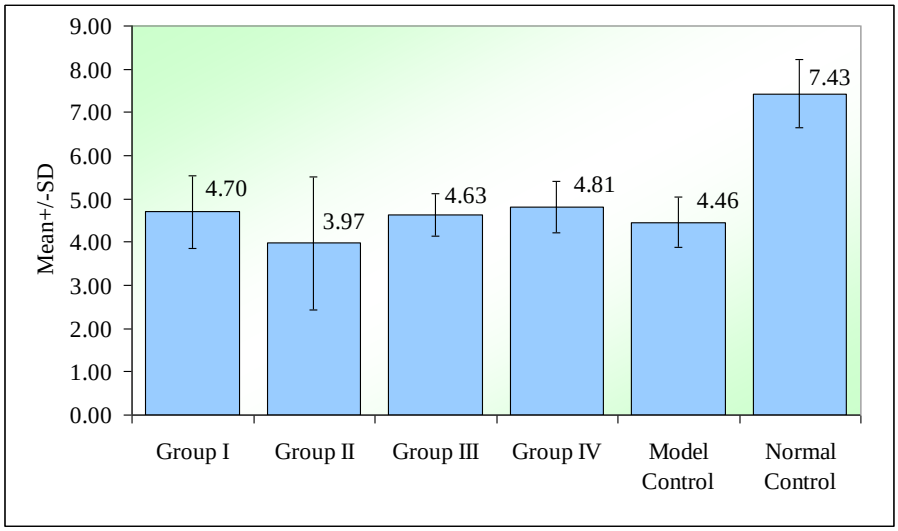
Among the treatments, PYB II (1.32 ± 0.19 g) and PYB I (1.28 ± 0.17 g) showed partial restorative effects, while CYB I (1.19 ± 0.13 g) demonstrated slight improvement. CYB II (0.95 ± 0.46 g) was the least effective.

Table No. 49 Comparison of testis coefficient between groups

Groups	Mean±SD	F	Significance
Group I (CYB I)	4.70 ±0.84	25.796	0.05*
Group II (CYB II)	3.97 ±1.53		
Group III (PYB I)	4.63 ±0.49		
Group IV (PYB II)	4.81 ±0.59		
Model Control	4.46 ±0.59		
Normal Control	7.43 ±0.79		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 20 Comparison of six groups with Testis Coefficient (gm/kg) scores



The ANOVA test ($F = 25.796$, $p < 0.05$) showed significant differences in testis coefficients. The Normal Control group had the highest coefficient (7.43 ± 0.79 g/kg), while the Model Control group had a reduced value (4.46 ± 0.59 g/kg). Among the treatments, PYB II (4.81 ± 0.59 g/kg) and PYB I (4.63 ± 0.49 g/kg) showed the highest coefficients, suggesting restorative effects, while CYB II (3.97 ± 1.53 g/kg) showed the lowest, indicating lesser effectiveness.

c) Biochemical parameters

Table No. 50 Rat hormonal levels in varied group (n=18) before and after intervention

Groups /Hormonal parameters	FSH (mlU/ml)		Sig.	LH (mlU/ml)		Sig.	Testosterone (ng/ml)		Sig.
	BT Mean± SD	AT Mean± SD		BT Mean± SD	AT Mean± SD		BT Mean± SD	AT Mean± SD	
Group I (CYB I)	2.89 ± 0.23	4.87 ± 0.25	<0. 05*	0.83 ± 0.05	1.93 ± 0.67	0. 098	1.60 ± 0.10	3.33 ± 0.68	0. 039*
Group II (CYB II)	3.33 ± 0.59	5.17 ± 1.07	0.187	0.94 ± 0.14	3.10 ± 0.30	0. 009*	0.94 ±0.113	2.80 ± 0.22	0. 004*
Group III (PYB I)	2.84 ± 0.19	5.50 ± 0.62	0. 018*	0.85 ± 0.08	3.13 ± 0.25	0. 005*	1.40 ± 0.10	4.47 ± 0.65	0. 019*
Group IV (PYB II)	2.87 ± 0.206	3.87 ±0.205	<0.05	1.01 ± 0.17	1.70 ± 0.26	0.084	1.57 ± 0.31	3.23 ± 0.31	0. 038*
Model Control	5.87 ± 1.79	6.87 ± 1.79	0.065	0.96 ± 0.15	1.20 ± 0.26	0.122	1.50 ± 0.26	2.17 ± 0.32	0. 184
Normal Control	1.78 ± 0.20	4.03 ± 1.20	0. 065*	1.10 ± 0.10	2.57 ± 0.67	0.1	2.80 ± 0.17	4.63 ± 0.38	0. 004*

*<0.05 significance is obtained by paired t test

The paired t-test results show significant changes in FSH and testosterone levels in several treatment groups (CYB I, CYB II, PYB I, and PYB II) compared to baseline, with improvements in testosterone levels, especially in PYB I and CYB I. However, Model Control and Normal Control groups showed less significant hormonal fluctuations.

Table No. 51 Comparison of FSH between the groups

Group/Parameter	FSH (mlU /ml)	F	Significance
Group I ±CYB I)	4.87 ±0.25	3.41	0.038*
Group II ±CYB II)	5.17 ±1.07		
Group III ±PYB I)	5.50 ±0.62		
Group IV ±PYB II)	3.87 ±0.205		
Model Control	6.87 ±1.79		
Normal Control	4.03 ±1.20		

*<0.05 significance is obtained by ANOVA TEST

The ANOVA results for FSH revealed significant differences (p = 0.038) among the groups. Group III (PYB I) exhibited the highest mean FSH levels (5.50 ± 0.62 mlU/ml), while Group IV (PYB II) had the lowest (3.87 ± 0.205 mlU/ml). Group I (CYB I) (4.87 ± 0.25 mlU/ml) and Group II (CYB II) (5.17 ± 1.07 mlU/ml) showed moderate increases in FSH levels compared to the Normal Control group (4.03 ± 1.20 mlU/ml). In contrast, the Model Control group had the highest FSH levels (6.87 ± 1.79 mlU/ml).

Table No. 52 Comparison of LH between the groups

Group/Parameter	LH (mlU/ml)	F	Significance
Group I (CYB I)	1.93 ±0.67	9.474	0.01*
Group II (CYB II)	3.10 ±0.30		
Group III (PYB I)	3.13 ±0.25		
Group IV (PYB II)	1.70 ±0.26		
Model Control	1.20 ±0.26		
Normal Control	2.57 ±0.67		

*<0.05 significance is obtained by ANOVA TEST

The ANOVA results for LH revealed significant differences (p = 0.01) among the groups. Group III (PYB I) and Group II (CYB II) exhibited the highest LH levels, with means of 3.13 ± 0.25 mlU/ml and 3.10 ± 0.30 mlU/ml, respectively. In contrast, Group IV (PYB II) (1.70 ± 0.26 mlU/ml) and the Model Control group (1.20 ± 0.26 mlU/ml) had the lowest LH levels. Group I (CYB I) showed slightly higher levels (1.93 ± 0.67 mlU/ml) compared to the Model Control group.

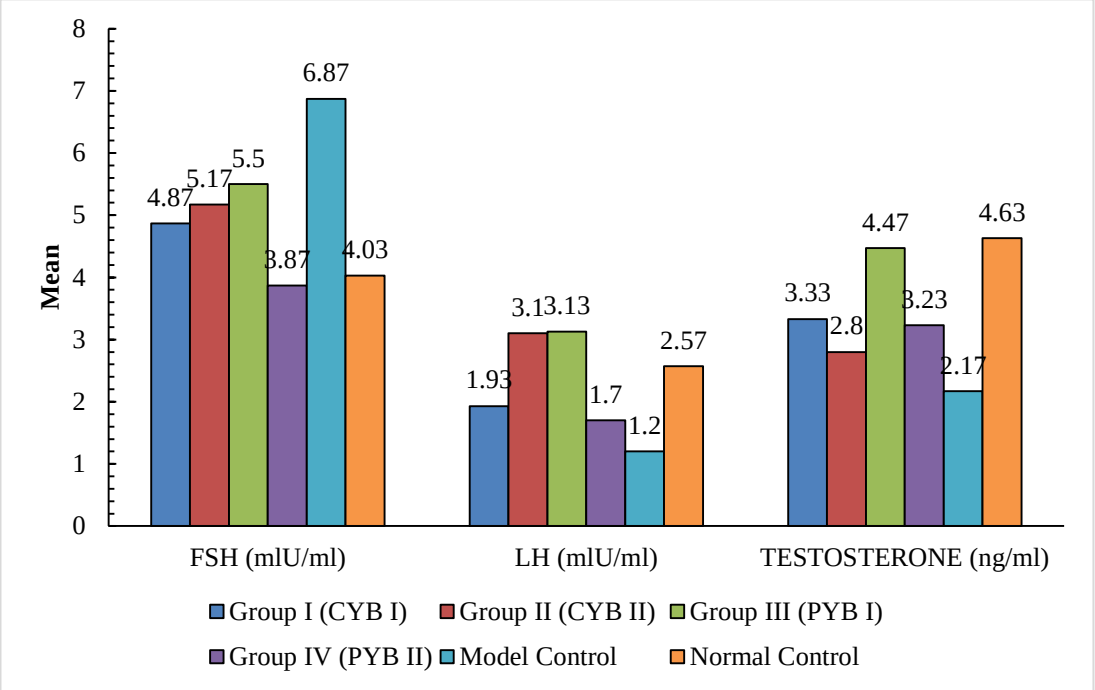
Table No. 53 Comparison of Testosterone between the groups

Group/Parameter	Testosterone (ng/ml)	F	Significance
Group I (CYB I)	3.33 ±0.68	12.669	<0.05*
Group II (CYB II)	2.80 ±0.26		
Group III (PYB I)	4.47 ±0.65		
Group IV (PYB II)	3.23 ±0.31		
Model Control	2.17 ±0.32		
Normal Control	4.63 ±0.38		

*<0.05 significance is obtained by ANOVA TEST

The ANOVA results for testosterone revealed significant differences (p < 0.05) among the groups. Group III (PYB I) had the highest testosterone levels (4.47 ± 0.65 ng/ml), followed by Group I (CYB I) (3.33 ± 0.68 ng/ml) and Group IV (PYB II) (3.23 ± 0.31 ng/ml). The Model Control group exhibited the lowest testosterone levels (2.17 ± 0.32 ng/ml), which were significantly lower than those in the Normal Control group (4.63 ± 0.38 ng/ml).

Graph No. 21 Comparison of hormonal level in rats between the groups



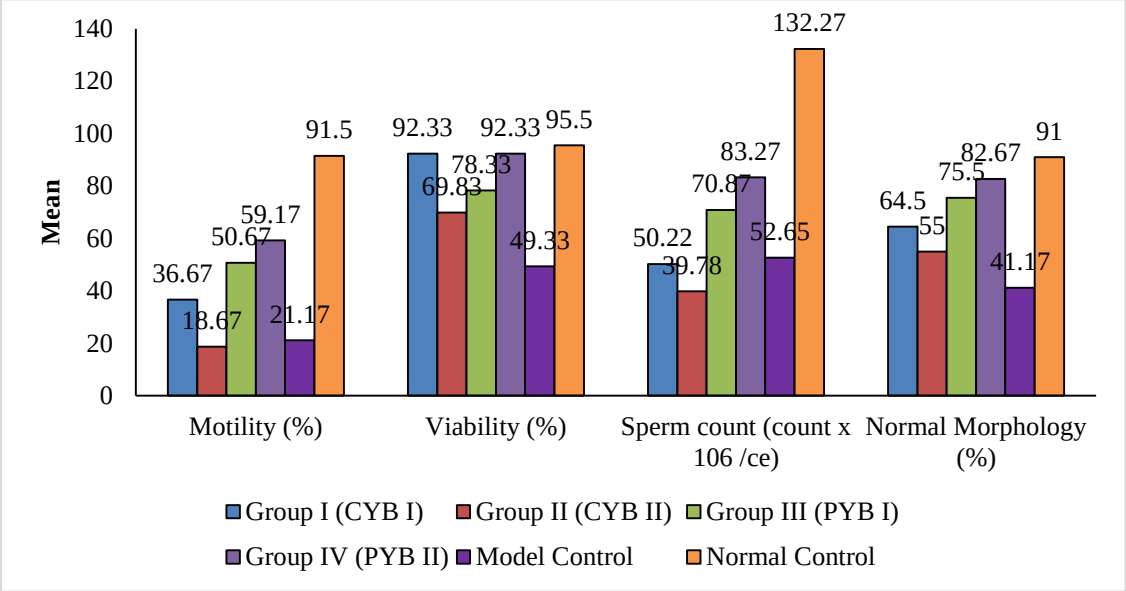
d) Sperm Parameters:

Table No. 54 Rat sperm counts, motility, Viability and Morphology of varied groups with reference to normal control group

Groups / Sperm parameters	Motility (%) Mean ± SD	Viability (%) Mean ± SD	Sperm count (count x 106 /ce) Mean ± SD	Normal Morphology (%) Mean ± SD
Group I (CYB I)	36.67 ±14.88 *	92.33±8.98 °	50.22±8.06 *	64.50±24.68
Group II (CYB II)	18.67 ±18.69 *	69.83±23.60	39.78 ±24.37 *	55.00±17.85 *
Group III (PYB I)	50.67 ±22.84 **	78.33±38.33	70.87±15.77 *	75.50±11.02 °
Group IV (PYB II)	59.17 ±9.60*	92.33±4.08 °	83.27±20.31 **	82.67±8.64 °
Model Control	21.17 ±16.86*	49.33±11.27*	52.65±14.50 *	41.17±15.66 *
Normal Control	91.50 ±3.27	95.50±3.83	132.27±9.55	91.00±2.61

*<0.05 in comparison to normal control group & **<0.05 in comparison to normal and model control group ° < 0.05 in comparison to model control group is obtained by ANOVA

Graph No. 22 Comparison of sperm parameters between groups



The sperm parameters of experimental groups showed significant variations compared to the normal control. Motility, viability, sperm count and normal morphology were significantly reduced in the CYB groups and model control, while PYB groups exhibited improvement. Specifically, PYB II demonstrated the highest recovery, with sperm motility (59.17 ±9.60%), viability (92.33±4.08%), sperm count (83.27±20.31 x 10^6/ce) and morphology (82.67±8.64%) significantly better than the model control.

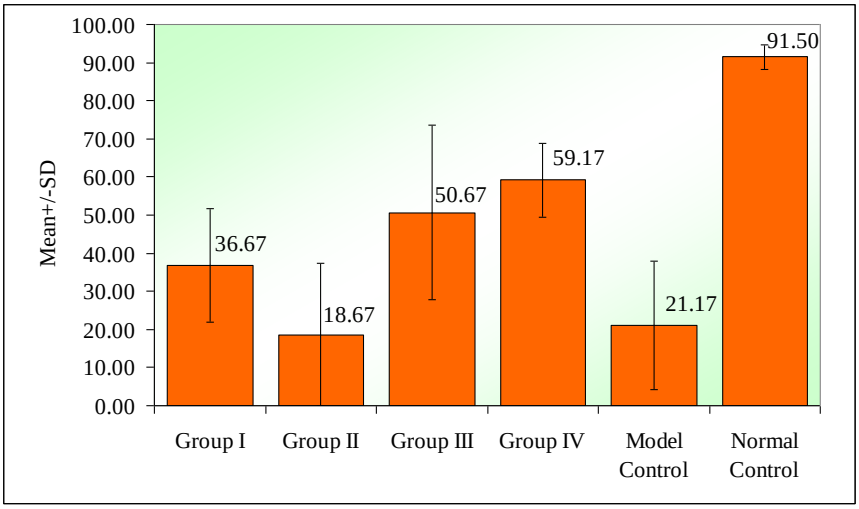
These findings suggest potential protective effects of PYB treatment against sperm damage. The individual parameter comparison between the groups is done as below.

Table No. 55 Comparison of Motility between the groups

Group	Mean ± SD	F	Significance
Group I (CYB I)	36.67 ±14.88	18.079	<0.05*
Group II (CYB II)	18.67 ±18.69		
Group III (PYB I)	50.67 ±22.84		
Group IV (PYB II)	59.17 ±9.60		
Model Control	21.17 ±16.86		
Normal Control	91.50 ±3.27		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 23 Comparison of Motility between the groups



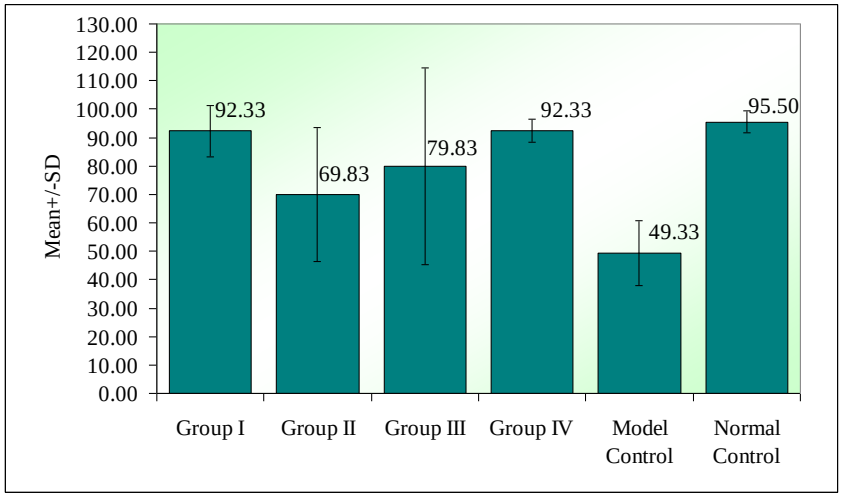
The motility (%) of spermatozoa varied significantly among the groups (ANOVA: F = 18.079, p < 0.05). The Normal Control group had the highest motility (91.50 ± 3.27), significantly higher (p < 0.05) than all other groups. The Model Control group showed reduced motility (21.17 ± 16.86). Among treatments, PYB II demonstrated the greatest improvement (59.17 ± 9.60), followed by PYB I (50.67 ± 22.84), both significantly better than the Model Control (p < 0.05). CYB I (36.67 ± 14.88) and CYB II (18.67 ± 18.69) were less effective, with CYB II showing the lowest motility among the treated groups.

Table No. 56 Comparison of Viability between the groups

Group	Mean ± SD	F	Significance
Group I (CYB I)	92.33±8.98	5.054	0.02*
Group II (CYB II)	69.83±23.60		
Group III (PYB I)	78.33±38.33		
Group IV (PYB II)	92.33±4.08		
Model Control	49.33±11.27		
Normal Control	95.50±3.83		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 24 Comparison of Viability between the groups



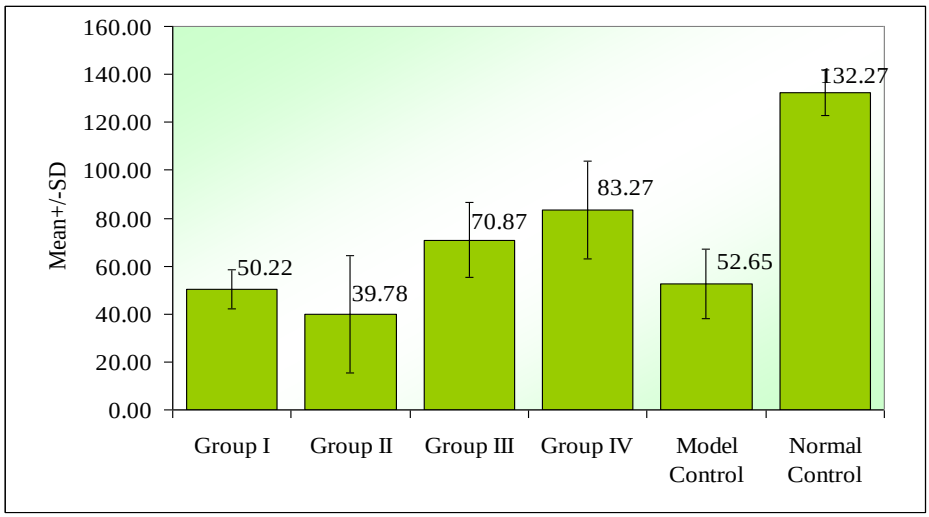
The viability (%) of spermatozoa varied significantly among the groups (ANOVA: F = 5.054, p = 0.02). The Normal Control group exhibited the highest viability (95.50 ± 3.83), significantly higher ($p < 0.05$) than most other groups, while the Model Control group showed the lowest viability (49.33 ± 11.27). Among the treated groups, CYB I (92.33 ± 8.98) and PYB II (92.33 ± 4.08) showed the most improvement, with viability significantly higher ($p < 0.05$) than the Model Control. PYB I (78.33 ± 38.33) demonstrated moderate improvement, while CYB II (69.83 ± 23.60) had relatively lower viability, being closer to the Model Control group.

Table No. 57 Comparison of Sperm count between the groups

Group	Mean ± SD	F	Significance
Group I (CYB I)	50.22±8.06	25.067	<0.05*
Group II (CYB II)	39.78 ±24.37		
Group III (PYB I)	70.87±15.77		
Group IV (PYB II)	83.27±20.31		
Model Control	52.65±14.50		
Normal Control	132.27±9.55		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 25 Comparison of Sperm count between the groups



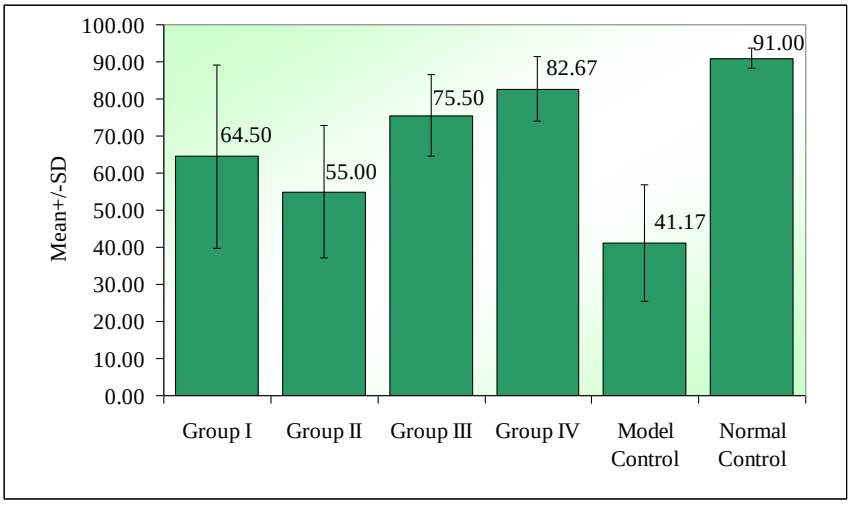
The sperm count (x10⁶/ce) varied significantly among the groups (ANOVA: F = 25.067, p < 0.05). The Normal Control group exhibited the highest sperm count (132.27 ± 9.55), significantly higher (p < 0.05) than all other groups. The Model Control group showed reduced sperm count (52.65 ± 14.50). Among the treated groups, PYB II (83.27 ± 20.31) demonstrated the greatest improvement, followed by PYB I (70.87 ± 15.77), both significantly higher than the Model Control. CYB I (50.22 ± 8.06) exhibited comparable results to the Model Control, while CYB II (39.78 ± 24.37) had the lowest sperm count among the treated groups.

Table No. 58 Comparison of Normal morphology between the groups

Group	Mean ± SD	F	Significance
Group I (CYB I)	64.50±24.68	8.899	<0.05*
Group II (CYB II)	55.00±17.85		
Group III (PYB I)	75.50±11.02		
Group IV (PYB II)	82.67±8.64		
Model Control	41.17±15.66		
Normal Control	91.00±2.61		

*<0.05 significance is obtained by ANOVA TEST

Graph No. 26 Comparison of Normal morphology between the groups



The normal morphology (%) of spermatozoa varied significantly among the groups (ANOVA: F = 8.899, p < 0.05). The Normal Control group exhibited the highest normal morphology (91.00 ± 2.61), significantly higher (p < 0.05) than all other groups. The Model Control group showed the lowest normal morphology (41.17 ± 15.66). Among the treated groups, PYB II (82.67 ± 8.64) demonstrated the greatest improvement in normal morphology, followed by PYB I (75.50 ± 11.02), both significantly higher than the Model Control. CYB I (64.50 ± 24.68) showed moderate improvement, while CYB II (55.00 ± 17.85) had the lowest normal morphology among the treated groups, closer to the Model Control.

e) Histopathological findings

In this study, histopathological analysis was conducted on the testicular tissue. The data from six experimental groups (CYB I, CYB II, PYB I, PYB II, MC, NC) were analyzed for several histological parameters of testis, including seminiferous tubule diameter, height of seminiferous epithelium, Johnsen's mean testicular biopsy score, Leydig cell count, and various pathological changes (e.g., congestion, distortion of architecture, germ cell detachment, and germ cell death). The photoplates of testicular histopathology asre attached as photoplates in annexure.

1. Seminiferous Tubule Diameter: The average diameter of seminiferous tubules was significantly different across the groups, reflecting the impact of adenine-induced infertility and subsequent interventions.

- **CYB I (259 µm) and CYB II (279 µm):** These intervention groups showed moderate reductions in tubule diameter compared to the normal control group, indicating partial preservation of testicular architecture after treatment with Yashada bhasma.
- **PYB I (306 µm) and PYB II (307 µm):** These groups exhibited a larger tubule diameter compared to CYB I and CYB II, suggesting a slightly better restoration of testicular structure following intervention.
- **MC (148 µm):** The model control group showed a significant reduction in tubule diameter, highlighting the adverse effects of adenine-induced infertility on testicular morphology.
- **NC (342 µm):** The normal control group maintained a healthy seminiferous tubule diameter, serving as the baseline for comparison.

These results indicate that the intervention groups had varying levels of success in mitigating the structural damage caused by adenine treatment with CYB I and PYB II showing more pronounced outcomes.

2. Height of Seminiferous Epithelium: The height of the seminiferous tubule epithelium was also measured and varied across the experimental groups, reflecting the impact of the treatments.

- **CYB I (91 µm) and CYB II (101 µm):** Both intervention groups showed improved epithelial height compared to the MC group, with CYB II exhibiting a more pronounced recovery.
- **PYB I (71 µm) and PYB II (120 µm):** PYB II had the highest epithelium height, suggesting a more effective intervention, while PYB I showed some improvement but less so than CYB II.
- **MC (56 µm):** The model control group had the lowest epithelial height, reflecting significant damage due to adenine treatment.
- **NC (62 µm):** The normal control group showed a relatively healthy epithelium height, serving as the standard for comparison.

The intervention with Yashada bhasma appears to have had a positive effect on the height of the seminiferous epithelium, with PYB II showing the most significant restoration.

3. Johnsen's Mean Testicular Biopsy Score: Johnsen's score is a key indicator of spermatogenesis. The scores across the groups provide insights into the functional state of the seminiferous epithelium.

- **CYB I (9.8) and CYB II (9.5):** These intervention groups had scores close to the normal control, indicating that spermatogenesis was largely preserved.

- **PYB I (8.5) and PYB II (9.3):** PYB II had a score closer to the normal control, suggesting better recovery of spermatogenesis compared to PYB I.
- **MC (5.5):** The model control group showed a significantly lower score, indicating poor spermatogenesis and severe damage to the germinal epithelium due to adenine treatment.
- **NC (9.7):** The normal control group maintained near-normal spermatogenesis.

These findings indicate that the intervention with Yashada bhasma helped preserve spermatogenesis, with CYB I showing the best results among the interventional groups.

4. Leydig Cell Count: Leydig cells are essential for testosterone production, and their count can provide insights into endocrine function.

- **CYB I (18), CYB II (20), PYB I (24), PYB II (19):** These intervention groups showed a normal range of Leydig cells, suggesting that the treatments did not significantly disrupt the endocrine function of the testes.
- **MC (42):** The model control group had a higher number of Leydig cells, possibly as a compensatory response to the infertility induced by adenine treatment.
- **NC (20):** The normal control group showed a typical number of Leydig cells.

Although Leydig cell counts in the model control group were higher, the interventional treatments did not drastically affect the Leydig cell population, suggesting no major alterations to testosterone production.

5. Pathological Changes: Several pathological changes were observed in the testicular tissue, including congestion, distortion of architecture, and germ cell detachment and death.

- **Congestion (+):** All groups showed some level of congestion, indicating mild vascular disturbance, which could be a consequence of adenine-induced infertility or a side effect of treatment.

- **Distortion of Architecture of Testis (-):** No distortion was observed in any group, suggesting that the overall architecture of the testes was preserved, especially in the intervention groups.

- **Distortion of Architecture of Germinal Epithelium (+/++):** Only CYB II showed mild distortion of the germinal epithelium (+), while the MC group showed significant distortion (++).

- **Multinucleated Giant Cells (-):** No multinucleated giant cells were observed in any group, suggesting no severe inflammatory reactions or testicular damage.

- **Detachment of Germ Cells (+/++):** Detachment of germ cells was observed in CYB II (+), with MC group (++) showing more severe detachment. This indicates a degree of germ cell injury, particularly in model control.

- **Germ Cell Death (+):** Germ cell death was observed in the MC group (+), but no significant death was observed in the intervention groups, indicating a lesser degree of cell damage in the treated groups.

5. DISCUSSION

The present study demonstrated that additional size-reduction techniques applied in classical method of Yashada bhasma preparation resulted in a potentiated form with significantly smaller particle size, evident by DLS particle size analysis. The acute oral toxicity study confirmed that, the safety of the potentiated formulation, at a significantly higher dose of 2000 mg/kg providing a strong foundation for its application in therapeutic settings. Though, bioavailability study showed no significant difference in zinc absorption between CYB and PYB groups, but the trend of slight increase in mean zinc concentration suggests that the reduced particle size of PYB might offer subtle advantages in zinc bioavailability.

Furthermore, the *in vivo* evaluation of spermatogenic activity in an adenine-induced rat model highlighted the potential of PYB in Group IV (PYB II – half of therapeutic dose), which showed the most notable improvements among other interventional groups, with significantly enhanced sperm motility, viability, count, and normal morphology. This highlights the potentiated form, to achieve superior therapeutic effects at reduced doses. These findings underline the efficacy of combining traditional Ayurvedic Bhasmikarana with modern instrumental techniques, paving the way for advancements in the development of nano-formulations with optimized dosing strategies and improved therapeutic outcomes.

5.1 Discussion on Pharmaceutical Part:

Bhasmikarana is the proven and well recognized procedure for the preparation of mineral and metallic bhasmas of nano dimension. It is a very systematic procedure which involves various steps like shodhana, jarana and marana which will bring the changes at their physical and chemical composition with reduction of size of the

particles at each level. This concept of size reduction raises the question of whether additional size reduction techniques, applied at various stages of processing with the aid of modern instruments and equipment, can further decrease particle size in bhasma, thereby improving bioavailability and therapeutic efficacy.

The present study aimed to investigate this concept of size reduction at various levels of Classical Yashadha bhasma (zinc calyx) preparation and analyzing it through appropriate instrumental techniques. Potentiation by means of size reduction was performed at four stages of bhasmikarana. The first and second being, ball milling of jarita yashada followed by PYB kajjali for 24 and 48 hours respectively. This technique is similar to classical method of mardana and physical method of nano particle synthesis. Additionally, milling is widely adopted in pharmaceutical applications to modify the supramolecular structure of active pharmaceutical ingredients (API), converting them into polymorphs, co-amorphous or cocrystal forms, which can lead to increased solubility and absorption.[146] Since, most bhasma in Ayurveda are insoluble in water, the use of ball milling can logically improve efficacy by increasing the surface area and solubility of the active pharmaceutical ingredient (API).[147]

The third distinct size reduction technique is wet grinding of PYB with bhavana dravya (kumari and nimbu swarasa) in a wet grinder for 24 hours before each Puta (incineration) in an electric muffle furnace (EMF). Wet grinding is known to be more efficient and effective than dry milling, providing advantages like reduced energy usage, minimized agglomeration and the production of finer particles. These benefits contribute to improved process efficiency and lower operational costs.[148] In contemporary science, wet media milling, followed by drying, is a proven approach for enhancing the bioavailability of poorly water-soluble drugs. It offers benefits like

tunable, high drug loading and applicability to various poorly soluble drugs.[149] In present study fixed duration of wet grinding with herbal juices contributed to addition of more organic compounds in the final bhasma sample compared to classical. These additional size reduction techniques are not exactly followed in classical bhasmikarana of Yashada. The fourth step intervened was additional number of incinerations even after attainment of bhasma siddhi lakshanas after the second Puta of PYB. It was given two more Putas with sequential wet grinding, palletization and incineration for further reduction in the particle size.

Enhancing the particle size reduction of PYB for oral administration can improve its absorption by promoting efficient diffusion through physiological barriers like the gut mucin layer and enterocyte membranes.[150] When comparing the preparation methods of CYB and PYB, it is evident that a significantly higher amount of bhavana dravya (kumari and nimbu swarasa) was used during the first incineration of PYB compared to CYB. The only notable difference at this point is the ball milling of jarita yashada in the PYB method. This suggests that ball milling of jarita yashada likely increased the surface area of the particles, which in turn led to greater liquid absorption during wet grinding before incineration.

In the PYB preparation, the grinding time was fixed to 24 hours, which resulted in a higher consumption of bhavana dravya compared to CYB. This longer grinding period also provided more contact time between the particles and the grinding surfaces, facilitating better particle size reduction. Notably, after the second incineration, both samples had a soft texture, but by the third and fourth incinerations, the PYB became significantly softer, indicating further particle size reduction. This enhanced size reduction likely contributed to the amorphous nature of PYB, while CYB remained as a fine powder.

5.2 Discussion on Analytical Part

The preliminary physico chemical and phyto chemical analysis of raw drugs ensured optimum qualities by meeting with the standard values. The physico chemical analysis of both samples reveal, the total ash content of PYB (98.71%) is slightly higher than that of CYB (96.27%), indicating greater incorporation of inorganic content during its preparation. This difference may be attributed to PYB's extended contact with bhavana dravyas, which could introduce more organic and inorganic matter. Conversely, CYB exhibits a higher acid-insoluble ash content (52.34% vs. 46.12% for PYB), suggesting the presence of more non-soluble minerals, potentially due to differences in processing especially number of incinerations. Higher acid-insoluble ash content, often composed of non-digestible minerals like silica, is typically considered undesirable. It may reflect contamination or may result from inadequate processing.[151] Additionally, these non-digestible minerals can hinder the bioavailability of active ingredients, negatively impacting drug efficacy.[152]

The loss on drying is greater in PYB (0.298% vs. 0.09% for CYB), indicating higher moisture or volatile content, likely due to the use of larger quantities of bhavana dravya and prolonged grinding.

The qualitative inorganic element analysis and NPST revealed similar findings. Quantitative zinc analysis using ICPAES revealed a stepwise reduction in zinc concentration across various stages: raw yashada (99.88%), shodhana (92.92%), jarita yashada (82.21%), classical Yashada bhasma (CYB, 77.13%), and potentiated Yashada bhasma (PYB, 70.29%). This progressive decrease indicates the transformation of elemental zinc into its oxides and sulphides during successive processes of shodhana, jarana, and marana. These specialized Ayurvedic techniques, performed at high temperatures in the presence of oxygen and sulfur (from kajjali),

play a significant role in altering the chemical composition and physicochemical properties of zinc, highlighting the importance of traditional methods in bhasma preparation.[153]

The particle size analysis of CYB and PYB, conducted using dynamic light scattering (DLS), revealed a stepwise reduction in particle size throughout the preparation process for both formulations. However, PYB showed a more significant size reduction in the intermediate steps, resulting in a final particle size approximately two and a half times smaller than CYB. The polydispersity index (PDI) of PYB was 0.412, indicating a more uniform and homogeneous particle size distribution, whereas CYB exhibited a PDI of 1, suggesting greater particle size heterogeneity.

Zeta potential analysis further confirmed that both CYB and PYB samples displayed negative zeta potential values ranging from -13 mV to -28 mV, ensuring good colloidal stability by promoting electrostatic repulsion between particles and preventing aggregation.[154] The minimal difference in zeta potential between the final PYB and CYB samples suggests that both formulations would remain well-dispersed in solution, which is crucial for maintaining their integrity and therapeutic effectiveness during storage and application.

X-ray diffraction (XRD) analysis of the shodita sample showed strong alignment with the zinc reference pattern, particularly at 2θ values of 36°, 43° and 54° confirming high crystallinity and minimal impurities, indicating the sample is predominantly zinc.[155] The XRD analysis of the jarita sample revealed zinc oxide (ZnO) formation, with significant peaks at 2θ values of 31.8°, 34.4° and 36.3° suggesting the conversion of zinc into ZnO during processing, with excellent crystallinity and minimal impurities. For both CYB and PYB, XRD confirmed ZnO as the primary crystalline phase, with sharp, well-defined peaks at 31.8°, 34.4° and 36.3°

indicating high purity and well-ordered structures.[156] This confirms that both preparations successfully lead formation of ZnO. The alignment of PYB XRD peaks with CYB further validates the formation of ZnO, supporting the effectiveness of the preparation methods. This observation is consistent with Pareek et al.'s study, which also identified hexagonal ZnO structures in Yashada bhasma prepared traditionally

SEM analysis showed that jarita yashada initially exhibited porous and agglomerated particles, while the particles in all bhasma samples eventually exhibited rectangular, spherical and hexagonal shapes. In the final PYB sample, the hexagonal wuzurite structure was more distinct compared to CYB after the second Puta as evidenced in earlier work.[157] The CYB sample displayed heterogeneous, agglomerated particles, with sizes ranging from 200 to 702 nm, whereas the PYB sample showed homogeneous, well-dispersed particles, ranging from 20 to 263 nm.

ICPMS analysis revealed that processing significantly reduced toxic elements like cadmium, mercury, lead, and copper, with complete removal of cadmium and mercury and near-total elimination of lead and copper in the final PYB sample. In contrast, beneficial elements such as zinc, sodium, magnesium, calcium, and copper were retained or increased in concentration in the later stages of preparation (Jarana, CYB, and PYB). These findings indicate that while undesirable elements are effectively removed, beneficial elements are concentrated in the final product, with PYB showing significantly lower levels of toxic elements and higher concentrations of beneficial elements like sodium, calcium, magnesium, and copper.

5.3 Experimental Study Discussion

5.3.1 Discussion on Acute Oral Toxicity Study

The present study evaluated the acute oral toxicity of PYB, as it was subjected to additional size reduction in classical method, using OECD Guidelines 420. This

globally recognized protocol ensures scientific validity, animal welfare and regulatory compliance while minimizing the number of animals used. Female rats were employed due to their slightly higher sensitivity in toxicity studies, as supported by existing literature.[143] Eighteen animals were sanctioned for ethical approval; however, no mortality was observed during the sighting study, allowing completion of the study with only first group of five animals.

Previous studies by Ashish Arora et al. (2023) and Rinku D. Umrani et al. (2013) demonstrated the non-toxic nature of Yashada bhasma at doses up to 300 mg/kg in rodents. Based on these findings, the current study utilized a dose of 2000 mg/kg, approximately ten times the therapeutic range (125–250 mg) of human, for the sighting study. Body weight changes, a sensitive indicator of toxicity, showed a steady increase, potentially due to nontoxic nature of Yashada bhasma and the use of sodium carboxymethyl cellulose (CMC) as a vehicle. While CMC has been linked to dose-dependent lipid accumulation in zebrafish embryos (Alper Baran et al., 2020), no such effects were evident in the present study, as supported by normal liver histopathology.[158]

Hematological and biochemical parameters were predominantly within the established physiological ranges for female Wistar rats.[159,160] Hemoglobin concentrations ranged between 13–15 mg%, while total WBC counts varied from 7×10^3 to 10×10^3 cells/cu.mm, reflecting overall stability. Notably, one animal exhibited elevated WBC and neutrophil levels, aligning with inflammatory and congestive changes identified in brain tissue during histopathological analysis, suggesting a localized inflammatory response.[161,162] Minor deviations in biochemical parameters, including slightly elevated total bilirubin (0.4 -0.5 mg/dl), were noted but

did not result in clinically significant symptoms. Stool discoloration, often associated with increased bilirubin levels, but it was transient and did not indicate jaundice.

Histopathological examination revealed mild congestion across major organs, with notable findings in the brain, liver, kidneys, and spleen. The brain exhibited mild edema, inflammation, and neuronal eosinophilia, indicative of neuroinflammation. The liver showed mild inflammation, ballooning degeneration, spotty necrosis (in one animal), and mild cholestasis, likely linked to elevated zinc exposure.[163] The kidneys displayed mild tubular desquamation, suggesting early renal stress, while the heart maintained normal architecture. Spleen findings indicated lymphoid hyperplasia, consistent with immune system activation and leukocytosis was observed in one animal.

These findings are consistent with the study by Ashish Arora et al., which highlighted alterations in liver and kidney function tests and histopathological changes, suggesting that high doses of Yashada Pushpa Bhasma (a zinc-containing compound) may cause liver and kidney damage. While zinc compounds have therapeutic benefits, especially in managing chronic kidney disease, their caustic nature can induce renal injuries such as interstitial nephritis and tubular necrosis when administered in high doses.[163] Additionally, certain structural changes could be partially attributed to the euthanasia method (diethyl ether), which is known to cause tissue necrosis in the brain, liver and kidneys.[164]

Importantly, the dose selected for this study (2000 mg/kg) was 10 times the human therapeutic dose and 100 times the animal therapeutic dose, a level unlikely to occur in clinical practice. Nonetheless, the findings suggest that PYB at this high dose exhibits mild to moderate toxicity, reinforcing its safety at therapeutic doses.

5.3.2 Discussion on Bioavailability Study:

The pharmacokinetic analysis of CYB and PYB showed similar bioavailability, with no significant difference between the two groups ($p = 0.9434$). The peak concentration (Cmax) of CYB was 9.79 ppm, and for PYB it was 10.15 ppm, both at 2 hours (Tmax). The AUC values for CYB and PYB were also comparable, indicating that both compounds have similar absorption profiles. A slight increase in the mean zinc concentration in Group II (PYB) was noted, but this difference was not statistically significant.

These results align with previous studies on zinc bioavailability: Balkrishna et. al. (2023) demonstrated that zinc nanoparticles, like JB cycle 17, are efficiently absorbed in the gastrointestinal tract without cytotoxicity, supporting their potential for improved bioavailability. Similarly, Pareek et al. (2013) reported increased serum zinc levels in rats administered zinc ash, with a peak concentration at 2 hours, followed by a rapid decline. This transient rise suggested limited overall intestinal uptake of zinc from zinc ash.

In comparison to these studies, the findings in the current study suggest that both CYB and PYB are absorbed similarly, with a marginally higher absorption observed for PYB, though this difference is not statistically significant. This indicates that while zinc compounds can be absorbed effectively, the formulation and delivery method may play a role in enhancing bioavailability, which warrants further exploration for optimizing therapeutic applications.

5.3.3 Discussion on Spermatogenic activity

Infertility animal model

In the present study, an adenine-induced rat model is used to investigate male infertility. Male infertility rat models are essential for understanding the underlying

causes and potential treatments for infertility, as these models can be induced through various methods targeting different mechanisms. Among these, adenine-induced infertility models are particularly useful for studying testicular damage and exploring therapeutic interventions. Previous studies have shown that adenine administration leads to reduced sperm count, testicular atrophy, and hormonal imbalances. Additionally, zinc supplementation has been found to be beneficial in reversing these effects by improving sperm quality and reducing oxidative stress.

Spermatogenic Activity and Body Weight Changes

The significant increase in body weight in the Normal Control group (203.33 ± 15.20 to 252.17 ± 17.93) confirmed the physiological normalcy of these animals, while the infertility groups, including the Model Control, demonstrated a significant reduction in body weight ($p < 0.05$). This weight loss signifies the successful induction of infertility due to adenine toxicity, with the Model Control group showing the most pronounced decrease. Furthermore, in the groups treated with CYB and PYB (zinc calyx), there was a noticeable improvement in body weight, with Group III (CYB II) showing the most significant decrease ($p = 0.031$*), indicating partial recovery from the effects of adenine. The treatment groups (CYB and PYB) exhibited the largest percentage of change, particularly in Group III and Group IV, which had substantial improvements in body weight (around -28.84% and -28.86%, respectively). This suggests that zinc supplementation has a potential role in mitigating the weight loss associated with infertility.

Testicular and Epididymal Weights

The significant reduction in testicular and epididymal weights observed in the experimental groups (CYB I, CYB II, PYB I, PYB II, and Model Control) compared

to the Normal Control group further corroborates the negative impact of adenine on reproductive organs. The Model Control group exhibited the lowest epididymis weight (0.36 ± 0.13 g) and testis weight (1.10 ± 0.22 g), signifying severe infertility. However, the treatment groups showed some degree of recovery, especially in CYB I, PYB I, and PYB II, which displayed mitigated adverse effects compared to the Model Control group. These findings underscore the potential therapeutic value of zinc calyx in restoring testicular function and size.

Hormonal Levels

The hormonal analysis revealed significant fluctuations in FSH and testosterone levels following treatment. Paired t-tests demonstrated that several treatment groups (CYB I, CYB II, PYB I, and PYB II) showed improvements in testosterone levels, with PYB I and CYB I displaying the most marked increases. The results suggest that zinc supplementation, particularly through PYB I and CYB I, can restore hormonal balance, which is often disrupted in adenine-induced infertility models. The Model Control group, however, exhibited minimal hormonal fluctuations, reflecting the sustained dysfunction in the reproductive endocrine system.

Sperm Motility, Viability, and Count

Sperm motility and viability were significantly impacted in the Model Control group, with motility decreasing to 21.17 ± 16.86% and viability dropping to 49.33 ± 11.27%, indicative of severe infertility. In contrast, the Normal Control group exhibited high motility (91.50 ± 3.27%) and viability (95.50 ± 3.83%). Among the treated groups, PYB II showed the most remarkable improvement in sperm motility (59.17 ± 9.60%) and viability (92.33 ± 4.08%), followed by PYB I, which also

showed significant improvement. These findings suggest that zinc supplementation via PYB II and PYB I could effectively improve sperm function, particularly motility and viability, in adenine-induced infertility models. On the other hand, CYB I and CYB II were less effective, with CYB II showing the lowest sperm motility and count, highlighting the relative inefficacy of PYB at half of the therapeutic dose.

Sperm Morphology

Sperm morphology also varied significantly across the groups. The Normal Control group exhibited the highest percentage of normal sperm morphology (91.00 ± 2.61%), while the Model Control group had the lowest (41.17 ± 15.66%). Among the treatment groups, PYB II demonstrated the greatest improvement in sperm morphology (82.67 ± 8.64%), followed by PYB I (75.50 ± 11.02%). This suggests that the Yashada bhasma formulations, particularly PYB II, can substantially improve sperm structure, which is essential for fertility.

Histopathological Analysis

Histopathological analysis of testicular tissue provided further insights into the structural damage caused by adenine and the potential restorative effects of zinc supplementation. The results revealed a significant reduction in seminiferous tubule diameter in the Model Control group (148 µm), indicative of severe testicular damage. However, the intervention groups (CYB I, CYB II, PYB I, and PYB II) showed varying degrees of recovery. PYB II exhibited the largest seminiferous tubule diameter (307 µm), suggesting better preservation of testicular architecture compared to the Model Control group. Similarly, the height of the seminiferous epithelium, a key marker of spermatogenesis, was notably improved in PYB II (120 µm), with CYB II showing a moderate recovery. Johnsen's mean testicular biopsy scores confirmed

that CYB I and PYB II (9.3) helped preserve spermatogenesis, with CYB I showing the most favorable score (9.8).

The Leydig cell count, essential for testosterone production, was not significantly altered in the treatment groups, suggesting that the interventions did not disrupt endocrine function. However, the Model Control group exhibited a compensatory increase in Leydig cells, possibly in response to the hormonal imbalance induced by adenine. Pathological changes such as congestion, distortion of testicular architecture,

and germ cell detachment were observed more pronouncing in MC group followed by CYB II group. Although mild congestion was seen in all groups, the intervention groups (especially PYB II) showed more restorative effect on testicular tissue.

6. SUMMARY

The study focused on the potentiation of Yashada bhasma (zinc calyx) through additional size-reduction techniques, comparing the classical Yashada bhasma (CYB) with a potentiated form (PYB) to assess its bioavailability, safety and therapeutic effects.

The study was done using Yashada (zinc) as a raw material, selected on based on grahya laxanas. This raw Yashada was transformed into bhasma by pharmaceutical processing techniques using samanya shodhana, vishesha shodhana, jarana and marana as per classical standard operating procedures, to yield CYB. Similarly, by following additional size reduction techniques using intermittent ball milling and wet grinding for extended hours in classical bhasmikarana with added number of two puta after attainment of bhasma siddha laxanas was adopted to prepare PYB. The herbal drugs (kumarai and nimbuka) used in bhavana processes were fresh and quantity sufficient each time. The whole pharmaceutical processing was carried out in well equipped laborotary under aseptic precautions. Both the samples were subjected for comparative classical bhasma pareeksha, physicochemical parameters and high-end instrumental characterization as per standard guidelines in recognized laboratories. The study summarized several key findings across various domains:

PYB showed significantly smaller particle sizes compared to CYB, as evidenced by Dynamic Light Scattering (DLS) and other particle size analyses. The process involved multiple stages of size reduction, including ball milling, wet grinding with herbal juices and additional incinerations, which enhanced the surface area and solubility of the particles. PYB exhibited a more homogeneous particle size distribution (PDI of 0.412) compared to CYB (PDI of 1), indicating better uniformity

and potentially better bioavailability. The particle size reduction was linked to a decrease in zinc concentration, suggesting the transformation of zinc into its oxide form during the preparation process, which is important for enhancing its bioavailability.

Various analytical techniques like X-ray diffraction (XRD), scanning electron microscopy (SEM) and inductively coupled plasma mass spectrometry (ICPMS) revealed the transformation of zinc into its oxide form, with PYB showing a more homogenous distribution of particles compared to CYB. PYB also had lower levels of harmful elements (e.g., cadmium, mercury & lead etc.) and retained beneficial elements like sodium, magnesium and calcium etc.

An acute oral toxicity study conducted on PYB at a dose of 2000 mg/kg (10 times the human therapeutic dose) showed no significant mortality with mild toxicity symptoms. Toxicity related changes included mild congestion in major organs like liver, kidney and brain inflammation but no clinically significant symptoms were noted at highest test dose. The findings suggest that PYB is safe for therapeutic use, with potential for mild to moderate toxicity at very high doses that are unlikely to occur in clinical practice.

Both CYB and PYB showed similar absorption profiles, with no significant difference in bioavailability between the two formulations. However, a slight increase in zinc concentration was observed in PYB, indicating subtle advantages of smaller particle size in enhancing zinc absorption, though this difference was not statistically significant.

The study used an adenine-induced rat model to evaluate the potential of CYB and PYB in improving male infertility. The results demonstrated that PYB

significantly improved sperm motility, viability and morphology compared to CYB, particularly at lower doses (PYB II), indicating the potentiated formulation's enhanced therapeutic effects at reduced doses.

PYB II also showed improved testicular and epididymal weights, hormonal levels (FSH and testosterone) and seminiferous tubule structure in histopathological analysis, suggesting better preservation of spermatogenesis and testicular function compared to CYB. PYB II exhibited the most notable improvements in sperm motility (59.17%), sperm count (83.27%) and sperm morphology (82.67%). The histopathological analysis showed significant testicular damage in the model control (MC) group, while intervention groups exhibited varying degrees of recovery. PYB II demonstrated the most improvement in seminiferous tubule structure and epithelial height. Spermatogenesis was largely preserved, especially in CYB I followed by PYB II and Leydig cell counts remained stable.

7. CONCLUSION

Additional size reduction methods (ball milling and wet grinding) in classical bhasmikarana significantly reduced particle size in Potentiated Yashada bhasma (PYB) sample, as evident by instrumental charecterization.

Acute oral toxicity studies showed that PYB is safe even at high doses (10 times the therapeutic dose), with mild biochemical and histopathological variations.

PYB and CYB showed similar zinc absorption levels, but PYB demonstrated slightly better mean zinc concentrations, suggesting potential for improved bioavailability.

PYB II significantly enhanced sperm quality (motility, viability, count & morphology) and testicular recovery, with better outcomes than CYB, even at half the therapeutic dose.

7.1 LIMITATION OF STUDY

1. Lacks detailed pharmacokinetic (ADME) profiling of the drugs.
2. Short term safety assessment.
3. Short term Spermatogenic activity evaluation.

7.2 RECOMMENDATIONS FOR FURTHER STUDY

1. Development of standardized protocols for the preparation of Potentiated Yashada bhasma to ensure consistency.
2. Chronic toxicity studies for long-term validation of its safety.
3. Clinical trials to validate the therapeutic efficacy and bioavailability of PYB in comparison to CYB.
4. Exploring the molecular mechanisms behind the improved spermatogenic efficacy of PYB.

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9. ANNEXURE 01: PHOTOPLATES

1. PARADA SHODHANA



Ashodhita Parada



Parada with Sudha churna



Parada with Lashuna and Saindhava

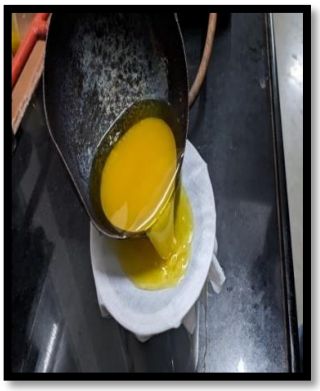
2. GANDHAKA SHODHANA



Ashodhita Gandhaka



Dravikarana of Gandhaka



Dhalana in Godugdha





Shodhita Gandhaka

3. YASHADA SHODHANA



Ashodhita Yashada



Kanji



Takra



Kulattha kwatha



Gomutra



Tilataila



Churnodaka



Dravikarana of Yashada



Dhalana



Samanya Shodhita yashada



Vishesha Shodhita yashada

4. YASHADA JARANA



Apamarga panchanga churna



Jarana yashada



Jarita yashada

5. CLASSICAL YASHADA BHASMA (CYB) PREPARATION



Jarita yashada with Shuddha Parada



Jarita yashada, Shodhita Parada and Shodhita Gandhaka



Bhavana with Kumari swarasa and nimbu swarasa

CYB 1st Puta(before):



Chakrika – Chaya shoshana



Chakrika arranged in sharava



Sandhi bandhana

Yashada in Marana 1st Puta (After):



Sharava in Muffle furnace



CYB - After 1st Puta

Yashada in Marana 2nd Puta (Before):



1st Puta bhasma with Parada



Mardana with Gandhaka



Bhavana with Kumari and Nimbu swarasa



Dry chakrika in sharava



Chakrika after 2nd Puta

6. POTENTITED YASHADA BHASMA (PYB) PREPARATION



Ballmilling of Jarita yashada



Mixing of Parada and Gandhaka in khalvayantra





Ballmilling of PYB Kajjali



Wet grinding with Kumari and Nimbu swarasa



Chakrika before 1st Puta of PYB



Incineration in EMF



PYB after 1st Puta





Mixing of Parada and Gandhaka in successive PYB puta



Chakrika before successive PYB puta

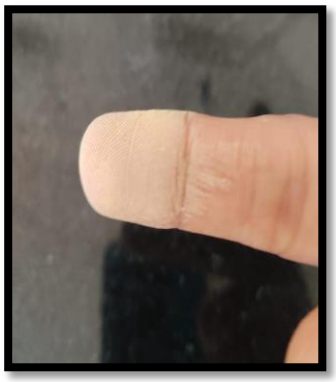


PYB after 4th Puta

7. BHASMA PAREEKSHAS:



Varitara



Rekhapurna



Unama

Niruttha Pareeksha



1gm Rajata with Yashada bhasma in a crucible



Subjected to incineration



Silver weighed for - 1gm after incineration

Apunarbhava Pareeksha



Panchadravaka Dravya with Yashada bhasma



After 950°C heat, no shiny particles observed



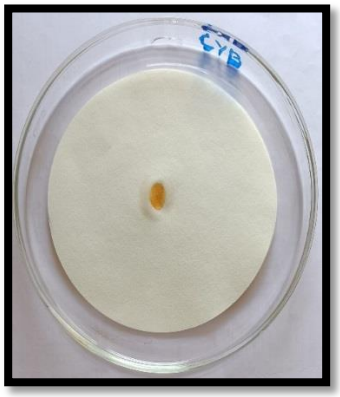
Nirdhuma

8. **NPST**

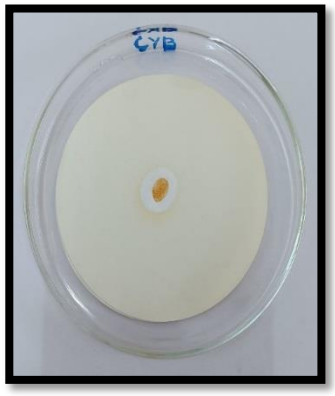
CLASSICAL YASHADA BHASMA



1st Phase (0 -5min)

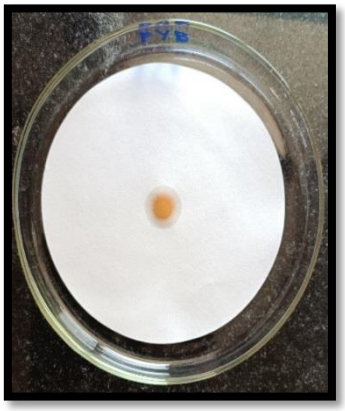


2nd Phase (5min -20min)



3rd Phase (20min -1day)

POTENTIATED YASHADA BHASMA



1st Phase (0 -5min)



2nd Phase (5min -20min)



3rd Phase (20min -1day)

9. ACUTE TOXICITY STUDY



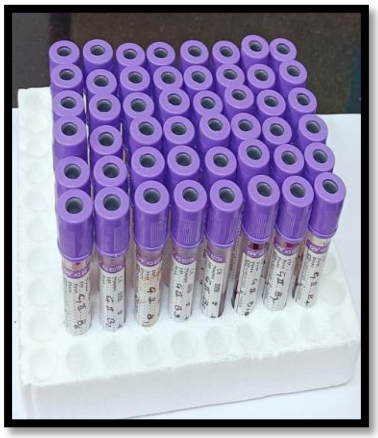
Dosing of the animal





Dissection of animal for major organ collection

10.BIOAVAILABILITY STUDY



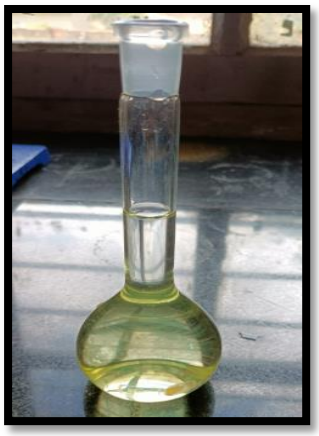
Collected blood samples



Wet acid digestion



Filtration



Volume made to 100 ml



Acid digested samples



AAS analysis

11. SPERMATOGENIC ACTIVITY



Animals housing in cages



Dosing of the animals



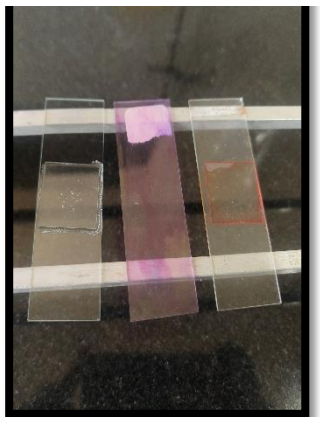
Dissection for Testis and epidydimis



Testis and epididymis



Sperm suspension preparation



Sperm viability and Morphology slides



Sperm motility



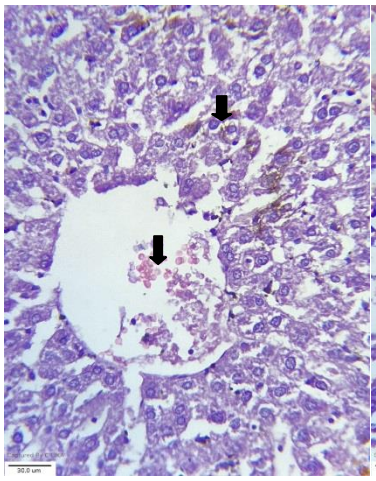
Sperm count



Sperm normal morphology

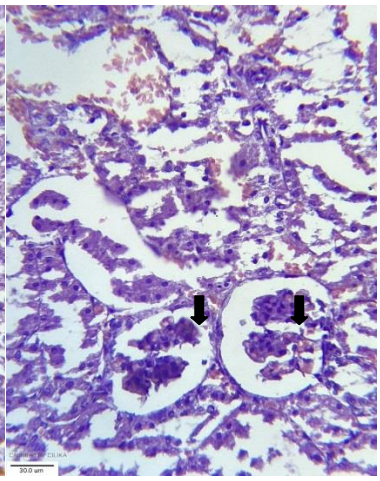
12. ACUTE TOXICITY STUDY: MICROPHOTOGRAPHS OF MAJOR ORGANS

Liver 40 X



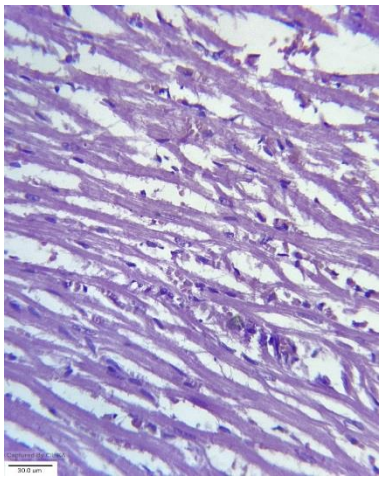
Venous and sinusoidal congestion Lymphocyte infiltration

Kidney 40 X



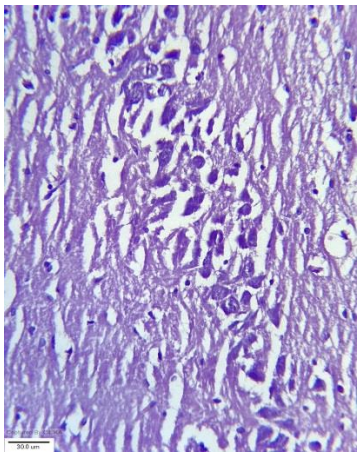
Congestion around the tubules and glomerular congestion

Heart 40 X



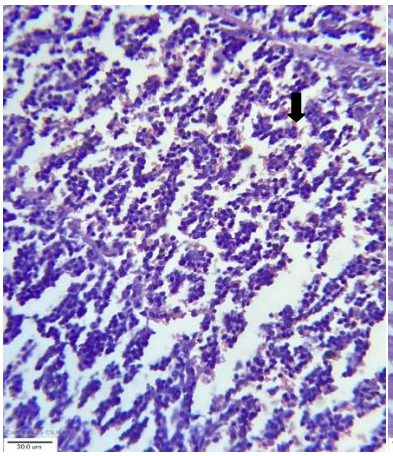
Mild congestion

Brain 40 X



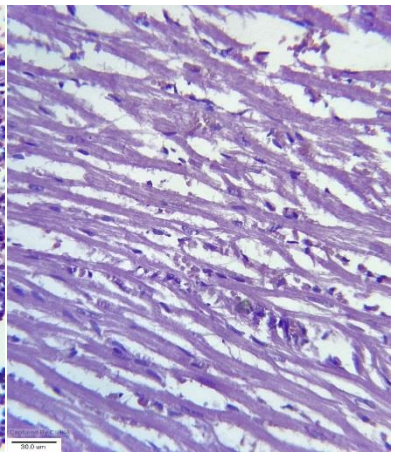
Cerebral & meningeal congestion, edema

Spleen 40 X



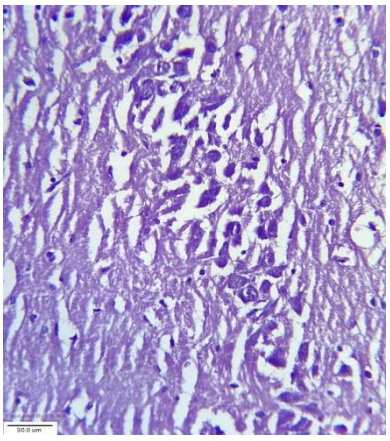
Mild congestion & lymphoid hyperplasia

Pancreas 40 X



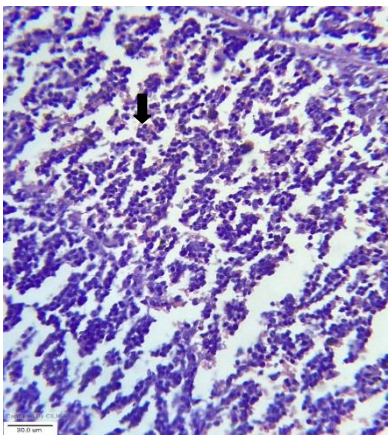
Mild congestion

Uterus 40 X



Mild congestion

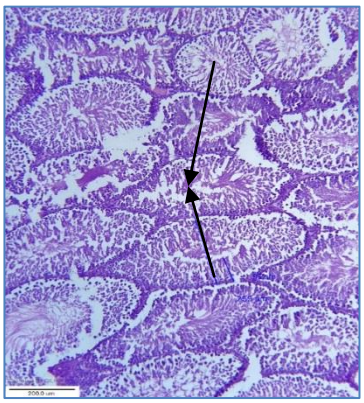
Ovary 40 X



Mild congestion

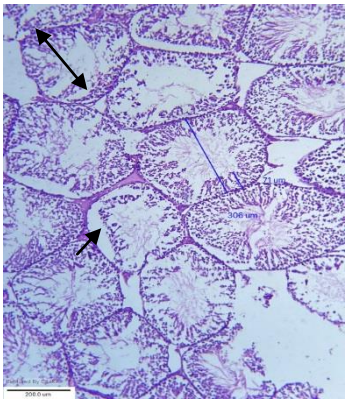
13. SPERMATOGENIC ACTIVITY: MICROPHOTOGRAPHS OF TESTIS

GI – CYB I Testis 10 X



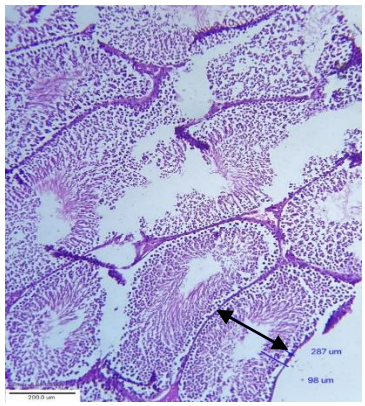
Seminiferous tubules diameter 259µm, Highest Johnson's score (9.8), Sperms and spermatids present.

GII – CYB II Testis 10 X



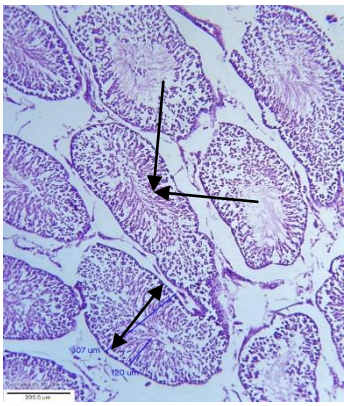
279 µm Seminiferous tubules diameter, Mild congestion and distortion of the germinal epithelium, Detachment of germ cells.

GIII: PYB I Testis 10 X



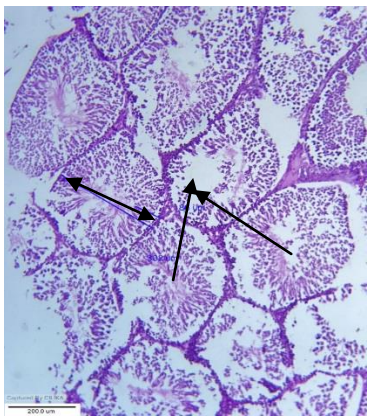
Seminiferous tubules diameter 306µm, sperms noted, lesser jonsons score (8.5)

GIV: PYB II Testis 10 X



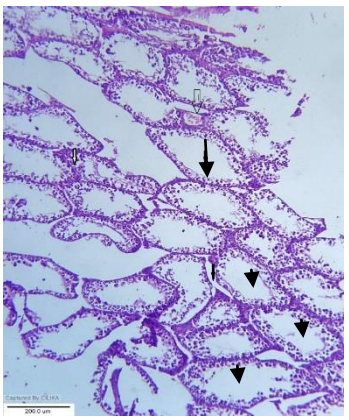
Seminiferous tubules diameter 307µm highest epithelium height, sperms noted

G V: Normal Control Testis 10 X



Highest (342 µm) seminiferous tubules diameter, visible sperms and spermatids

G VI: Model Control Testis 10 X



Lowest (148 µm) diameter, congestion, distortion of architecture of germinal epithelium, no sperms, detachment of germ cells

ANNEXURE 02: IAEC CERTIFICATE

SHRI BM KANKANAWADI AYURVED MAHAVIDYALAYA
Post Graduate Studies & Reseach Centre
(Approved by Central Council of Indial Medicine, New Delhi & M/o AYUSH, GoI)
A Constituent Unit of
KLE ACADEMY OF HIGHER EDUCATION & RESEARCH
(DEEMED-TO-BE- UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)

First AYUSH Institution having NAAC & NABH Accreditation

File No.25/369/2010-AWD Government of India Ministry of Environment & Forests & Climate Change (Animal Welfare Division)
1017/PO/Re/S/06/CPCSEA Dated: 24/11/2016

Certificate

This is to certify that the project entitled:

"Evaluation of Potentiated Yashada Bhasma for Safety, Bioavailability and Spermatogenic Activity"

Submitted by: **Dr. Geeta G. Gadad**,dated **6th August 2021**

Has been approved by the IAEC having IAEC approval No **BMK/IAEC/Res.No.-21/2021- 11.**

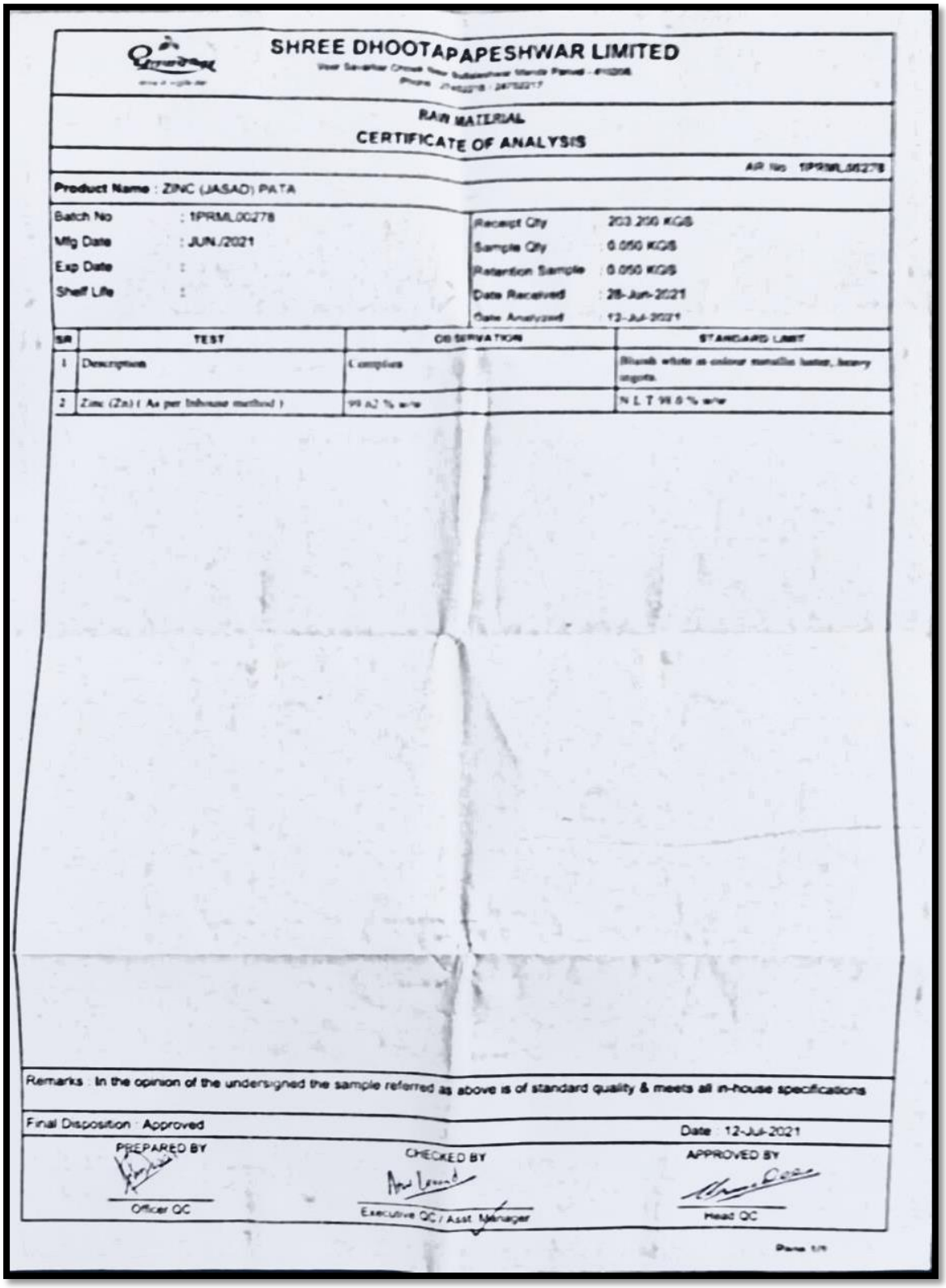
Dr.Rudramma R. Hiremath
MEMBER SECRETARY
Institutional Animal Ethics Committee
KAHER Shri B.M. Kankanwadi Ayurveda
Mahavidyalaya Shahapur, Belagavi.

Mr. Mallikarjun Kolhar
CPCSEA Nominee
Institutional Animal Ethics Committee
KAHER Shri B.M. Kankanwadi Ayurveda
Mahavidyalaya Shahapur, Belagavi.

Note:- Meeting was conducted online due to Covid-19 lockdown Reference letterF.No25/2/2020-CPCSEA-DADF dated 13th April 2020. The proposal has been approved and noted in meeting minutes

Shahapur, Belagavi – 590 003, Karnataka, India
Phone: +91 831 2486286; 7204969289 Fax: +91 831 2424157
Website. www. kleayurworld.edu.in, Email. bmkprincipal.kaher@kleayurworld.edu.in

ANNEXURE 03: RAW DRUG ZINC COA FROM SDL



SHREE DHOOTAPAPESHWAR LIMITED

RAW MATERIAL
CERTIFICATE OF ANALYSIS

Product Name : ZINC (JASAD) PATA

Batch No	: 1PRMLO0278	Receipt Qty	203.200 KGS
Mfg Date	: JUN./2021	Sample Qty	0.050 KGS
Exp Date	:	Retention Sample	0.050 KGS
Shelf Life	:	Date Received	: 28-Jun-2021
		Date Analyzed	: 12-Jul-2021

SR	TEST	OBSERVATION	STANDARD LIMIT
1	Description	Complies	Bluish white or colour metallic luster, heavy ingots.
2	Zinc (Zn) (As per Inhouse method)	99.62 % w/w	N.L.T 98.0 % w/w

Remarks : In the opinion of the undersigned the sample referred as above is of standard quality & meets all in-house specifications

Final Disposition : Approved

Date : 12-Jul-2021

PREPARED BY — Officer QC

CHECKED BY — Executive QC / Asst. Manager

APPROVED BY — Head QC

ANNEXURE 04: RAW DRUG ANALYSIS

Ref: -ICP-AES-159

Date: 12/04/2022

IITB - SAIF

Analytical report of the samples submitted by **Dr. Geeta G. Gadad, Ph.D Scholar**, **Ayurved Mahavidyalaya,** using Inductively Coupled Plasma Atomic Emission Spectroscopy.

SAMPLE	Hg	S	Zn
	%	%	%
SAMPLE-1	99.991	80.6	99.88

ND MEANS LESS THAN 0.01PPM

Note: Content of this report is meant for your information only and should not be quoted as certificate to third party for advertisement, evidence or litigation.

To,

Principal,

Shri B.M. Kankanawadi

A Constituent Unit of KAHER

Shahapur, Belagavi-03

GEOLOGICAL IDENTIFICATION OF MAJOR METAL AND MINERALS

Dr.P.T.Hanamgond
Associate Professor & Head

Department of Geology
G.S.Science Degree College,
Tilakwadi, BELGAUM 590 006
Phone: 0831-2485193, M: 9480275757
Email:hanamgondpt@gmail.com

5 Jan 2022

To whomsoever it may concern

Authentication of the given sample

The given sample has the following Physical properties

Color: Silver or Silvery White.

Lustre: Metallic

Density : Heavy. Standard value is 13.6 $g.cm^{-3}$

Form & Habit: It can take any form. Droplets are circular and exhibit convex surface

Name: MERCURY (Element)

Chemistry: Hg

Occurrence: Mercury is derived from the ore Cinnabar. The Cinnabar releases mercury as a vapour when heated; after the vapour is cooled liquid mercury is collected.

Diagnostic Properties: Silvery white liquid metal.

The above observations are made with routine classical methods of mineralogy. However, for confirmation, AAS or XRD or XRF methods can be used.

Dr.P.T.Hanamgond

To,
Dr Geeta G Gadad
Asst Prof. Dept of RSBK,
BMK Ayurveda Mahavidyalaya,
Belagavi

Dr.P.T.Hanamgond
Associate Professor & Head

Department of Geology
G.S.Science Degree College,
Tilakwadi, BELGAUM 590 006
Phone: 0831-2485193, M: 9480275757
Email:hanamgondpt@gmail.com

5 Jan 2022

To whomsoever it may concern

Authentication of the given sample

The given sample has the following Physical properties

Color: Lemon-Yellow

Streak: Pale yellow

Lustre: Resinous or Greasy

Hardness: 1.5 - 2.0 (Soils the finger, Scratched with finger nail)

Specific Gravity: 2.095 (Standard value range 2.0 – 2.1)

Fracture: Uneven, Bladed, Sectile.

Cleavage: None. Striations are seen

Form & Habit: irregular, granular or encrusting

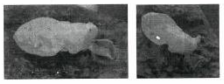
Name: SULFUR (Native)

Chemistry: S (Sulfur)

Crystal system: Orthorhombic (Standard, not analysed)

Occurrence: In natural form It forms near volcanic vents and fumaroles, where it sublimates from a stream of hot gases. Small amounts of native sulfur also form during the weathering of sulfate and sulfide minerals.

Diagnostic Properties: Yellow color, low hardness, low specific gravity, extremely flammable burning with a blue flame, low melting temperature and insoluble in Water.



The above observations are made with routine classical methods of mineralogy. However, for confirmation, AAS or XRD or XRF methods can be used.

Dr.P.T.Hanamgond

To,
Dr Geeta G Gadad
Asst Prof. Dept of RSBK,
BMK Ayurveda Mahavidyalaya,
Belagavi

Dr.P.T.Hanamgond
Associate Professor & Head

Department of Geology
G.S.Science Degree College,
Tilakwadi, BELGAUM 590 006
Phone: 0831-2485193, M: 9480275757
Email:hanamgondpt@gmail.com

5 Jan 2022

To whomsoever it may concern

Authentication of the given sample

The given sample has the following Physical properties

Color: Silvery White.

Lustre: Metallic

Specific Gravity: 6.67 (Standard Value 7)

Density : Heavy. Standard value is 7.11 $g.cm^{-3}$

Form & Habit: Massive

Name: ZINC (Metal)

Chemistry: Zn

Occurrence: Zinc metal is derived from Sphalerite ore.

Diagnostic Properties: Silvery white metal. Heavy and Tough. Cannot cut easily.

The above observations are made with routine classical methods of mineralogy. However, for confirmation, AAS or XRD or XRF methods can be used.

Dr.P.T.Hanamgond

To,
Dr Geeta G Gadad
Asst Prof. Dept of RSBK,
BMK Ayurveda Mahavidyalaya,
Belagavi

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA
A constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University
Central Research Facility
DRUG AUTHENTICATION REPORT

Submitted By: Dr. Geeta G. Gadad
Submitted Date:27/11/2021

Date of Issue:29/11/2021

SL No	Sample Name	Scientific Name	Varga (Category)	CRF Code	Authenticated as		
					Common Name	Scientific Name	Varga (Category)
1.	Parada (Mercury	Hydrarigium (Hg)	Rasa	CRF/Auth 40/2021	Parada	Hydrarigium (Hg)	Rasa
2.	Gandhaka	Sulphur (S)	Uparasa	CRF/Auth 41/2021	Gandhaka	Sulphur (S)	Uparasa
3.	Yashada	Zinc (Zn)	Putiloha	CRF/Auth 42/2021	Yashada	Zinc (Zn)	Putiloha
4.	Sudha.(Lime stone	Calcium Hydroxide $Ca(OH)_2$	Sudha Varga	CRF/Auth 43/2021	Sudha	Calcium Hydroxide $Ca(OH)_2$	Sudha Varga
5.	Saindhava Lavana	Sodium chloride (NaCl)	Lavana Varga	CRF/Auth 44/2021	Saindhava Lavana	Sodium chloride (NaCl)	Lavana Varga

Signature:
Authentication Expert Name: Dr. Veena B. Kupati
Date:29/11/2021

CRF SHAHAPUR BELGAUM

Signature of Coordinator
ASU Drug Testing Laboratory

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA
A constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University
Central Research Facility
DRUG AUTHENTICATION REPORT

Submitted By: Dr. Geeta G. Gadad
Submitted Date:27/11/2021

Date of Issue:29/11/2021

SL No	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Common Name	Scientific Name	Family	Part Authenticated
1.	Lashuna	Allium sativum L.	Liliaceae	Bulb	CRF/Auth 38/2021	Lashuna	Allium sativum L.	Liliaceae	Bulb

SL No	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Common Name	Scientific Name	Family	Part Authenticated
1.	Kulattha	Dolichos biflorus Linn.	Leguminosae	Seed	CRF/Auth 39/2021	Kulattha	Dolichos biflorus Linn.	Leguminosae	Seed

SL No	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Common Name	Scientific Name	Family	Part Authenticated
1.	Apamarga	Achyranthes aspera L.	Amaranthaceae	Whole Plant	CRF/Auth 37/2021	Apamarga	Achyranthes aspera L.	Amaranthaceae	Whole Plant

CRF SHAHAPUR BELGAUM

Signature:
Authentication Expert Name: Dr. Divya Khare
Date:29/11/2021

Signature of Coordinator
ASU Drug Testing Laboratory

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD Gol)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/~~139~~/2022-23

Reference No:CRF/RM/169/2022-23
Submitted by:Dr.Geeta Gadad
Sample : Sudha
Product : Mineral
(* N/A - Not Available)
Batch No. : NA
Sample Qty: 50gm
Registration Dt:-13/07/2022
Requisition No:--------
Part/Form:Powder
Report Date: 26/07/2022

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	RESULTS
PART	: Fine powder
COLOUR	: White
TASTE	: Slightly Chalky
ODOUR	: Odourless

Physico Chemical Standards :

TESTS	RESULTS
Foreign Matter	: Nil
Loss on Drying	: 61.457%
Ash Value	:59.088 %
Acid insoluble Ash	:11.165 %
Water soluble Ash	:25.012%
Water soluble extractive	:9.612 %
Alcohol soluble extractive	:0.319 %

Note : **API** Standards are not Available.Given results are of the submitted sample.

ANALYST

CRF SHAHAPUR BELGAUM

AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
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CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/~~139~~/2022-23

Reference No:CRF/RM/169/2022-23
Submitted by:Dr.Geeta G.Gadad
Sample : Sudha
Product : Mineral
(* N/A - Not Available)
Batch No. : NA
Sample Qty: 50gm
Registration Dt:-13/07/2022
Requisition No:--------
Part/Form:Powder
Report Date: 26/07/2022

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	WATER	ALCOHOL
Test for Carbohydrates	Positive	Positive
Test for Reducing sugar	Negative	Negative
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Proteins	Negative	Negative
Test for Amino Acids	Negative	Negative
Test for Steroids	Negative	Positive
Test for Flavonoids	Negative	Negative
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Negative
Test for Glycosides:		
A.Cardiac Glycosides	Negative	Positive
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Negative	Negative

ANALYST

VERIFIED BY

CRF SHAHAPUR BELGAUM

AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
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CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/187/2022-23

Reference No:CRF/RM/169/2022-23 | Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad | Requisition No:--------
Sample : Sudha | Batch No. : NA | Part/Form:Powder
Product : Mineral | Sample Qty: 50gm | Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Inorganic element Analysis

TESTS		RESULTS
Calcium	-	Positive
Magnesium	-	Positive
Sodium	-	Positive
Potassium	-	Negative
Iron	-	Negative
Sulphate	-	Positve
Phosphate	-	Negative
Chloride	-	Negative
Carbonate	-	Positive
Nitrates	-	Negative

ANALYST VERIFIED BY AUTHORISED SIGNATORY

CRF SHAHAPUR, BELGAUM

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/82/2022-23

Reference No:CRF/RM/46/2022-23 | Registration Dt:-22/02/2022
Submitted by:KLE Ayurved Pharmacy | Requisition No:---------
Sample : Saindhav Lavana | Batch No. : I | Part/Form:Hard mass
Product : Mineral | Sample Qty: 20gm | Report Date:08/03/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	RESULTS
FORM	:Irregular shaped hard masses
COLOUR	: Whitish
TASTE	: Salty
ODOUR	: Not Specific

Physico Chemical Standards :

TESTS	RESULTS
pH (10% Solution)	: 5.96
Test For Sodium (Na+)	: Present
Test For Potassium(K+)	: Present

Note : API Standards are not Available.Given results are of the submitted sample.

ANALYST AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
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Outward No:-BMK/CRF/183/2022-23

Reference No:CRF/RM/170/2022-23 | Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad | Requisition No:--------
Sample : Lasuna | Batch No. : NA | Part/Form:Bulb
Product : PLANT | Sample Qty: 50gm | Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	LIMITS	RESULTS
PART	Bulb	: Bulb
COLOUR	Whitish	: Whitish
TASTE	Acrid	: Acrid
ODOUR	Pungent and disagreeable	: Pungent and disagreeable

Physico Chemical Standards :

TESTS	LIMITS	RESULTS
Foreign Matter	Not more than 2%	:Nil
Loss on Drying	Not less than 60%	: 61.457%
Ash Value	Not more than 4%	:2.283 %
Water soluble Ash	NA	: 1.567%
Acid insoluble Ash	Not more than 1%	:0.245 %
Water soluble extractive	NA	:15.522 %
Alcohol soluble extractive	Not less than 2.5%	:3.940 %

(Standards reffered above are as per **API**)
* In my opinion the Sample is standard quality

ANALYST AUTHORISED SIGNATORY

CRF SHAHAPUR, BELGAUM

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
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CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/183/2022-23

Reference No:CRF/RM/170/2022-23 | Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad | Requisition No:--------
Sample : Lasuna | Batch No. : NA | Part/Form:Bulb
Product : PLANT | Sample Qty: 50gm | Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	WATER	ALCOHOL
Test for Carbohydrates	Negative	Positive
Test for Reducing sugar	Positive	Positive
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Proteins	Negative	Negative
Test for Amino Acids	Positive	Negative
Test for Steroids	Positive	Positive
Test for Flavonoids	Negative	Negative
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Negative
Test for Glycosides:		
A.Cardiac Glycosides	Positive	Positive
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Positive	Positive

ANALYST VERIFIED BY AUTHORISED SIGNATORY

CRF SHAHAPUR, BELGAUM

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
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CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/193/2022-23

Reference No:CRF/RM/170/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad — Requisition No:--------
Sample : Lasuna — Batch No. : NA — Part/Form:Bulb
Product : PLANT — Sample Qty: 50gm — Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Inorganic element Analysis

TESTS		RESULTS
Calcium	-	Negative
Magnesium	-	Negative
Sodium	-	Negative
Potassium	-	Negative
Iron	-	Negative
Sulphate	-	Positve
Phosphate	-	Negative
Chloride	-	Negative
Carbonate	-	Negative
Nitrates	-	Negative

ANALYST — VERIFIED BY — AUTHORISED SIGNATORY

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CENTRAL RESEARCH FACILITY
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Outward No:-BMK/CRF/193/2022-23

Reference No:CRF/RM/168/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad — Requisition No:--------
Sample : Kulatta — Batch No. : NA — Part/Form:Seed
Product : PLANT — Sample Qty: 50gm — Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

TESTS	LIMITS	RESULTS
PART	Seed	: Seed
COLOUR	Greyish to reddish brown	: Greyish to reddish brown
TASTE	Astringent	: Astringent

Physico Chemical Standards :

TESTS	LIMITS	RESULTS
Foreign Matter	Nil	: Nil
Loss on Drying	NA	: 0.334%
Ash Value	Not more than 5%	:3.626 %
Acid insoluble Ash	Not more than 1%	:0.483 %
Water soluble Ash	NA	: 2.089%
Water soluble extractive	Not less than 12%	:16.463 %
Alcohol soluble extractive	Not less than 3%	:4.150 %

(Standards reffered above are as per **API**)
* In my opinion the Sample is standard quality

ANALYST — AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/193/2022-23

Reference No:CRF/RM/168/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad — Requisition No:--------
Sample : Kulatta — Batch No. : NA — Part/Form:Seed
Product : PLANT — Sample Qty: 50gm — Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	WATER	ALCOHOL
Test for Carbohydrates	Positive	Positive
Test for Reducing sugar	Negative	Positive
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Proteins	Positive	Negative
Test for Amino Acids	Positive	Negative
Test for Steroids	Negative	Positive
Test for Flavonoids	Negative	Negative
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Negative
Test for Glycosides:		
A.Cardiac Glycosides	Negative	Positive
B.Anthraquinone glycosides	Positive	Negative
C.Saponin glycosides	Positive	Negative

ANALYST — VERIFIED BY — AUTHORISED SIGNATORY

SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
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Outward No:-BMK/CRF/193/2022-23

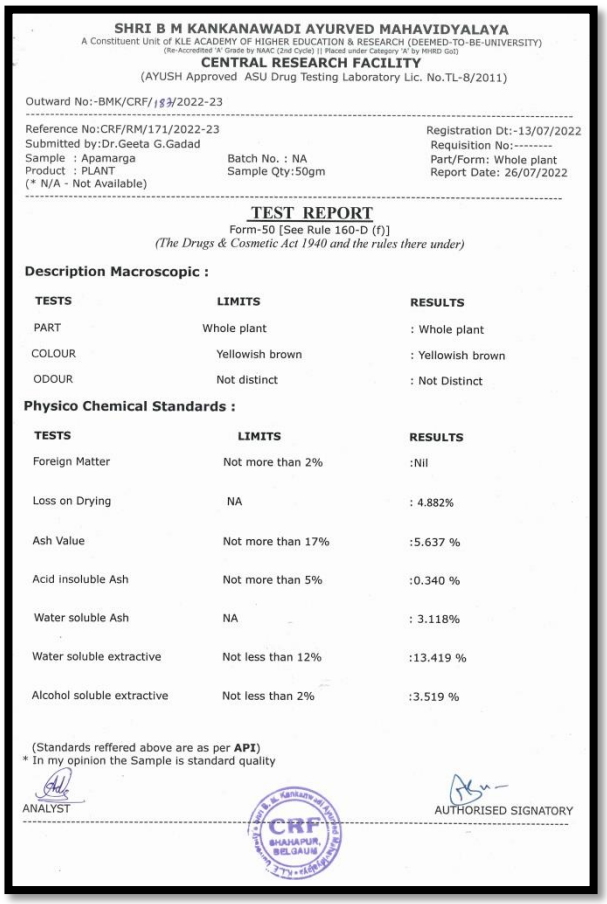
Reference No:CRF/RM/168/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad — Requisition No:--------
Sample : Kulatta — Batch No. : NA — Part/Form:Seed
Product : PLANT — Sample Qty: 50gm — Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Inorganic element Analysis

TESTS		RESULTS
Calcium	-	Negative
Magnesium	-	Negative
Sodium	-	Positive
Potassium	-	Negative
Iron	-	Positive
Sulphate	-	Negative
Phosphate	-	Negative
Chloride	-	Negative
Carbonate	-	Negative
Nitrates	-	Negative

ANALYST — VERIFIED BY — AUTHORISED SIGNATORY



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Outward No:-BMK/CRF/183/2022-23

Reference No:CRF/RM/171/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad — Requisition No:--------
Sample : Apamarga — Batch No. : NA — Part/Form: Whole plant
Product : PLANT — Sample Qty:50gm — Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

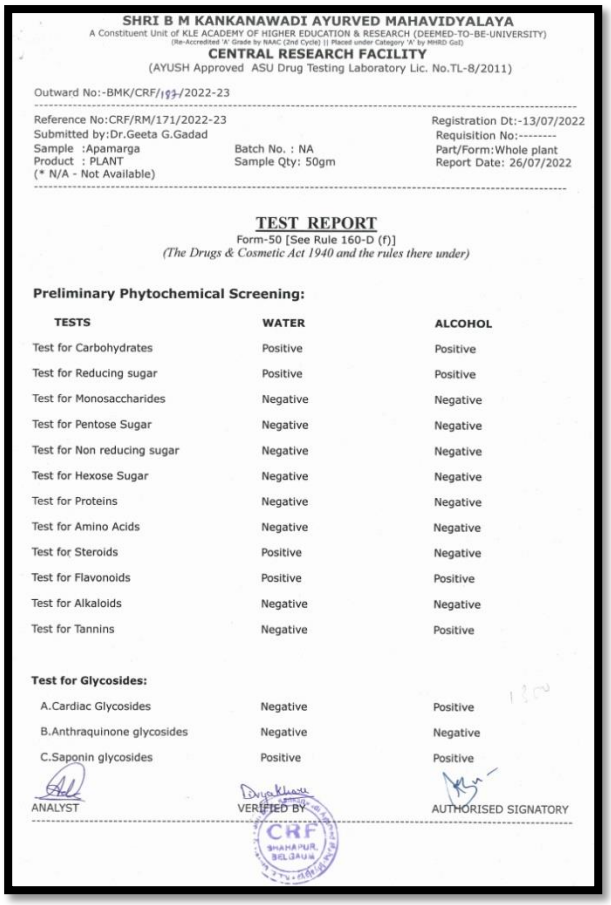
TESTS	LIMITS	RESULTS
PART	Whole plant	: Whole plant
COLOUR	Yellowish brown	: Yellowish brown
ODOUR	Not distinct	: Not Distinct

Physico Chemical Standards :

TESTS	LIMITS	RESULTS
Foreign Matter	Not more than 2%	:Nil
Loss on Drying	NA	: 4.882%
Ash Value	Not more than 17%	:5.637 %
Acid insoluble Ash	Not more than 5%	:0.340 %
Water soluble Ash	NA	: 3.118%
Water soluble extractive	Not less than 12%	:13.419 %
Alcohol soluble extractive	Not less than 2%	:3.519 %

(Standards reffered above are as per **API**)
* In my opinion the Sample is standard quality

ANALYST — AUTHORISED SIGNATORY



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A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
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CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/183/2022-23

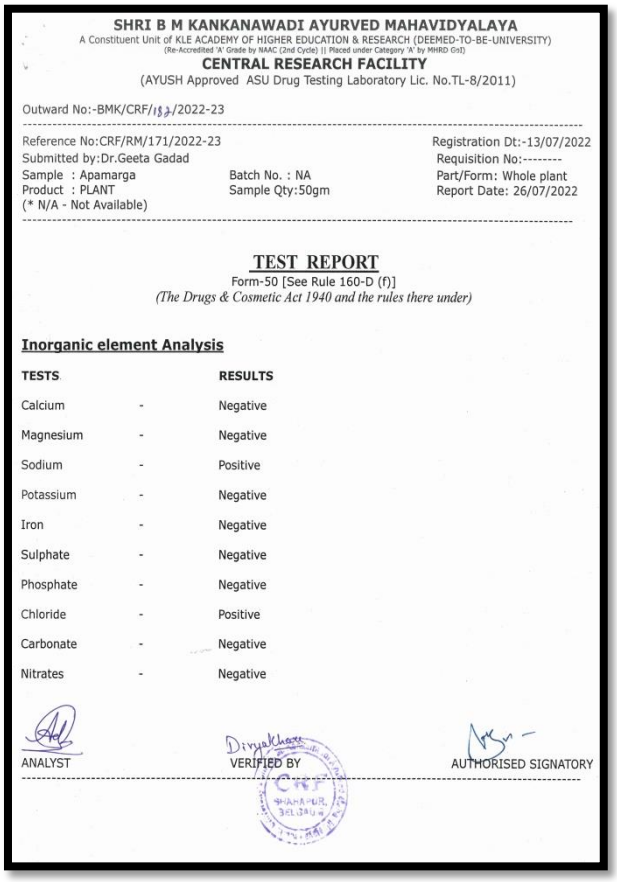
Reference No:CRF/RM/171/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta G.Gadad — Requisition No:--------
Sample :Apamarga — Batch No. : NA — Part/Form:Whole plant
Product : PLANT — Sample Qty: 50gm — Report Date: 26/07/2022
(* N/A - Not Available)

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Preliminary Phytochemical Screening:

TESTS	WATER	ALCOHOL
Test for Carbohydrates	Positive	Positive
Test for Reducing sugar	Positive	Positive
Test for Monosaccharides	Negative	Negative
Test for Pentose Sugar	Negative	Negative
Test for Non reducing sugar	Negative	Negative
Test for Hexose Sugar	Negative	Negative
Test for Proteins	Negative	Negative
Test for Amino Acids	Negative	Negative
Test for Steroids	Positive	Negative
Test for Flavonoids	Positive	Positive
Test for Alkaloids	Negative	Negative
Test for Tannins	Negative	Positive
Test for Glycosides:		
A.Cardiac Glycosides	Negative	Positive
B.Anthraquinone glycosides	Negative	Negative
C.Saponin glycosides	Positive	Positive

ANALYST — VERIFIED BY — AUTHORISED SIGNATORY



SHRI B M KANKANAWADI AYURVED MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A' Grade by NAAC (2nd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/183/2022-23

Reference No:CRF/RM/171/2022-23 — Registration Dt:-13/07/2022
Submitted by:Dr.Geeta Gadad — Requisition No:--------
Sample : Apamarga — Batch No. : NA — Part/Form: Whole plant
Product : PLANT — Sample Qty:50gm — Report Date: 26/07/2022
(* N/A - Not Available)

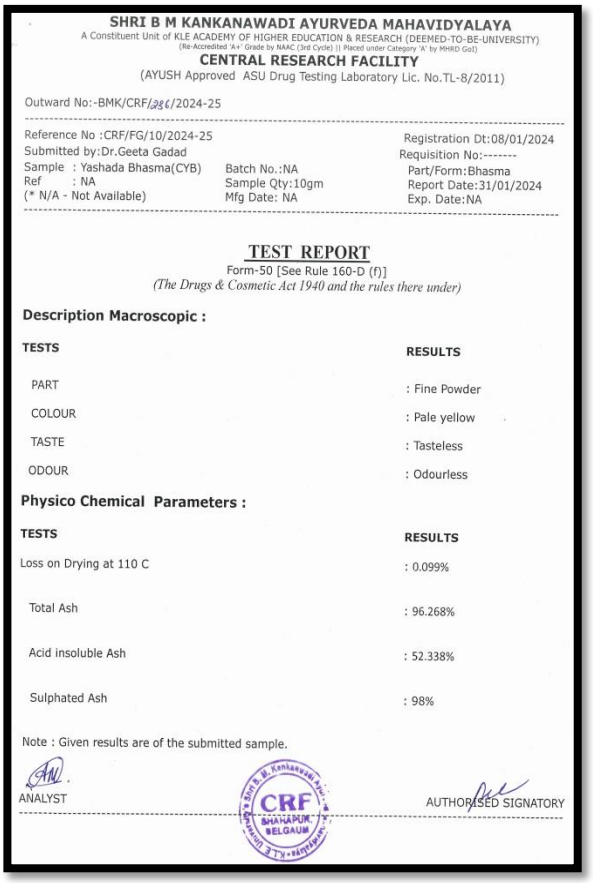
TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Inorganic element Analysis

TESTS		RESULTS
Calcium	-	Negative
Magnesium	-	Negative
Sodium	-	Positive
Potassium	-	Negative
Iron	-	Negative
Sulphate	-	Negative
Phosphate	-	Negative
Chloride	-	Positive
Carbonate	-	Negative
Nitrates	-	Negative

ANALYST — VERIFIED BY — AUTHORISED SIGNATORY

ANNEXURE 05: FINAL BHASMA SAMPLES ANALYSIS



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A+' Grade by NAAC (3rd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/236/2024-25

Reference No :CRF/FG/10/2024-25
Submitted by:Dr.Geeta Gadad
Sample : Yashada Bhasma(CYB)
Ref : NA
(* N/A - Not Available)
Batch No.:NA
Sample Qty:10gm
Mfg Date: NA
Registration Dt:08/01/2024
Requisition No:-------
Part/Form:Bhasma
Report Date:31/01/2024
Exp. Date:NA

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

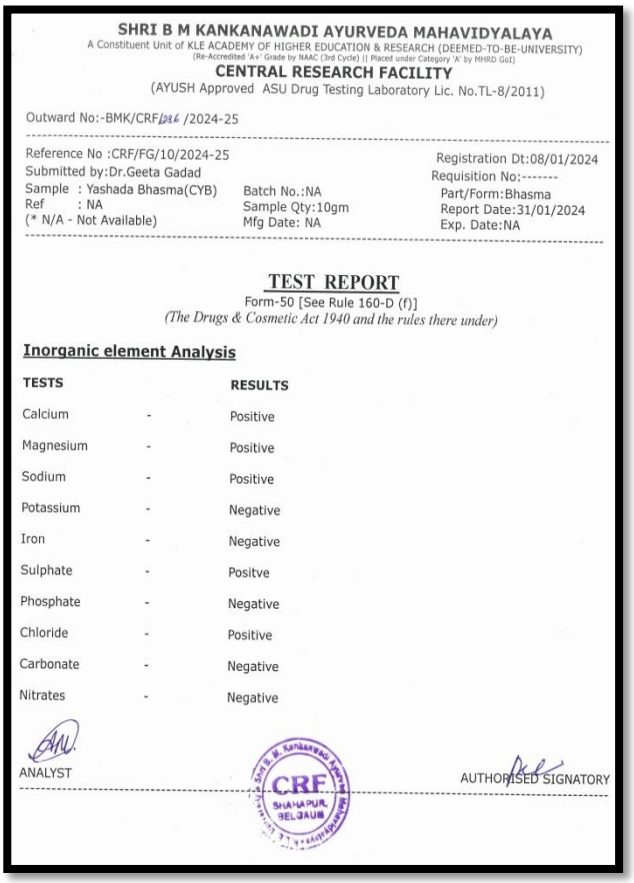
TESTS	RESULTS
PART	: Fine Powder
COLOUR	: Pale yellow
TASTE	: Tasteless
ODOUR	: Odourless

Physico Chemical Parameters :

TESTS	RESULTS
Loss on Drying at 110 C	: 0.099%
Total Ash	: 96.268%
Acid insoluble Ash	: 52.338%
Sulphated Ash	: 98%

Note : Given results are of the submitted sample.

ANALYST
AUTHORISED SIGNATORY



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A+' Grade by NAAC (3rd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/236 /2024-25

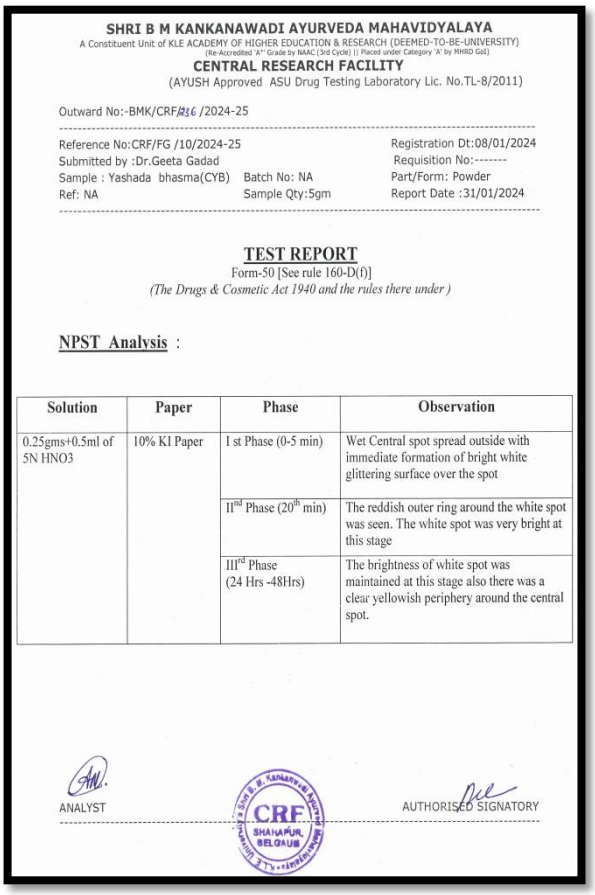
Reference No :CRF/FG/10/2024-25
Submitted by:Dr.Geeta Gadad
Sample : Yashada Bhasma(CYB)
Ref : NA
(* N/A - Not Available)
Batch No.:NA
Sample Qty:10gm
Mfg Date: NA
Registration Dt:08/01/2024
Requisition No:-------
Part/Form:Bhasma
Report Date:31/01/2024
Exp. Date:NA

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Inorganic element Analysis

TESTS		RESULTS
Calcium	-	Positive
Magnesium	-	Positive
Sodium	-	Positive
Potassium	-	Negative
Iron	-	Negative
Sulphate	-	Positve
Phosphate	-	Negative
Chloride	-	Positive
Carbonate	-	Negative
Nitrates	-	Negative

ANALYST
AUTHORISED SIGNATORY



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
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Outward No:-BMK/CRF/236 /2024-25

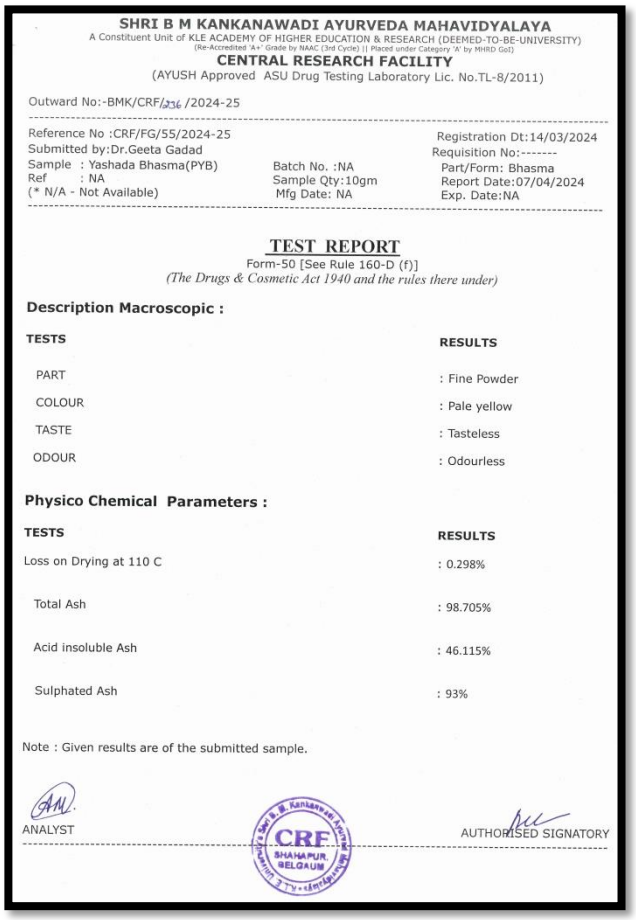
Reference No:CRF/FG /10/2024-25
Submitted by :Dr.Geeta Gadad
Sample : Yashada bhasma(CYB)
Ref: NA
Batch No: NA
Sample Qty:5gm
Registration Dt:08/01/2024
Requisition No:-------
Part/Form: Powder
Report Date :31/01/2024

TEST REPORT
Form-50 [See rule 160-D(f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

NPST Analysis :

Solution	Paper	Phase	Observation
0.25gms+0.5ml of 5N HNO3	10% KI Paper	I st Phase (0-5 min)	Wet Central spot spread outside with immediate formation of bright white glittering surface over the spot
		IInd Phase (20th min)	The reddish outer ring around the white spot was seen. The white spot was very bright at this stage
		IIIrd Phase (24 Hrs -48Hrs)	The brightness of white spot was maintained at this stage also there was a clear yellowish periphery around the central spot.

ANALYST
AUTHORISED SIGNATORY



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(Re-Accredited 'A+' Grade by NAAC (3rd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/336 /2024-25

Reference No :CRF/FG/55/2024-25 — Registration Dt:14/03/2024
Submitted by:Dr.Geeta Gadad — Requisition No:-------
Sample : Yashada Bhasma(PYB) — Batch No. :NA — Part/Form: Bhasma
Ref : NA — Sample Qty:10gm — Report Date:07/04/2024
(* N/A - Not Available) — Mfg Date: NA — Exp. Date:NA

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Description Macroscopic :

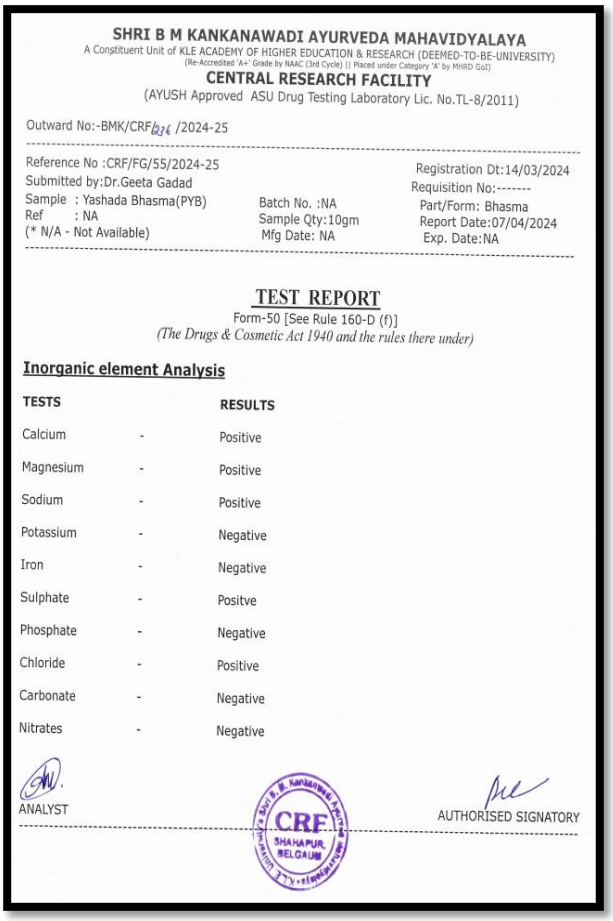
TESTS	RESULTS
PART	: Fine Powder
COLOUR	: Pale yellow
TASTE	: Tasteless
ODOUR	: Odourless

Physico Chemical Parameters :

TESTS	RESULTS
Loss on Drying at 110 C	: 0.298%
Total Ash	: 98.705%
Acid insoluble Ash	: 46.115%
Sulphated Ash	: 93%

Note : Given results are of the submitted sample.

ANALYST — CRF SHAHAPUR BELGAUM — AUTHORISED SIGNATORY



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A+' Grade by NAAC (3rd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/334 /2024-25

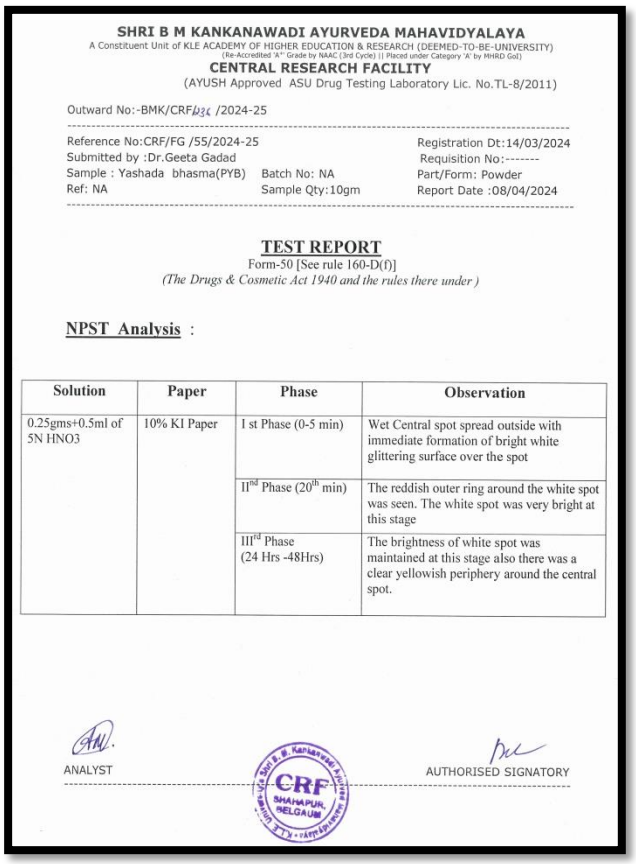
Reference No :CRF/FG/55/2024-25 — Registration Dt:14/03/2024
Submitted by:Dr.Geeta Gadad — Requisition No:-------
Sample : Yashada Bhasma(PYB) — Batch No. :NA — Part/Form: Bhasma
Ref : NA — Sample Qty:10gm — Report Date:07/04/2024
(* N/A - Not Available) — Mfg Date: NA — Exp. Date:NA

TEST REPORT
Form-50 [See Rule 160-D (f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

Inorganic element Analysis

TESTS		RESULTS
Calcium	-	Positive
Magnesium	-	Positive
Sodium	-	Positive
Potassium	-	Negative
Iron	-	Negative
Sulphate	-	Positve
Phosphate	-	Negative
Chloride	-	Positive
Carbonate	-	Negative
Nitrates	-	Negative

ANALYST — CRF SHAHAPUR BELGAUM — AUTHORISED SIGNATORY



SHRI B M KANKANAWADI AYURVEDA MAHAVIDYALAYA
A Constituent Unit of KLE ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED-TO-BE-UNIVERSITY)
(Re-Accredited 'A+' Grade by NAAC (3rd Cycle) || Placed under Category 'A' by MHRD GoI)
CENTRAL RESEARCH FACILITY
(AYUSH Approved ASU Drug Testing Laboratory Lic. No.TL-8/2011)

Outward No:-BMK/CRF/331 /2024-25

Reference No:CRF/FG /55/2024-25 — Registration Dt:14/03/2024
Submitted by :Dr.Geeta Gadad — Requisition No:-------
Sample : Yashada bhasma(PYB) — Batch No: NA — Part/Form: Powder
Ref: NA — Sample Qty:10gm — Report Date :08/04/2024

TEST REPORT
Form-50 [See rule 160-D(f)]
(The Drugs & Cosmetic Act 1940 and the rules there under)

NPST Analysis :

Solution	Paper	Phase	Observation
0.25gms+0.5ml of 5N HNO3	10% KI Paper	I st Phase (0-5 min)	Wet Central spot spread outside with immediate formation of bright white glittering surface over the spot
		IInd Phase (20th min)	The reddish outer ring around the white spot was seen. The white spot was very bright at this stage
		IIIrd Phase (24 Hrs -48Hrs)	The brightness of white spot was maintained at this stage also there was a clear yellowish periphery around the central spot.

ANALYST — CRF SHAHAPUR BELGAUM — AUTHORISED SIGNATORY

ANNEXURE 06: INSTRUMENTAL ANALYSIS OF INTERMEDIATE AND FINAL PRODUCTS

IITB – SAIF

Ref: ICP-AES-20

Date: 24/05/2024

Analytical report of the samples submitted by **Dr. Geeta Gadad, Shri B. M. Kankanawadi Ayurveda Mahavidyalaya, Karnataka-590003**, using Inductively Coupled Plasma Atomic Emission Spectroscopy.

Sample	**Zn**
	%
SHODHITA YASHADA	**92.92**
JARITA YASHADA	**82.219**
CLASSICAL YASHADA	**77.132**
POTENTIATED YASHADA	**70.29**

ND MEANS LESS THAN 0.01PPM

Note: Content of this report is meant for your information only and should not be quoted as certificate to third party for advertisement, evidence or litigation.

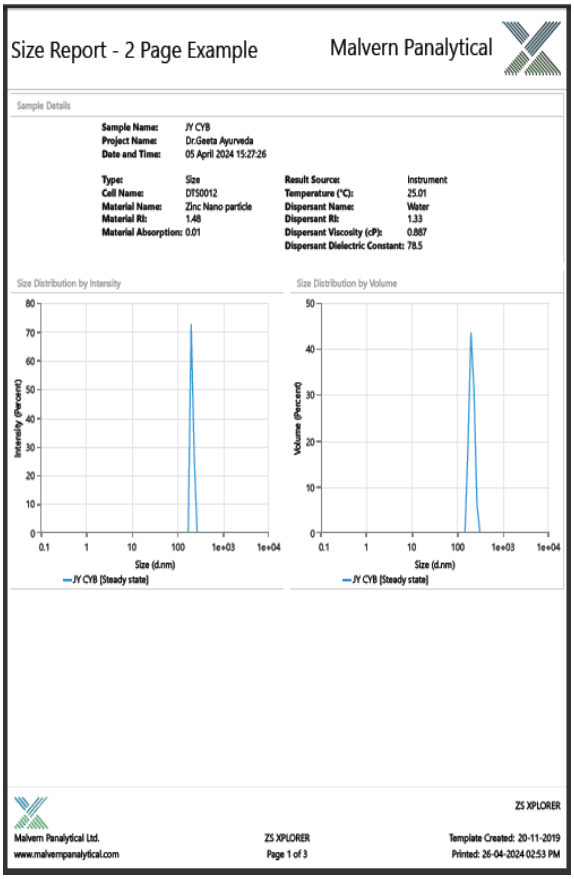
To,

Dr. P.G.Jadar,

B.M.Kankanawadi Ayurveda Mahavidyalaya,

Karnataka-590003

PARTICLE SIZE ANALYSIS


Size Report - 2 Page Example

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Sample Details

Sample Name: JY CYB
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:27:26

Type: Size
Cell Name: DTS0012
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 25.01
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Size Distribution by Intensity

Intensity (Percent)

Size (d.nm)

JY CYB [Steady state]

Size Distribution by Volume

Volume (Percent)

Size (d.nm)

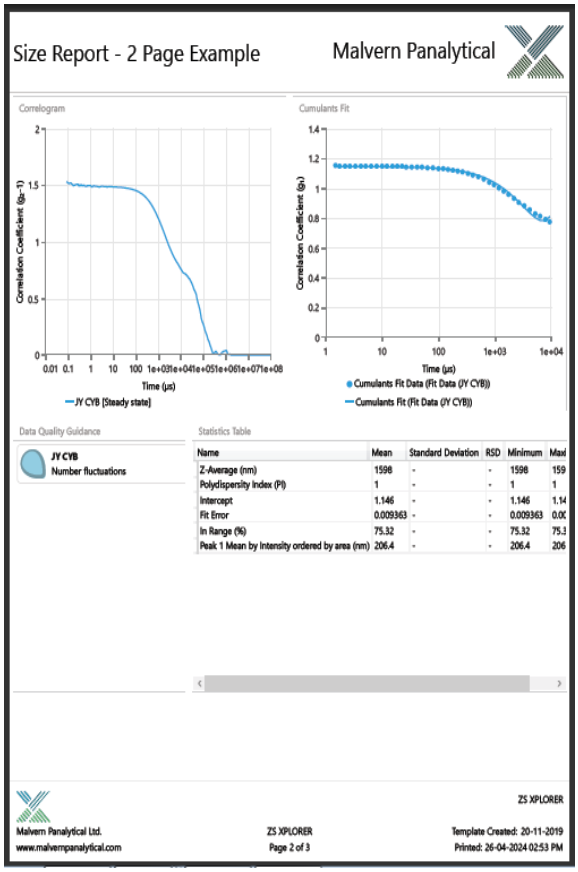
JY CYB [Steady state]

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Size Report - 2 Page Example

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Correlogram

Correlation Coefficient (g2-1)

Time (µs)

JY CYB [Steady state]

Cumulants Fit

Correlation Coefficient (g1)

Time (µs)

Cumulants Fit Data (Fit Data (JY CYB))

Cumulants Fit (Fit Data (JY CYB))

Data Quality Guidance

JY CYB
Number fluctuations

Statistics Table

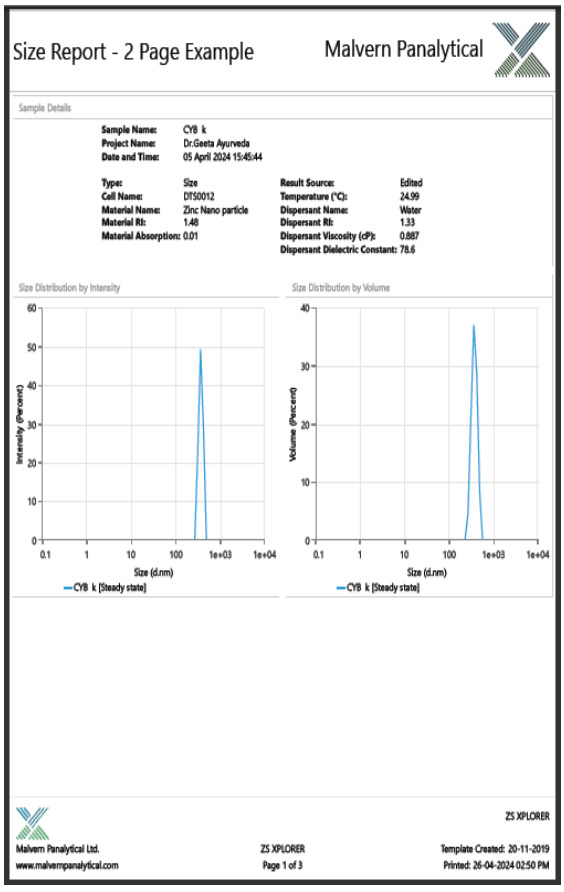
Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	1598	-	-	1598	159
Polydispersity Index (PI)	1	-	-	1	1
Intercept	1.146	-	-	1.146	1.14
Fit Error	0.009363	-	-	0.009363	0.0(
In Range (%)	75.32	-	-	75.32	75.3
Peak 1 Mean by Intensity ordered by area (nm)	206.4	-	-	206.4	206

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Size Report - 2 Page Example

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Sample Details

Sample Name: CYB k
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:45:44

Type: Size
Cell Name: DTS0012
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Edited
Temperature (°C): 24.99
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.6

Size Distribution by Intensity

Intensity (Percent)

Size (d.nm)

CYB k [Steady state]

Size Distribution by Volume

Volume (Percent)

Size (d.nm)

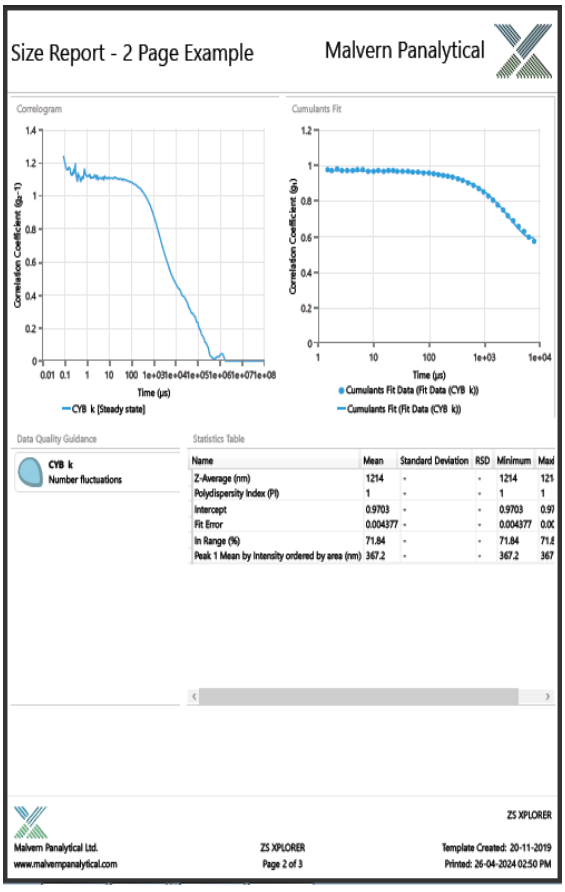
CYB k [Steady state]

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Size Report - 2 Page Example

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Correlogram

Correlation Coefficient (g2-1)

Time (µs)

CYB k [Steady state]

Cumulants Fit

Correlation Coefficient (g1)

Time (µs)

Cumulants Fit Data (Fit Data (CYB k))

Cumulants Fit (Fit Data (CYB k))

Data Quality Guidance

CYB k
Number fluctuations

Statistics Table

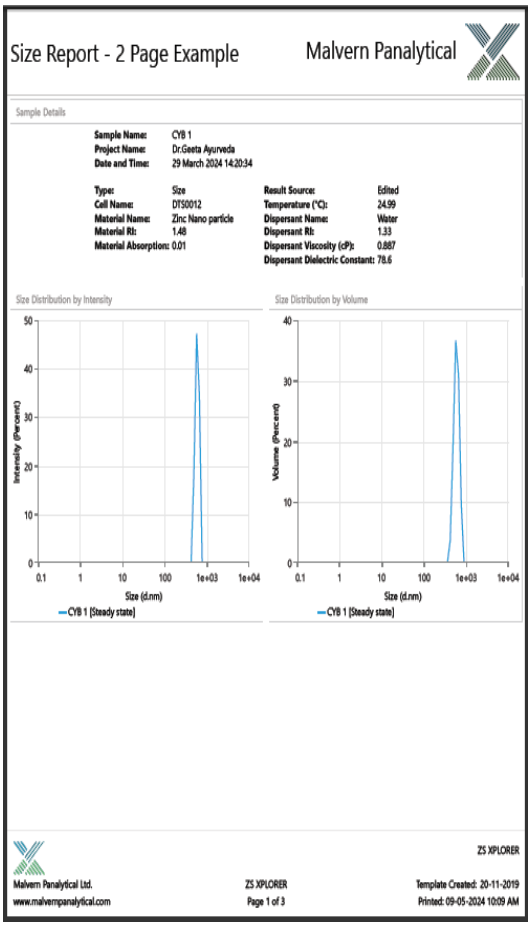
Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	1214	-	-	1214	121
Polydispersity Index (PI)	1	-	-	1	1
Intercept	0.9703	-	-	0.9703	0.97
Fit Error	0.004377	-	-	0.004377	0.0(
In Range (%)	71.84	-	-	71.84	71.8
Peak 1 Mean by Intensity ordered by area (nm)	367.2	-	-	367.2	367

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Size Report - 2 Page Example

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Sample Details

Sample Name: CYB 1
Project Name: Dr.Geeta Ayurveda
Date and Time: 29 March 2024 14:20:34

Type: Size
Cell Name: DTS0012
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Edited
Temperature (°C): 24.99
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.6

Size Distribution by Intensity

Intensity (Percent)

Size (d.nm)

CYB 1 [Steady state]

Size Distribution by Volume

Volume (Percent)

Size (d.nm)

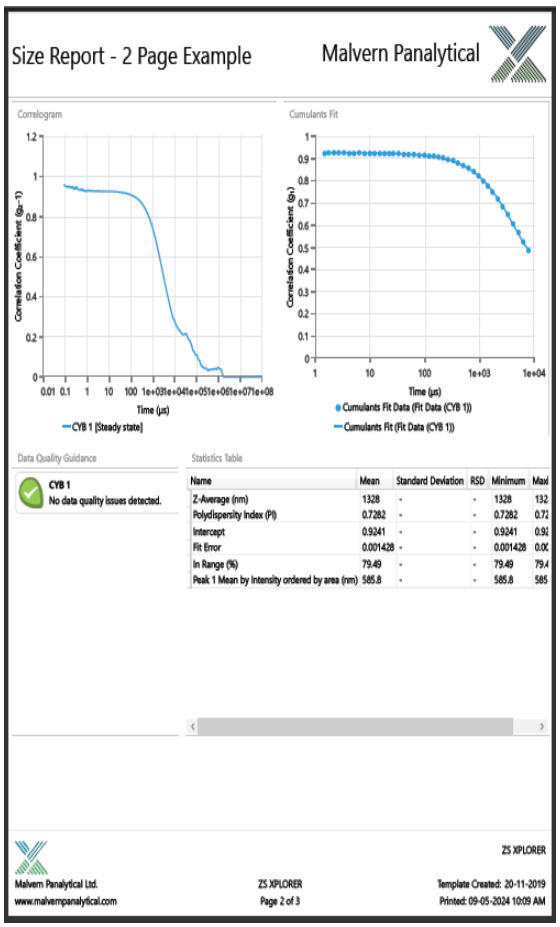
CYB 1 [Steady state]

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Correlogram

Correlation Coefficient (g2-1)

Time (µs)

CYB 1 [Steady state]

Cumulants Fit

Correlation Coefficient (g2)

Time (µs)

Cumulants Fit Data (Fit Data (CYB 1))

Cumulants Fit (Fit Data (CYB 1))

Data Quality Guidance

CYB 1
No data quality issues detected.

Statistics Table

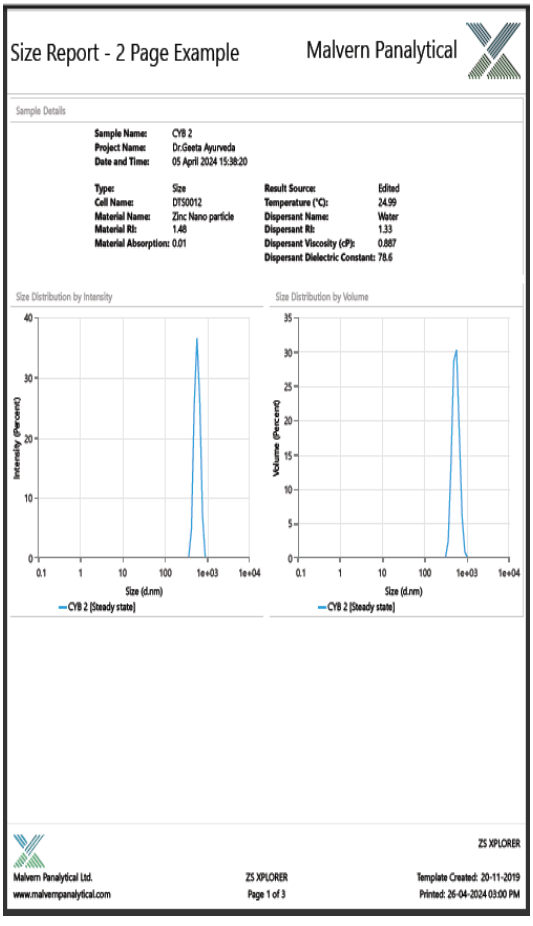
Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	1328	-	-	1328	132
Polydispersity Index (PI)	0.7282	-	-	0.7282	0.72
Intercept	0.9241	-	-	0.9241	0.92
Fit Error	0.001428	-	-	0.001428	0.00
In Range (%)	79.49	-	-	79.49	79.4
Peak 1 Mean by Intensity ordered by area (nm)	585.8	-	-	585.8	585

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Size Report - 2 Page Example

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Sample Details

Sample Name: CYB 2
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:38:20

Type: Size
Cell Name: DTS0012
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Edited
Temperature (°C): 24.99
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.6

Size Distribution by Intensity

Intensity (Percent)

Size (d.nm)

CYB 2 [Steady state]

Size Distribution by Volume

Volume (Percent)

Size (d.nm)

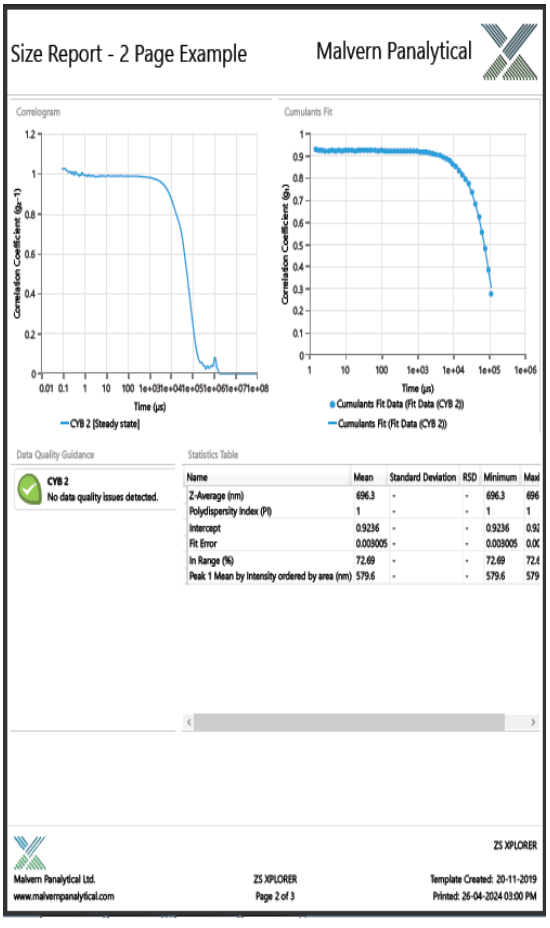
CYB 2 [Steady state]

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Size Report - 2 Page Example

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Correlogram

Correlation Coefficient (g2-1)

Time (µs)

CYB 2 [Steady state]

Cumulants Fit

Correlation Coefficient (g2)

Time (µs)

Cumulants Fit Data (Fit Data (CYB 2))

Cumulants Fit (Fit Data (CYB 2))

Data Quality Guidance

CYB 2
No data quality issues detected.

Statistics Table

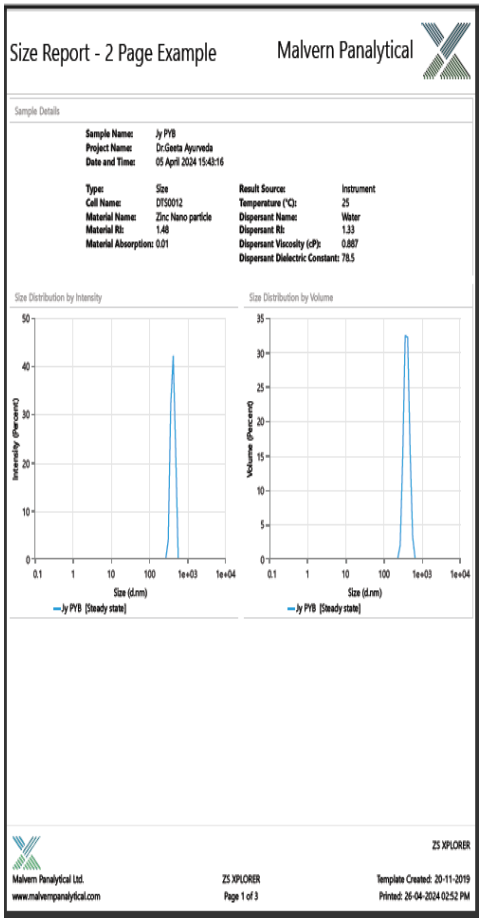
Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	696.3	-	-	696.3	696
Polydispersity Index (PI)	1	-	-	1	1
Intercept	0.9236	-	-	0.9236	0.92
Fit Error	0.003005	-	-	0.003005	0.00
In Range (%)	72.69	-	-	72.69	72.6
Peak 1 Mean by Intensity ordered by area (nm)	579.6	-	-	579.6	579

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Sample Details

Sample Name: Jy PYB
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:43:16

Type: Size
Cell Name: DTS0012
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

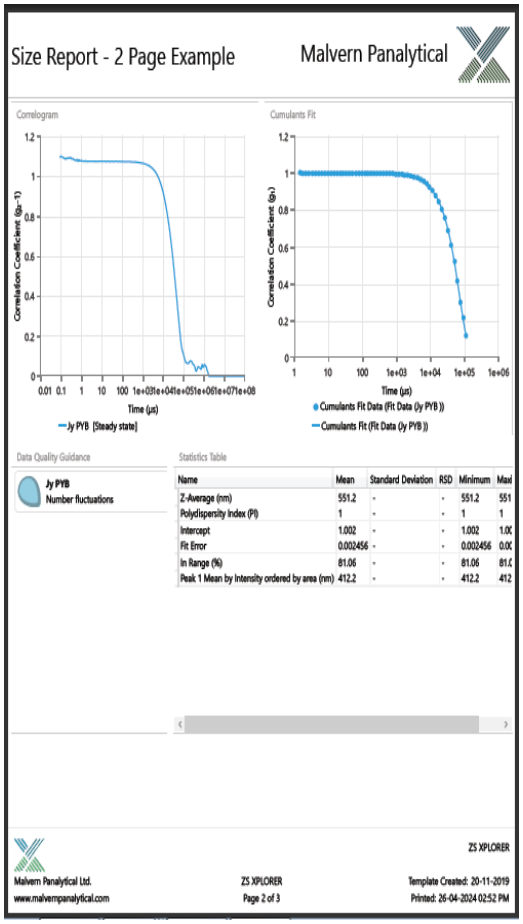
Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Size Distribution by Intensity — Intensity (Percent) — Size (d.nm) — Jy PYB [Steady state]

Size Distribution by Volume — Volume (Percent) — Size (d.nm) — Jy PYB [Steady state]

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Correlogram — Correlation Coefficient (g2-1) — Time (µs) — Jy PYB [Steady state]

Cumulants Fit — Correlation Coefficient (g1) — Time (µs) — Cumulants Fit Data (Fit Data (Jy PYB)) — Cumulants Fit (Fit Data (Jy PYB))

Data Quality Guidance

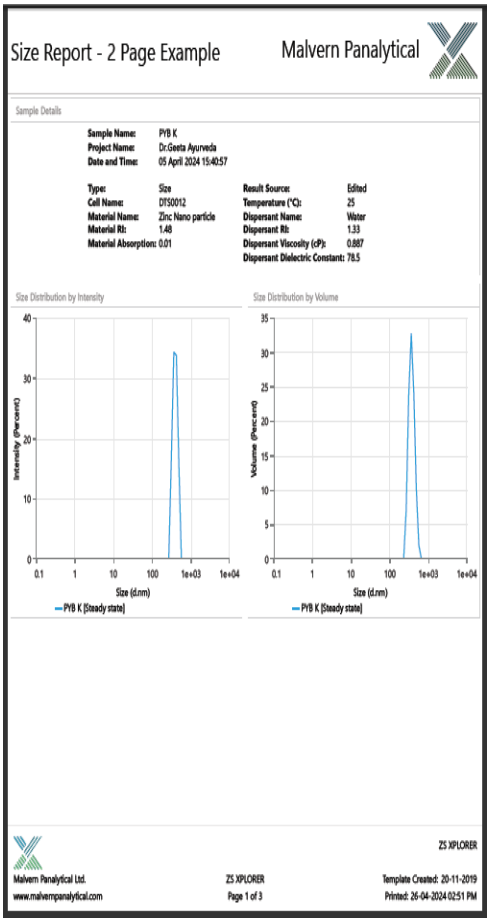
Jy PYB
Number fluctuations

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	551.2	-	-	551.2	551
Polydispersity Index (PI)	1	-	-	1	1
Intercept	1.002	-	-	1.002	1.00
Fit Error	0.002456	-	-	0.002456	0.00
In Range (%)	81.06	-	-	81.06	81.0
Peak 1 Mean by Intensity ordered by area (nm)	412.2	-	-	412.2	412

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Size Report - 2 Page Example — Malvern Panalytical

Sample Details

Sample Name: PYB K
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:40:57

Type: Size
Cell Name: DTS0012
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

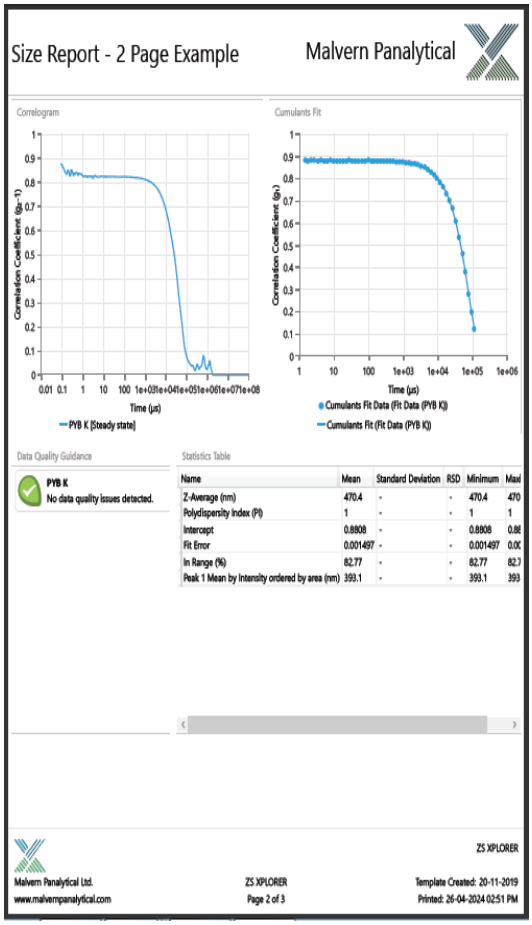
Result Source: Edited
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Size Distribution by Intensity — Intensity (Percent) — Size (d.nm) — PYB K [Steady state]

Size Distribution by Volume — Volume (Percent) — Size (d.nm) — PYB K [Steady state]

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Correlogram — Correlation Coefficient (g2-1) — Time (µs) — PYB K [Steady state]

Cumulants Fit — Correlation Coefficient (g1) — Time (µs) — Cumulants Fit Data (Fit Data (PYB K)) — Cumulants Fit (Fit Data (PYB K))

Data Quality Guidance

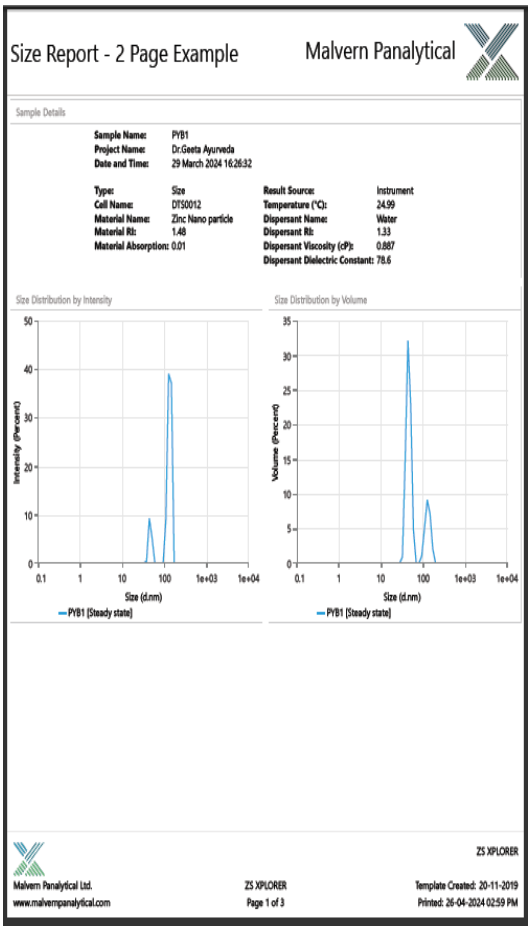
PYB K
No data quality issues detected.

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	470.4	-	-	470.4	470
Polydispersity Index (PI)	1	-	-	1	1
Intercept	0.8808	-	-	0.8808	0.88
Fit Error	0.001497	-	-	0.001497	0.00
In Range (%)	82.77	-	-	82.77	82.7
Peak 1 Mean by Intensity ordered by area (nm)	393.1	-	-	393.1	393

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Size Report - 2 Page Example

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Sample Details

Sample Name:	PYB1		
Project Name:	Dr.Geeta Ayurveda		
Date and Time:	29 March 2024 16:26:32		
Type:	Size	Result Source:	Instrument
Cell Name:	DTS0012	Temperature (°C):	24.99
Material Name:	Zinc Nano particle	Dispersant Name:	Water
Material RI:	1.48	Dispersant RI:	1.33
Material Absorption:	0.01	Dispersant Viscosity (cP):	0.887
		Dispersant Dielectric Constant:	78.6

Size Distribution by Intensity

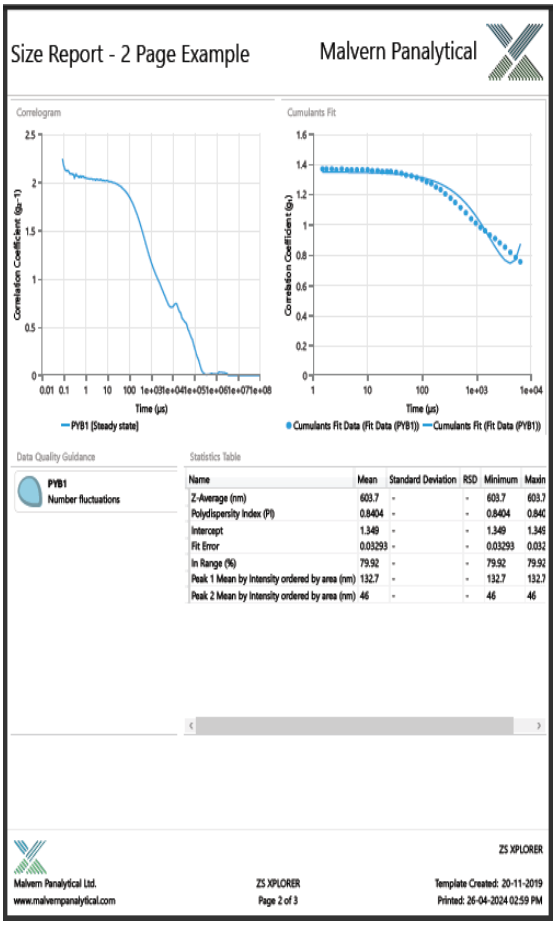
Size Distribution by Volume

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Size Report - 2 Page Example

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Correlogram

Cumulants Fit

Data Quality Guidance

PYB1
Number fluctuations

Statistics Table

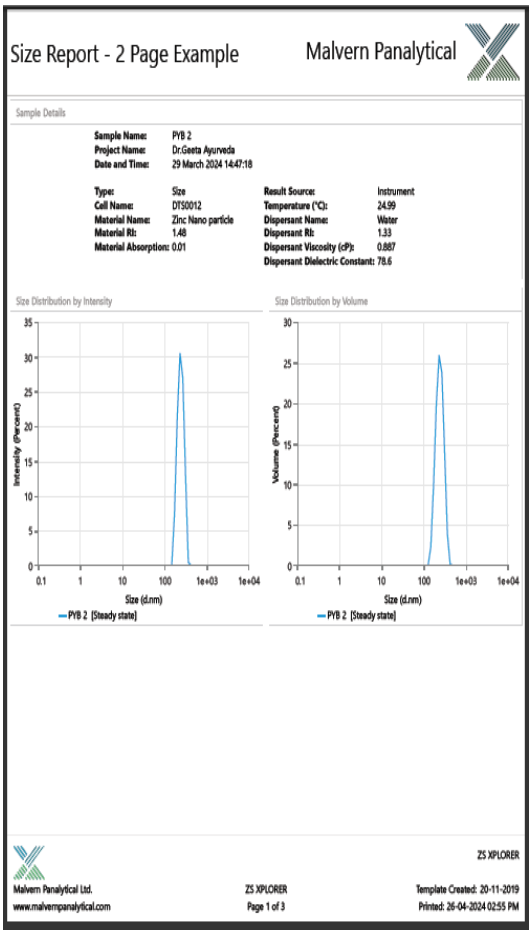
Name	Mean	Standard Deviation	RSD	Minimum	Maxin
Z-Average (nm)	603.7	-	-	603.7	603.7
Polydispersity Index (PI)	0.8404	-	-	0.8404	0.840
Intercept	1.349	-	-	1.349	1.349
Fit Error	0.03293	-	-	0.03293	0.032
In Range (%)	79.92	-	-	79.92	79.92
Peak 1 Mean by Intensity ordered by area (nm)	132.7	-	-	132.7	132.7
Peak 2 Mean by Intensity ordered by area (nm)	46	-	-	46	46

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Size Report - 2 Page Example

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Sample Details

Sample Name:	PYB 2		
Project Name:	Dr.Geeta Ayurveda		
Date and Time:	29 March 2024 14:47:18		
Type:	Size	Result Source:	Instrument
Cell Name:	DTS0012	Temperature (°C):	24.99
Material Name:	Zinc Nano particle	Dispersant Name:	Water
Material RI:	1.48	Dispersant RI:	1.33
Material Absorption:	0.01	Dispersant Viscosity (cP):	0.887
		Dispersant Dielectric Constant:	78.6

Size Distribution by Intensity

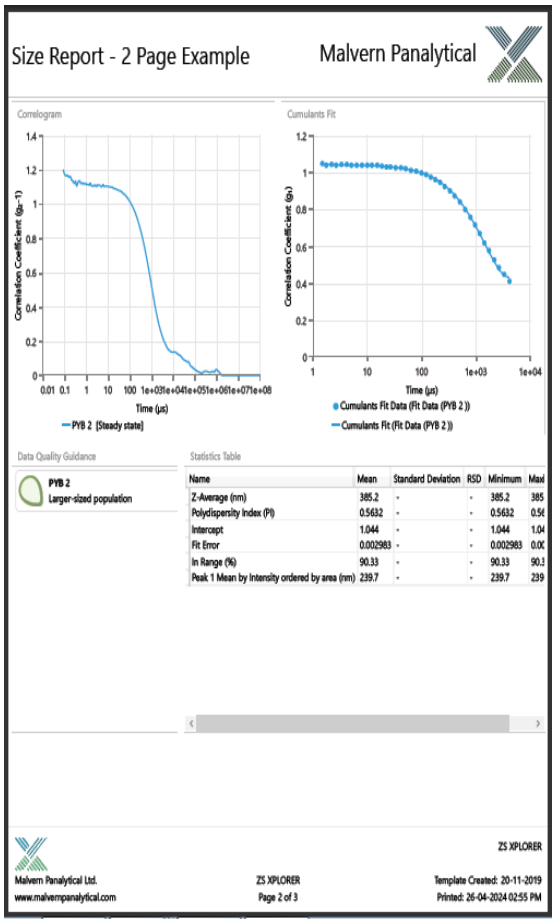
Size Distribution by Volume

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Size Report - 2 Page Example

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Correlogram

Cumulants Fit

Data Quality Guidance

PYB 2
Larger-sized population

Statistics Table

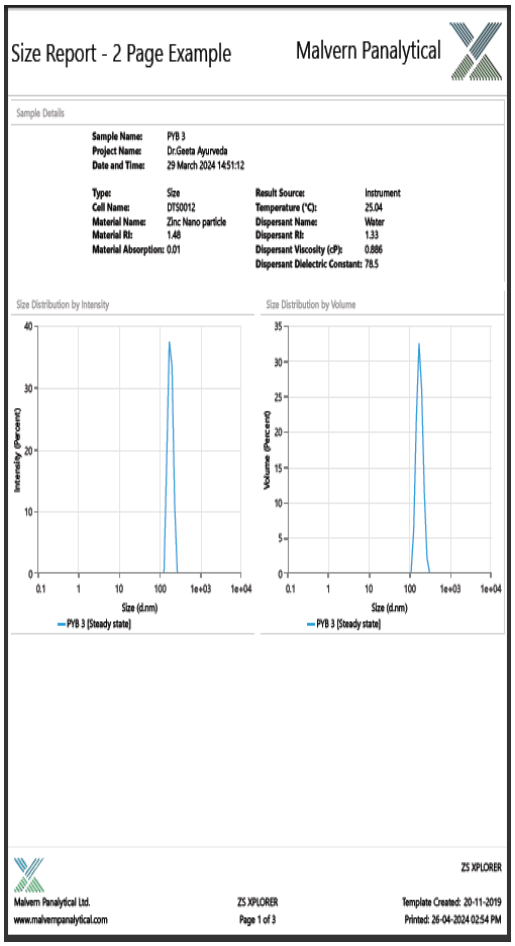
Name	Mean	Standard Deviation	RSD	Minimum	Maxi
Z-Average (nm)	385.2	-	-	385.2	385
Polydispersity Index (PI)	0.5632	-	-	0.5632	0.56
Intercept	1.044	-	-	1.044	1.04
Fit Error	0.002983	-	-	0.002983	0.00
In Range (%)	90.33	-	-	90.33	90.3
Peak 1 Mean by Intensity ordered by area (nm)	239.7	-	-	239.7	239

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Size Report - 2 Page Example — Malvern Panalytical

Sample Details

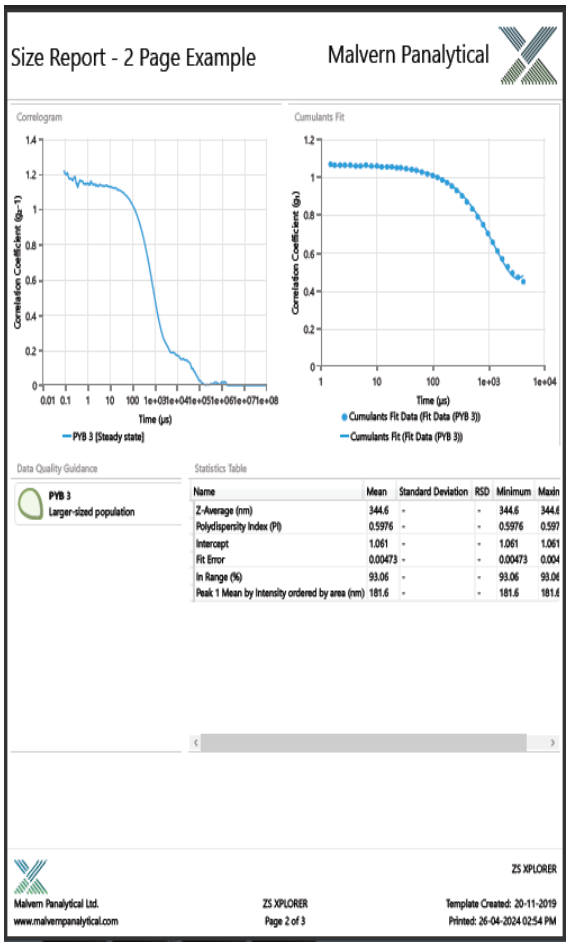
Sample Name:	PYB 3		
Project Name:	Dr.Geeta Ayurveda		
Date and Time:	29 March 2024 14:51:12		
Type:	Size	Result Source:	Instrument
Cell Name:	DTS0012	Temperature (°C):	25.04
Material Name:	Zinc Nano particle	Dispersant Name:	Water
Material RI:	1.48	Dispersant RI:	1.33
Material Absorption:	0.01	Dispersant Viscosity (cP):	0.886
		Dispersant Dielectric Constant:	78.5

Size Distribution by Intensity

Size Distribution by Volume

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Size Report - 2 Page Example — Malvern Panalytical

Correlogram

Cumulants Fit

Data Quality Guidance

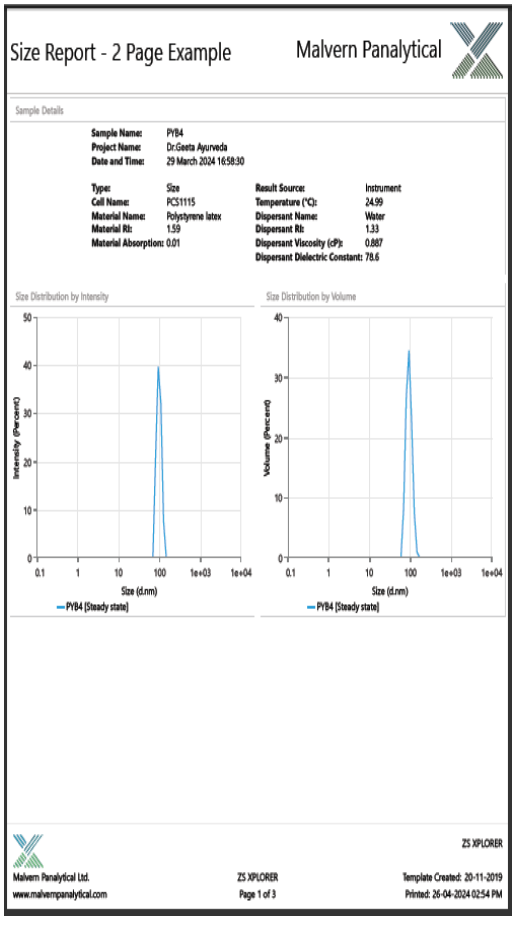
PYB 3
Larger-sized population

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maxin
Z-Average (nm)	344.6	-	-	344.6	344.6
Polydispersity Index (PI)	0.5976	-	-	0.5976	0.597
Intercept	1.061	-	-	1.061	1.061
Fit Error	0.00473	-	-	0.00473	0.004
In Range (%)	93.06	-	-	93.06	93.06
Peak 1 Mean by Intensity ordered by area (nm)	181.6	-	-	181.6	181.6

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Sample Details

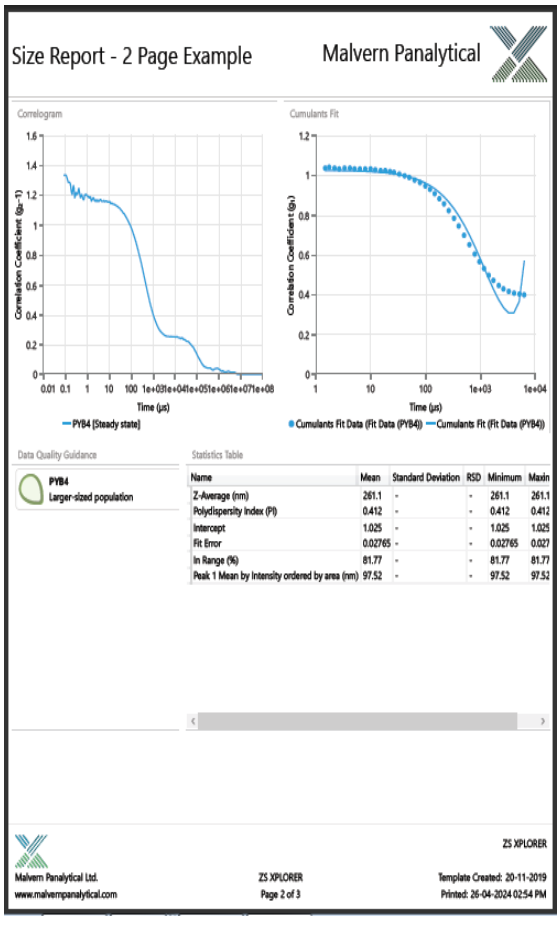
Sample Name:	PYB4		
Project Name:	Dr.Geeta Ayurveda		
Date and Time:	29 March 2024 16:58:30		
Type:	Size	Result Source:	Instrument
Cell Name:	PCS1115	Temperature (°C):	24.99
Material Name:	Polystyrene latex	Dispersant Name:	Water
Material RI:	1.59	Dispersant RI:	1.33
Material Absorption:	0.01	Dispersant Viscosity (cP):	0.887
		Dispersant Dielectric Constant:	78.6

Size Distribution by Intensity

Size Distribution by Volume

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Correlogram

Cumulants Fit

Data Quality Guidance

PYB4
Larger-sized population

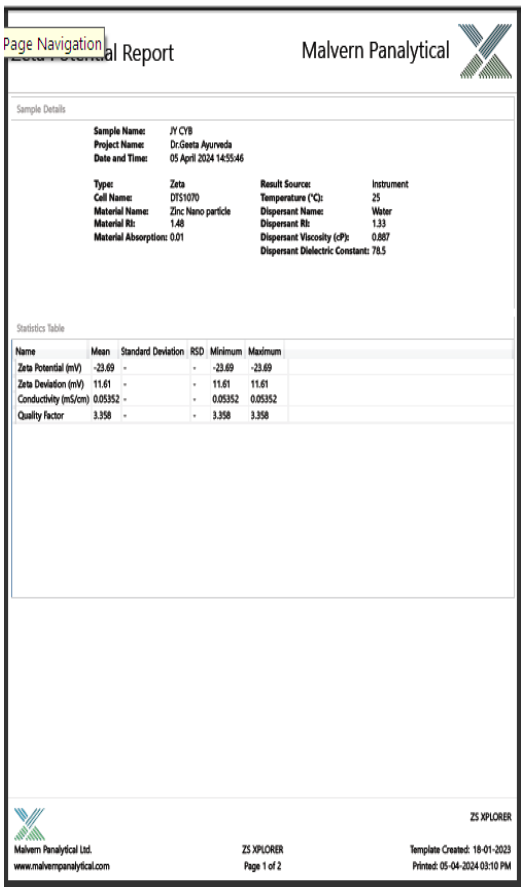
Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maxin
Z-Average (nm)	261.1	-	-	261.1	261.1
Polydispersity Index (PI)	0.412	-	-	0.412	0.412
Intercept	1.025	-	-	1.025	1.025
Fit Error	0.02765	-	-	0.02765	0.027
In Range (%)	81.77	-	-	81.77	81.77
Peak 1 Mean by Intensity ordered by area (nm)	97.52	-	-	97.52	97.52

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ZETA POTENTIAL ANALYSIS



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al Report

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Sample Details

Sample Name: JY CYB
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 14:55:46

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Statistics Table

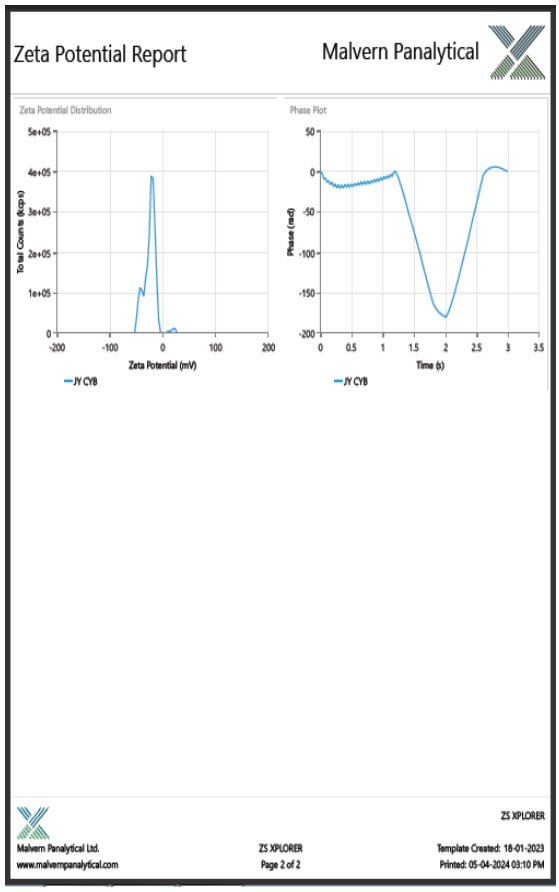
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-23.69	-	-	-23.69	-23.69
Zeta Deviation (mV)	11.61	-	-	11.61	11.61
Conductivity (mS/cm)	0.05352	-	-	0.05352	0.05352
Quality Factor	3.358	-	-	3.358	3.358

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Zeta Potential Report

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Zeta Potential Distribution

Phase Plot

Total Counts (kcps)

Zeta Potential (mV)

Phase (rad)

Time (s)

JY CYB

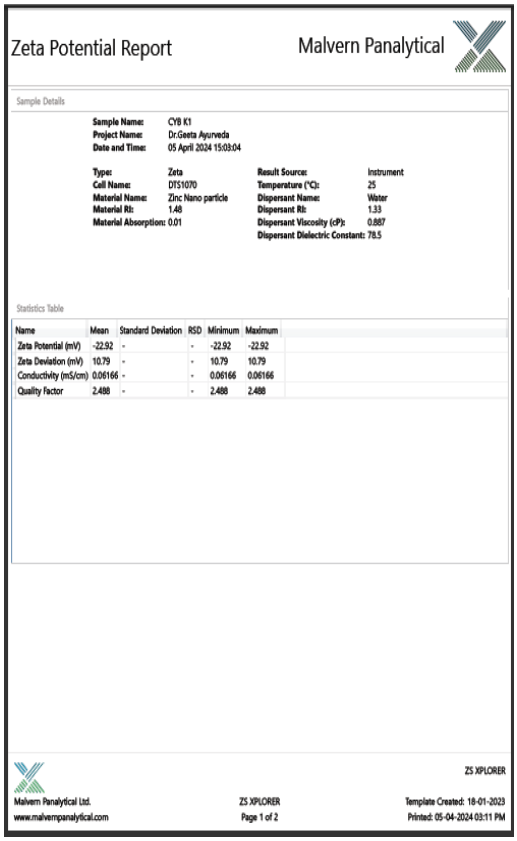
JY CYB

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Zeta Potential Report

Malvern Panalytical

Sample Details

Sample Name: CYB K1
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:03:04

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Statistics Table

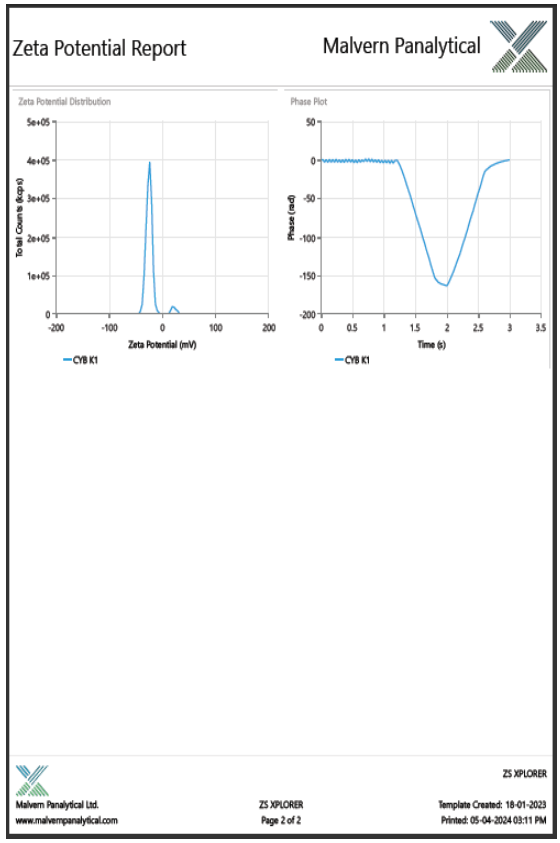
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-22.92	-	-	-22.92	-22.92
Zeta Deviation (mV)	10.79	-	-	10.79	10.79
Conductivity (mS/cm)	0.06166	-	-	0.06166	0.06166
Quality Factor	2.488	-	-	2.488	2.488

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Zeta Potential Report

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Zeta Potential Distribution

Phase Plot

Total Counts (kcps)

Zeta Potential (mV)

Phase (rad)

Time (s)

CYB K1

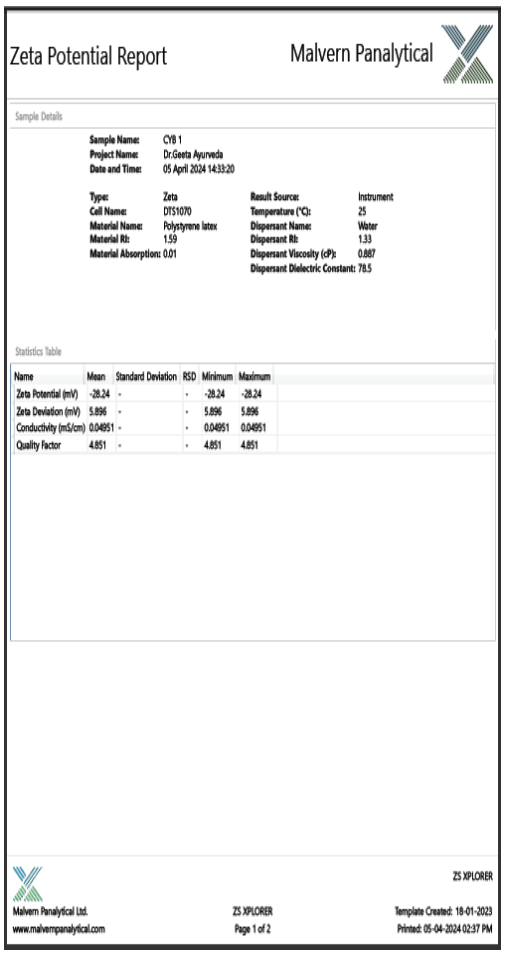
CYB K1

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Zeta Potential Report

Malvern Panalytical

Sample Details

Sample Name: CYB 1
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 14:33:20

Type: Zeta
Cell Name: DTS1070
Material Name: Polystyrene latex
Material RI: 1.59
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Statistics Table

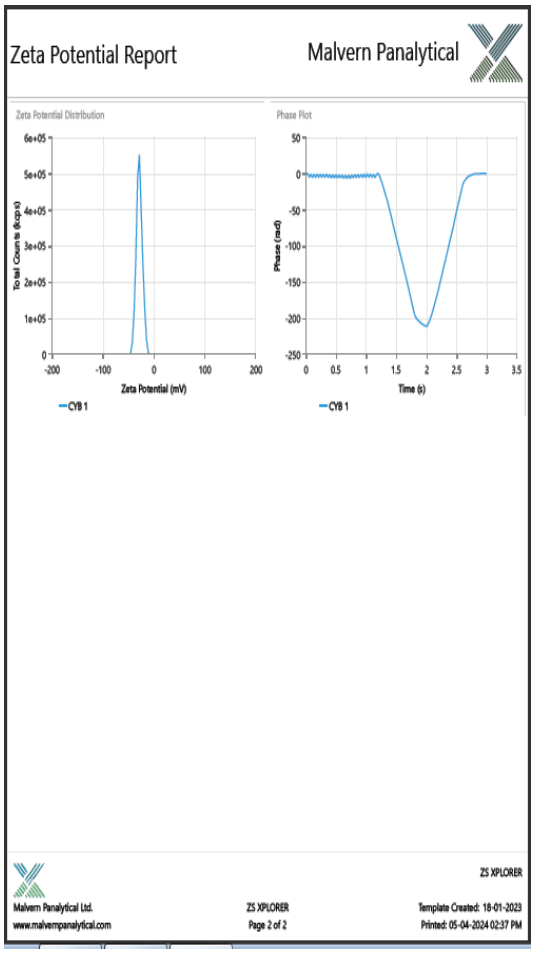
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-28.24	-	-	-28.24	-28.24
Zeta Deviation (mV)	5.896	-	-	5.896	5.896
Conductivity (mS/cm)	0.04951	-	-	0.04951	0.04951
Quality Factor	4.851	-	-	4.851	4.851

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Zeta Potential Report

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Zeta Potential Distribution

Total Counts (kcps)

Zeta Potential (mV)

CYB 1

Phase Plot

Phase (rad)

Time (s)

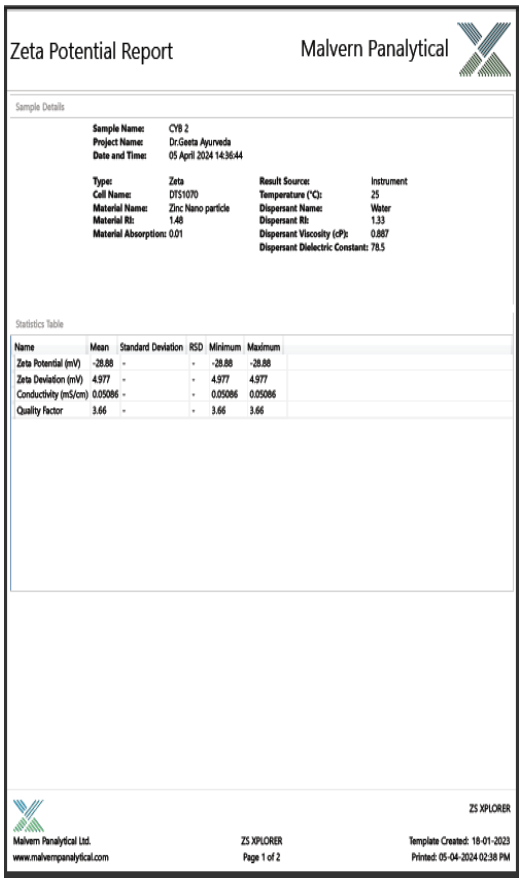
CYB 1

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Zeta Potential Report

Malvern Panalytical

Sample Details

Sample Name: CYB 2
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 14:36:44

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Statistics Table

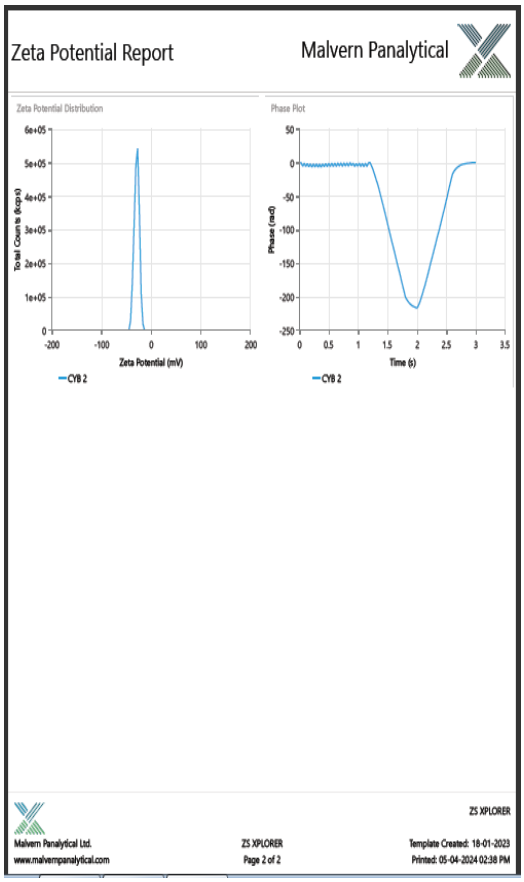
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-28.88	-	-	-28.88	-28.88
Zeta Deviation (mV)	4.977	-	-	4.977	4.977
Conductivity (mS/cm)	0.05086	-	-	0.05086	0.05086
Quality Factor	3.66	-	-	3.66	3.66

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Zeta Potential Report

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Zeta Potential Distribution

Total Counts (kcps)

Zeta Potential (mV)

CYB 2

Phase Plot

Phase (rad)

Time (s)

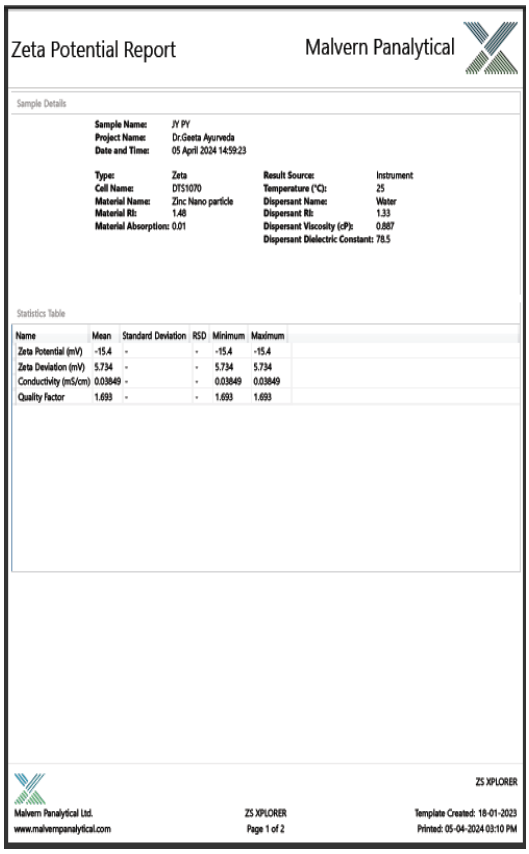
CYB 2

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Zeta Potential Report

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Sample Details

Sample Name: JY PY
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 14:59:23

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Statistics Table

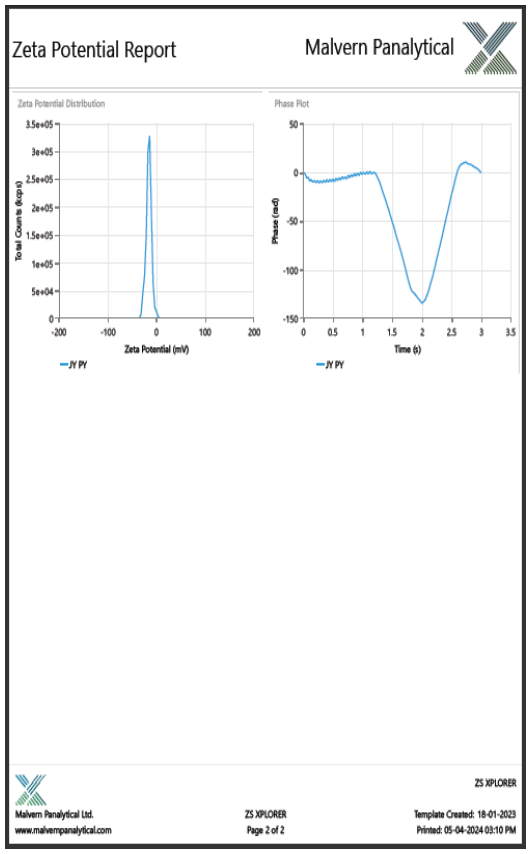
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-15.4	-	-	-15.4	-15.4
Zeta Deviation (mV)	5.734	-	-	5.734	5.734
Conductivity (mS/cm)	0.03849	-	-	0.03849	0.03849
Quality Factor	1.693	-	-	1.693	1.693

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Zeta Potential Report

Malvern Panalytical

Zeta Potential Distribution

Total Counts (kcps)

Zeta Potential (mV)

JY PY

Phase Plot

Phase (rad)

Time (s)

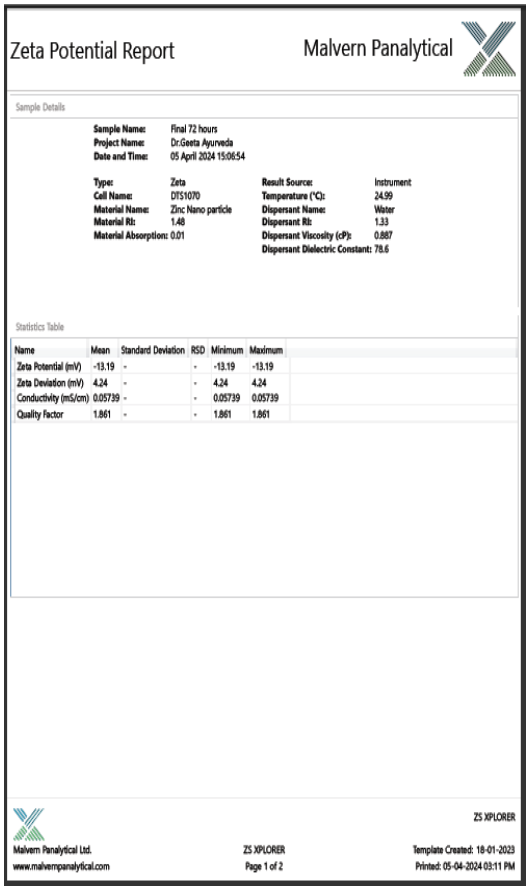
JY PY

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Zeta Potential Report

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Sample Details

Sample Name: Final 72 hours
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 15:06:54

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

Result Source: Instrument
Temperature (°C): 24.99
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.6

Statistics Table

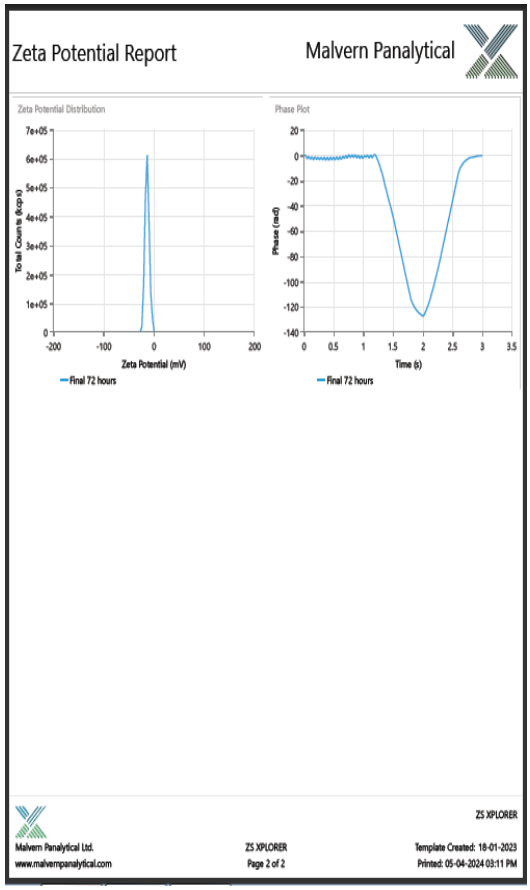
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-13.19	-	-	-13.19	-13.19
Zeta Deviation (mV)	4.24	-	-	4.24	4.24
Conductivity (mS/cm)	0.05739	-	-	0.05739	0.05739
Quality Factor	1.861	-	-	1.861	1.861

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Zeta Potential Report

Malvern Panalytical

Zeta Potential Distribution

Total Counts (kcps)

Zeta Potential (mV)

Final 72 hours

Phase Plot

Phase (rad)

Time (s)

Final 72 hours

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Zeta Potential Report

Malvern Panalytical

Sample Details

Sample Name:	PYB 1		
Project Name:	Dr.Geeta Ayurveda		
Date and Time:	05 April 2024 14:41:49		
Type:	Zeta	**Result Source:**	Instrument
Cell Name:	DTS1070	**Temperature (°C):**	24.99
Material Name:	Zinc Nano particle	**Dispersant Name:**	Water
Material RI:	1.48	**Dispersant RI:**	1.33
Material Absorption:	0.01	**Dispersant Viscosity (cP):**	0.887
		Dispersant Dielectric Constant:	78.6

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-25.27	-	-	-25.27	-25.27
Zeta Deviation (mV)	5.728	-	-	5.728	5.728
Conductivity (mS/cm)	0.1048	-	-	0.1048	0.1048
Quality Factor	4.391	-	-	4.391	4.391

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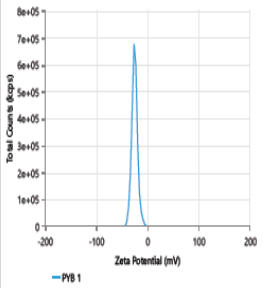
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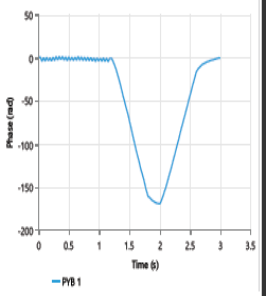
Zeta Potential Report

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Zeta Potential Distribution



Phase Plot



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Zeta Potential Report

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Sample Details

Sample Name:	PYB 2		
Project Name:	Dr.Geeta Ayurveda		
Date and Time:	05 April 2024 15:03:04		
Type:	Zeta	**Result Source:**	Edited
Cell Name:	DTS1070	**Temperature (°C):**	25
Material Name:	Zinc Nano particle	**Dispersant Name:**	Water
Material RI:	1.48	**Dispersant RI:**	1.33
Material Absorption:	0.01	**Dispersant Viscosity (cP):**	0.887
		Dispersant Dielectric Constant:	78.5

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-22.92	-	-	-22.92	-22.92
Zeta Deviation (mV)	10.79	-	-	10.79	10.79
Conductivity (mS/cm)	0.06166	-	-	0.06166	0.06166
Quality Factor	2.488	-	-	2.488	2.488

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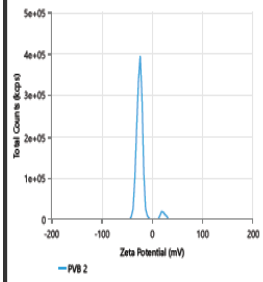
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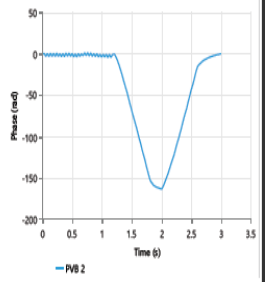
Zeta Potential Report

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Zeta Potential Distribution



Phase Plot

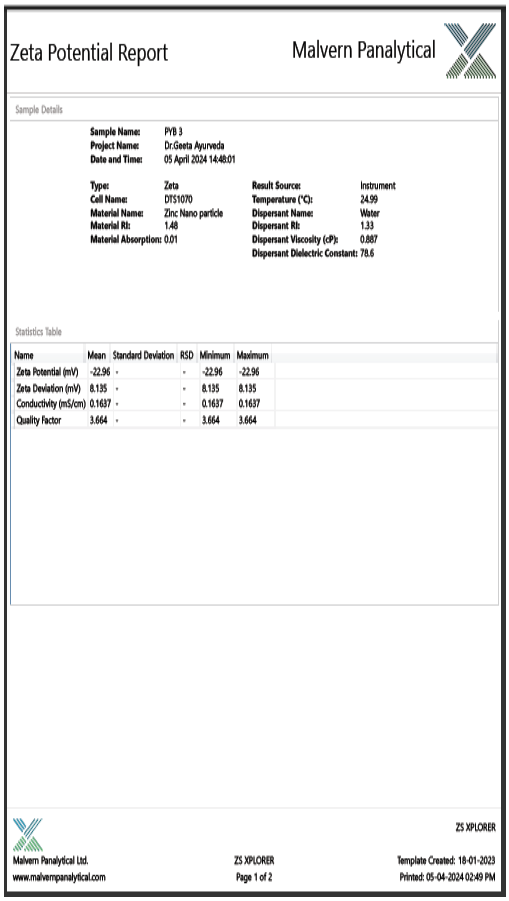


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Zeta Potential Report — Malvern Panalytical

Sample Details

Sample Name: PYB 3
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 14:48:01

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

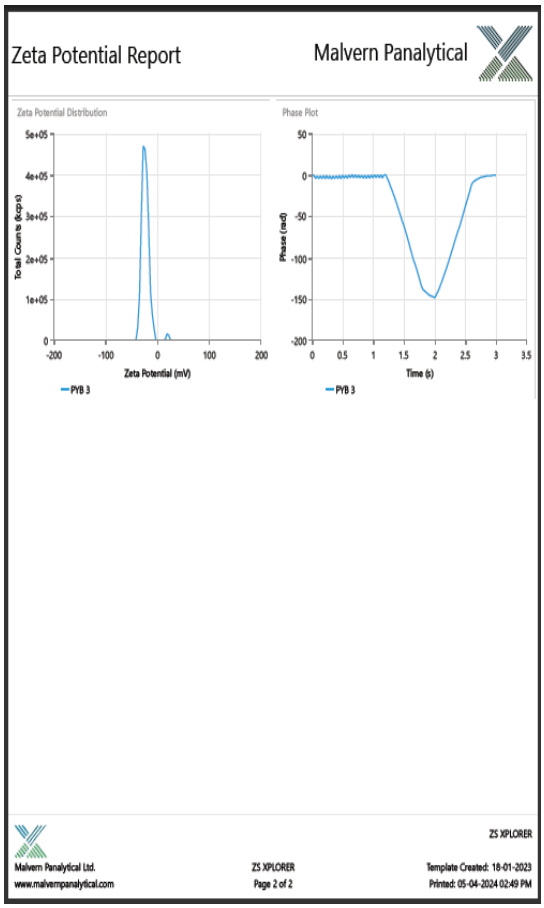
Result Source: Instrument
Temperature (°C): 24.99
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.6

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-22.96			-22.96	-22.96
Zeta Deviation (mV)	8.135			8.135	8.135
Conductivity (mS/cm)	0.1637			0.1637	0.1637
Quality Factor	3.664			3.664	3.664

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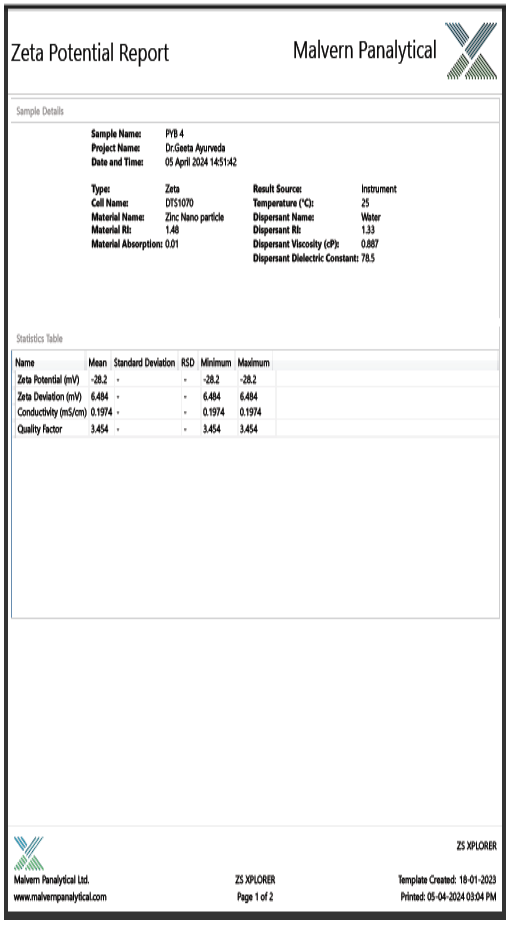
Zeta Potential Report — Malvern Panalytical

Zeta Potential Distribution

Phase Plot

ZS XPLORER

Malvern Panalytical Ltd. | ZS XPLORER | Template Created: 18-01-2023
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Zeta Potential Report — Malvern Panalytical

Sample Details

Sample Name: PYB 4
Project Name: Dr.Geeta Ayurveda
Date and Time: 05 April 2024 14:51:42

Type: Zeta
Cell Name: DTS1070
Material Name: Zinc Nano particle
Material RI: 1.48
Material Absorption: 0.01

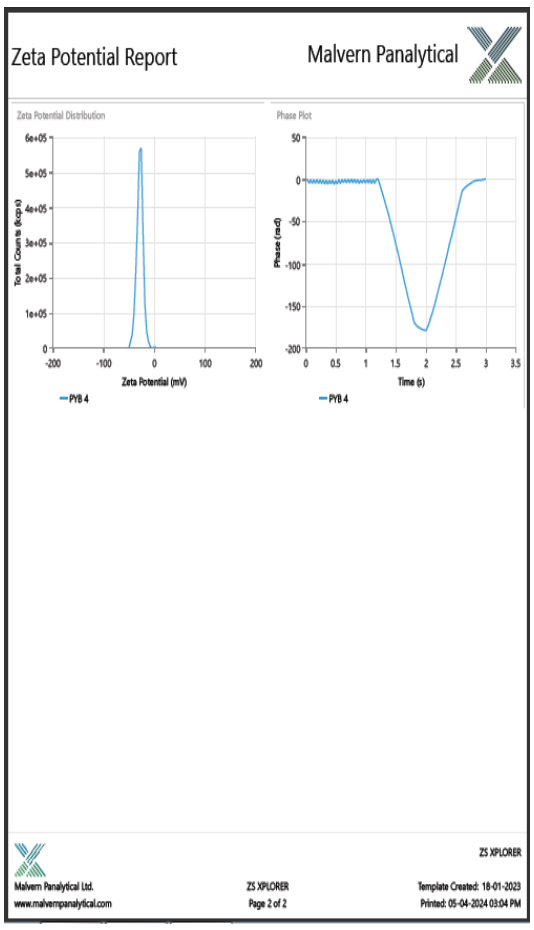
Result Source: Instrument
Temperature (°C): 25
Dispersant Name: Water
Dispersant RI: 1.33
Dispersant Viscosity (cP): 0.887
Dispersant Dielectric Constant: 78.5

Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-28.2			-28.2	-28.2
Zeta Deviation (mV)	6.484			6.484	6.484
Conductivity (mS/cm)	0.1974			0.1974	0.1974
Quality Factor	3.454			3.454	3.454

ZS XPLORER

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Zeta Potential Report — Malvern Panalytical

Zeta Potential Distribution

Phase Plot

ZS XPLORER

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XRD ANALYSIS OF INTERMEDIATE AND FINAL PRODUTS



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TEST NAME:
X-RAY DIFFRACTOMETER (XRD)

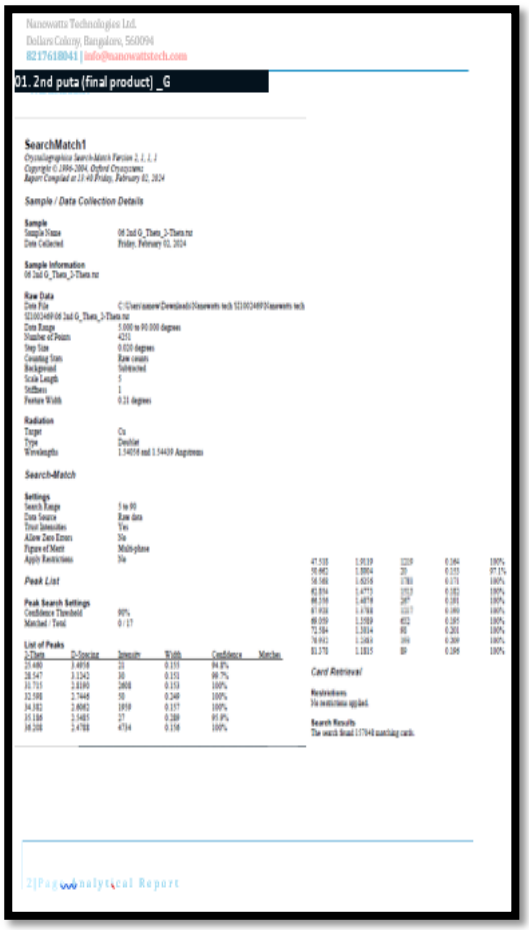
MATERIALS USED
1. XRD PALLET MAKER
2. GLASS SLIDES
3. GLASS XRD HOLDERS
4. 1 GM OF SAMPLE (TOTAL NO: 3 SAMPLES)

TESTING REQUESTOR: Dr. Geetha G Gubbi
Shri SMK ayurvedic College, Shikaripura, Belgavi-3

INSTRUMENT MAKE & MODEL:
RIGAKU SMART LAB 9200

STEPS	PROCEDURE
1	1 gm of air-dried samples was accurately weighed and loaded on glass slide
2	XRAY_CHAR = K-ALPHA *WAVE_LENGTH1= 1.54059 *WAVE_LENGTH2= 1.54441
3	2theta/theta = 5 to 80 Degree
4	XRD analysis is performed using Rigaku Instrument with 2 Avg measurement points to get least standard deviation.

Number	Sample name	Original name
1	06-2nd_G	2nd puta (final product)
2	07-J_G	Jaitha Yashada
3	08-S_G	Shobha yashada



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01. 2nd puta (final product) _G

SearchMatch1
Crystallographica Search-Match Version 2.1.1.1
Copyright © 1996-2004, Oxford Cryosystems
Report Compiled at 13:49 Friday, February 02, 2024

Sample / Data Collection Details

Sample
Sample Name: 06 2nd G_Theta_2-Theta.rsr
Date Collected: Friday, February 02, 2024

Sample Information
06 2nd G_Theta_2-Theta.rsr

Raw Data
Data File: C:\Users\nanow\Downloads\Nanowatts tech 5210024497\Nanowatts tech 5210024497\06 2nd G_Theta_2-Theta.rsr
Data Range: 5.000 to 90.000 degrees
Number of Points: 4251
Step Size: 0.020 degrees
Counting Time: Raw counts
Background: Subtracted
Scale Length: 5
Stiffness: 1
Feature Width: 0.21 degrees

Radiation
Target: Cu
Type: Double
Wavelengths: 1.54056 and 1.54439 Angstroms

Search-Match

Settings
Search Range: 5 to 90
Data Source: Raw data
Treat Intensities: Yes
Allow Zero Errors: No
Figure of Merit: Multi-phase
Apply Restrictions: No

Peak List

Peak Search Settings
Confidence Threshold: 80%
Matched / Total: 0 / 17

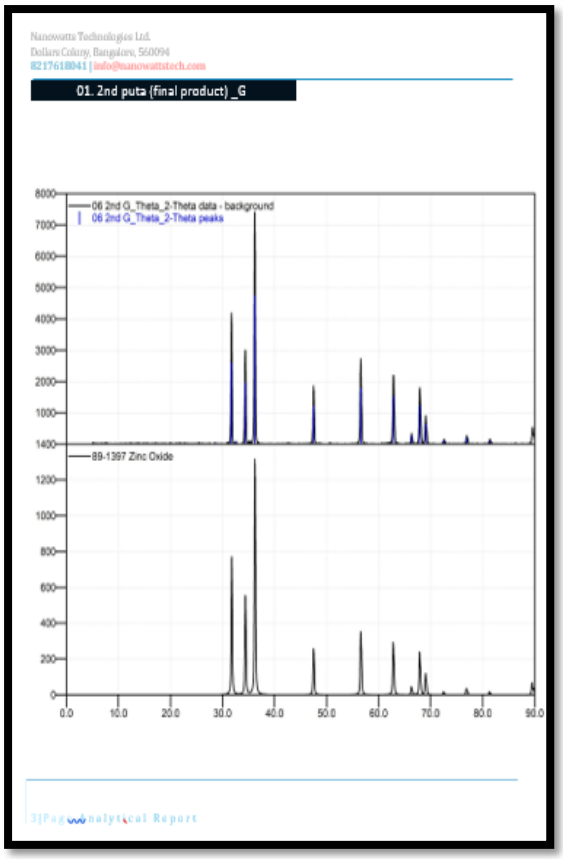
List of Peaks

2-Theta	D-Spacing	Intensity	Width	Confidence	Matches
25.480	3.4916	21	0.153	84.8%	
28.547	3.1242	30	0.153	99.7%	
31.715	2.8190	2608	0.153	100%	
32.588	2.7448	50	0.240	100%	
34.382	2.6062	1959	0.157	100%	
35.186	2.5485	27	0.288	91.9%	
46.206	1.9788	4734	0.156	100%	
47.535	1.9119	1219	0.164	100%	
55.982	1.8864	20	0.153	97.1%	
56.566	1.6265	1783	0.171	100%	
62.854	1.4773	1013	0.182	100%	
68.156	1.4074	2687	0.181	100%	
67.938	1.4768	1117	0.189	100%	
69.059	1.3589	402	0.195	100%	
72.584	1.3014	92	0.201	100%	
76.942	1.2484	196	0.209	100%	
81.270	1.1815	89	0.196	100%	

Card Retrieval

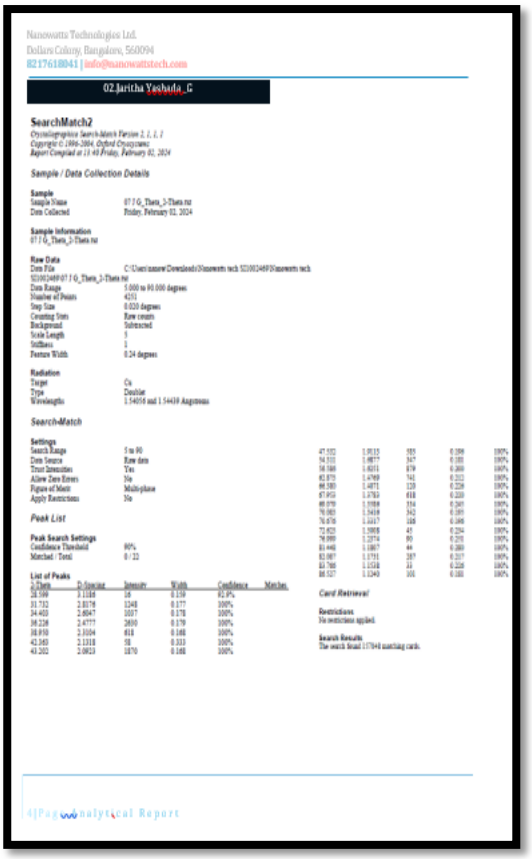
Restrictions
No restrictions applied.

Search Results
The search found 177948 matching cards.



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01. 2nd puta (final product) _G



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02.Jaritha Yashada _G

SearchMatch2
Crystallographica Search-Match Version 2.1.1.1
Copyright © 1996-2004, Oxford Cryosystems
Report Compiled at 13:49 Friday, February 02, 2024

Sample / Data Collection Details

Sample
Sample Name: 07 J G_Theta_2-Theta.rsr
Date Collected: Friday, February 02, 2024

Sample Information
07 J G_Theta_2-Theta.rsr

Raw Data
Data File: C:\Users\nanow\Downloads\Nanowatts tech 5210024497\Nanowatts tech 5210024497\07 J G_Theta_2-Theta.rsr
Data Range: 5.000 to 90.000 degrees
Number of Points: 4251
Step Size: 0.020 degrees
Counting Time: Raw counts
Background: Subtracted
Scale Length: 5
Stiffness: 1
Feature Width: 0.24 degrees

Radiation
Target: Cu
Type: Double
Wavelengths: 1.54056 and 1.54439 Angstroms

Search-Match

Settings
Search Range: 5 to 90
Data Source: Raw data
Treat Intensities: Yes
Allow Zero Errors: No
Figure of Merit: Multi-phase
Apply Restrictions: No

Peak List

Peak Search Settings
Confidence Threshold: 80%
Matched / Total: 0 / 22

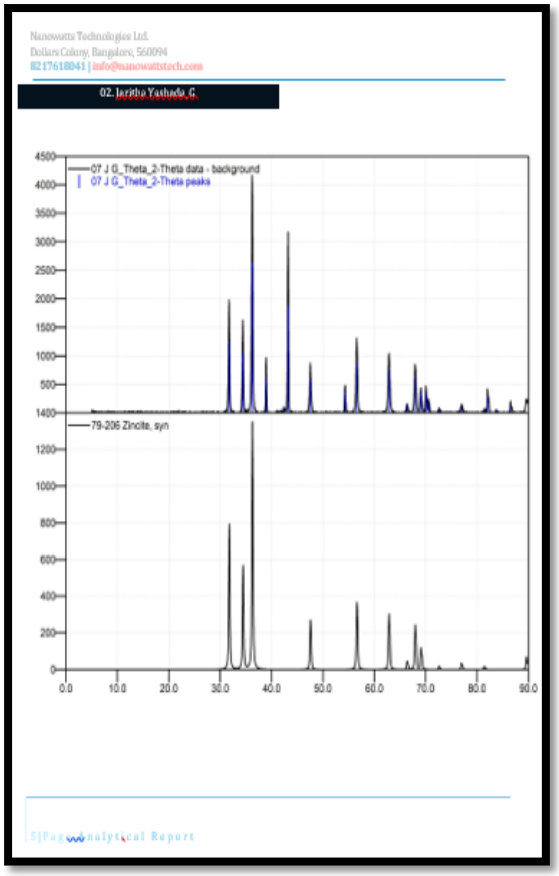
List of Peaks

2-Theta	D-Spacing	Intensity	Width	Confidence	Matches
26.599	3.3484	58	0.159	82.9%	
31.732	2.8178	1248	0.177	100%	
34.403	2.6047	1017	0.178	100%	
36.238	2.4777	2650	0.179	100%	
38.930	2.3104	618	0.188	100%	
42.340	2.1318	58	0.333	100%	
43.202	2.0923	1070	0.168	100%	
47.532	1.9115	355	0.198	100%	
56.555	1.6277	347	0.180	100%	
56.588	1.6251	679	0.200	100%	
62.870	1.4769	741	0.222	100%	
68.580	1.4071	229	0.228	100%	
67.952	1.3783	418	0.220	100%	
69.070	1.3588	334	0.245	100%	
76.002	1.2419	342	0.265	100%	
76.878	1.2317	168	0.249	100%	
72.820	1.2908	45	0.254	100%	
76.999	1.2374	90	0.255	100%	
81.448	1.1807	44	0.280	100%	
82.067	1.1731	287	0.217	100%	
82.768	1.1648	33	0.226	100%	
86.527	1.1249	101	0.188	100%	

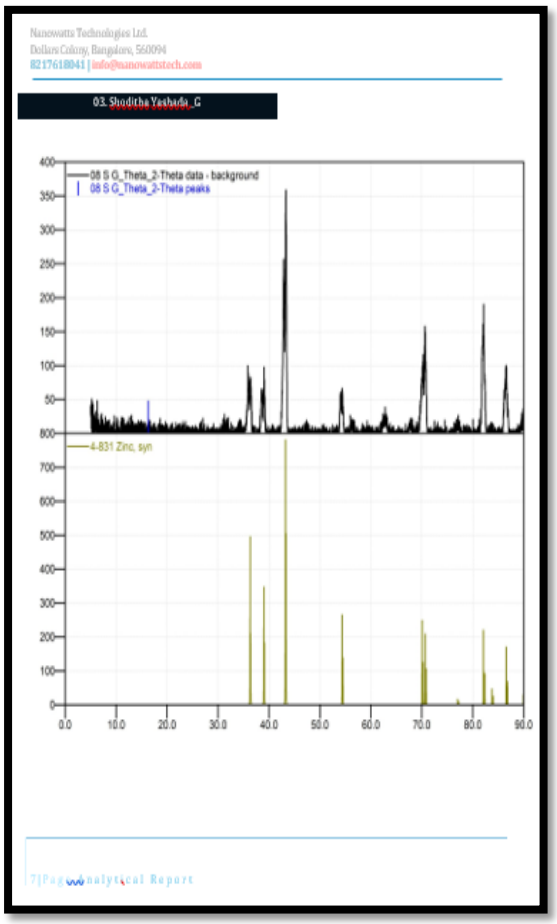
Card Retrieval

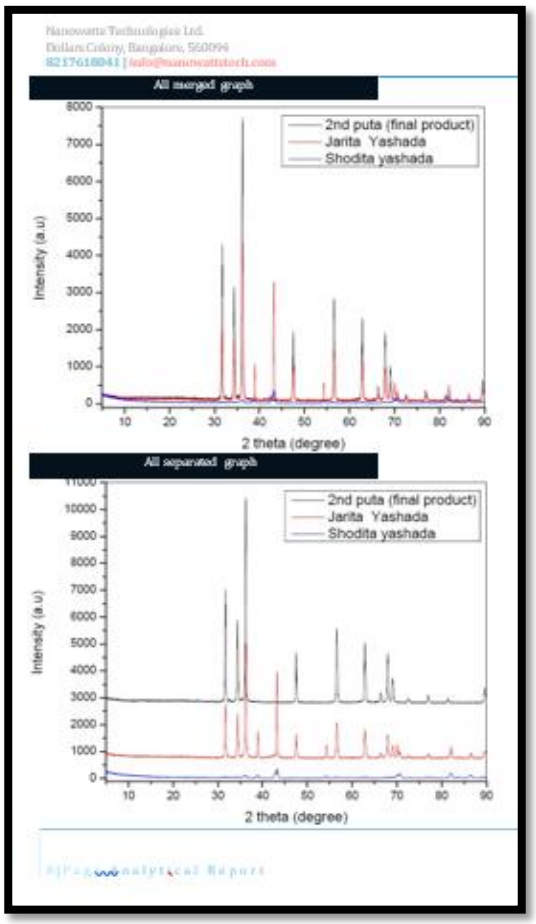
Restrictions
No restrictions applied.

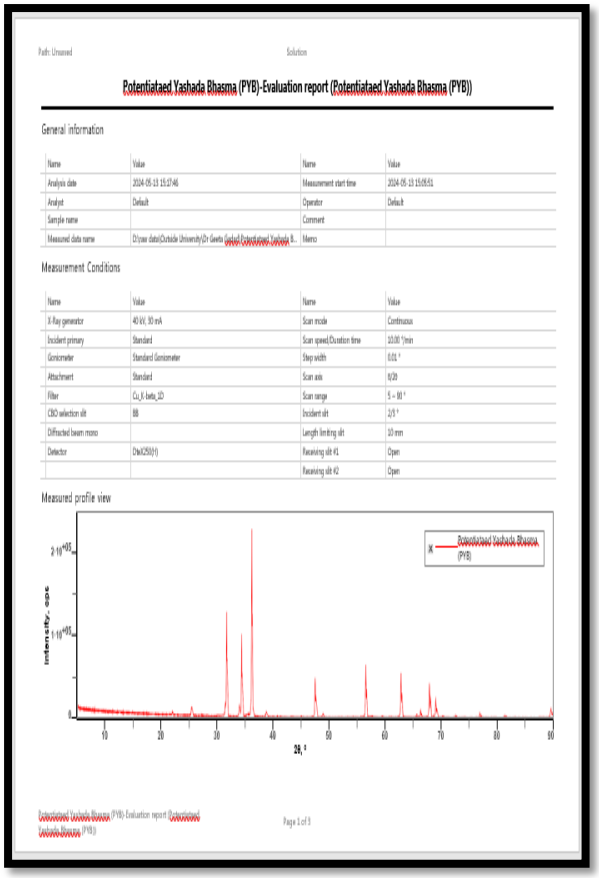
Search Results
The search found 177948 matching cards.


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02. Jaritha Yashada_G
07 J G_Theta_2-Theta data - background
07 J G_Theta_2-Theta peaks
79-206 Zincite, syn
5|Page Analytical Report


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03. Shoditha Yashada _G
SearchMatch3
Sample / Data Collection Details
Search-Match
Peak List
Card Retrieval
6|Page Analytical Report


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03. Shoditha Yashada_G
08 S G_Theta_2-Theta data - background
08 S G_Theta_2-Theta peaks
4-831 Zinc, syn
7|Page Analytical Report


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All merged graph
2nd puta (final product)
Jarita Yashada
Shodita yashada
Intensity (a.u)
2 theta (degree)
All separated graph
2nd puta (final product)
Jarita Yashada
Shodita yashada
Intensity (a.u)
2 theta (degree)
8|Page Analytical Report



Potentiataed Yashada Bhasma (PYB)-Evaluation report (Potentiataed Yashada Bhasma (PYB))

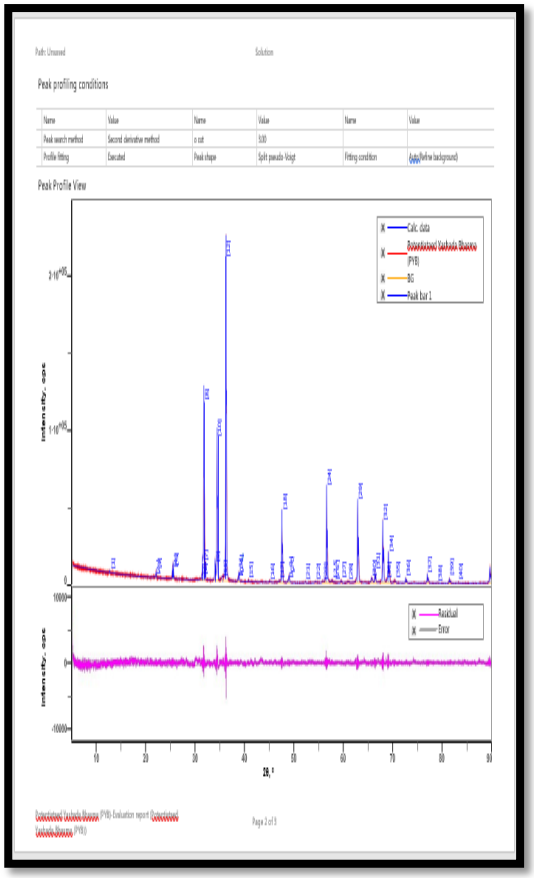
General information

Name	Value	Name	Value
Analysis date	2024-05-13 15:17:46	Measurement start time	2024-05-13 15:05:51
Analyst	Default	Operator	Default
Sample name		Comment	
Measured data name	D:\raw data\Outside University\Dr Geeta Gokul Potentiataed Yashada B...	Memo	

Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 30 mA	Scan mode	Continuous
Incident primary	Standard	Scan speed/Duration time	10.00 °/min
Goniometer	Standard Goniometer	Step width	0.01 °
Attachment	Standard	Scan axis	θ/2θ
Filter	Cu_K-beta_3D	Scan range	5 – 90 °
CBO selection slit	BB	Incident slit	2/3 °
Diffracted beam mono		Length limiting slit	10 mm
Detector	D/teX250/1	Receiving slit #1	Open
		Receiving slit #2	Open

Measured profile view

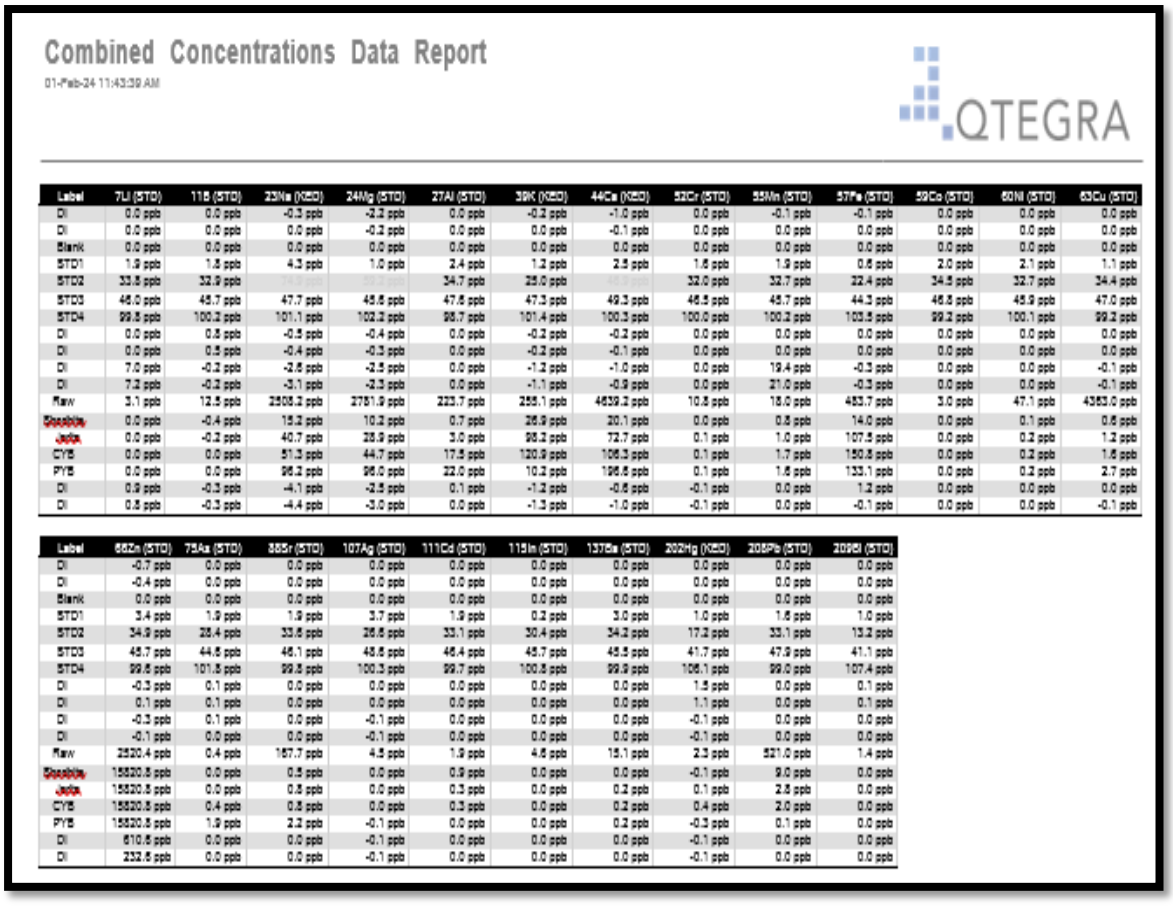


Peak profiling conditions

Name	Value	Name	Value	Name	Value
Peak search method	Second derivative method	α cut	0.00		
Profile fitting	Executed	Peak shape	Split pseudo-Voigt	Fitting condition	Auto(Refine background)

Peak Profile View

ICPMS ANALYSIS



Combined Concentrations Data Report

01-Feb-24 11:43:39 AM

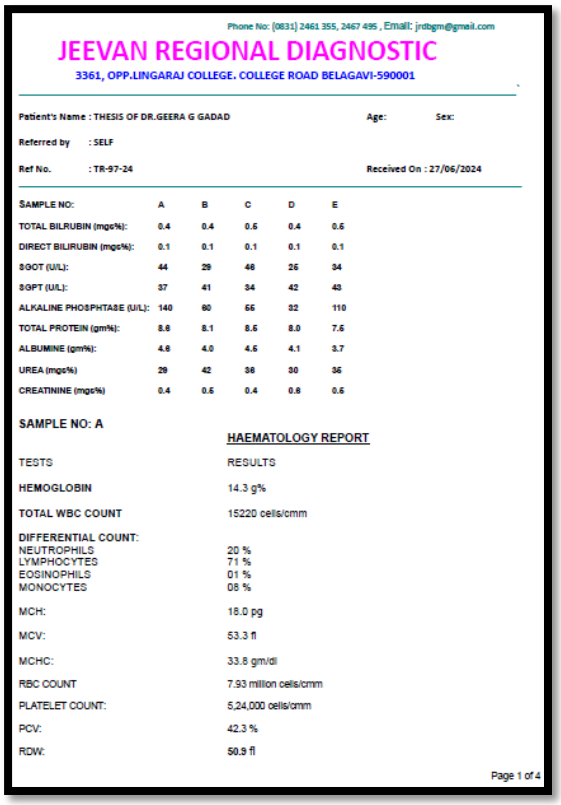
QTEGRA

Label	7Li (STD)	11B (STD)	23Na (KED)	24Mg (STD)	27Al (STD)	39K (KED)	44Ca (KED)	52Cr (STD)	55Mn (STD)	57Fe (STD)	59Co (STD)	60Ni (STD)	63Cu (STD)
DI	0.0 ppb	0.0 ppb	-0.3 ppb	-2.2 ppb	0.0 ppb	-0.2 ppb	-1.0 ppb	0.0 ppb	-0.1 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb
DI	0.0 ppb	0.0 ppb	0.0 ppb	-0.2 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
Blank	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
STD1	1.9 ppb	1.8 ppb	4.3 ppb	1.0 ppb	2.4 ppb	1.2 ppb	2.5 ppb	1.6 ppb	1.9 ppb	0.8 ppb	2.0 ppb	2.1 ppb	1.1 ppb
STD2	33.8 ppb	32.9 ppb			34.7 ppb	25.0 ppb		32.0 ppb	32.7 ppb	22.4 ppb	34.8 ppb	32.7 ppb	34.4 ppb
STD3	46.0 ppb	45.7 ppb	47.7 ppb	45.6 ppb	47.6 ppb	47.3 ppb	49.3 ppb	46.5 ppb	45.7 ppb	44.3 ppb	46.8 ppb	45.9 ppb	47.0 ppb
STD4	99.8 ppb	100.2 ppb	101.1 ppb	102.2 ppb	98.7 ppb	101.4 ppb	100.3 ppb	100.0 ppb	100.2 ppb	103.5 ppb	99.2 ppb	100.1 ppb	99.2 ppb
DI	0.0 ppb	0.8 ppb	-0.5 ppb	-0.4 ppb	0.0 ppb	-0.2 ppb	-0.2 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
DI	0.0 ppb	0.8 ppb	-0.4 ppb	-0.3 ppb	0.0 ppb	-0.2 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
DI	7.0 ppb	-0.2 ppb	-2.6 ppb	-2.5 ppb	0.0 ppb	-1.2 ppb	-1.0 ppb	0.0 ppb	19.4 ppb	-0.3 ppb	0.0 ppb	0.0 ppb	-0.1 ppb
DI	7.2 ppb	-0.2 ppb	-3.1 ppb	-2.3 ppb	0.0 ppb	-1.1 ppb	-0.9 ppb	0.0 ppb	21.0 ppb	-0.3 ppb	0.0 ppb	0.0 ppb	-0.1 ppb
Raw	3.1 ppb	12.5 ppb	2508.2 ppb	2751.9 ppb	223.7 ppb	255.1 ppb	4639.2 ppb	10.8 ppb	15.0 ppb	483.7 ppb	3.0 ppb	47.1 ppb	4363.0 ppb
Double	0.0 ppb	-0.4 ppb	15.2 ppb	10.2 ppb	0.7 ppb	26.9 ppb	20.1 ppb	0.0 ppb	0.8 ppb	14.0 ppb	0.0 ppb	0.1 ppb	0.6 ppb
Jaja	0.0 ppb	-0.2 ppb	40.7 ppb	28.9 ppb	3.0 ppb	98.2 ppb	72.7 ppb	0.1 ppb	1.0 ppb	107.5 ppb	0.0 ppb	0.2 ppb	1.2 ppb
CYB	0.0 ppb	0.0 ppb	51.3 ppb	44.7 ppb	17.5 ppb	120.9 ppb	106.3 ppb	0.1 ppb	1.7 ppb	150.8 ppb	0.0 ppb	0.2 ppb	1.6 ppb
PYB	0.0 ppb	0.0 ppb	96.2 ppb	96.0 ppb	22.0 ppb	10.2 ppb	196.6 ppb	0.1 ppb	1.6 ppb	133.1 ppb	0.0 ppb	0.2 ppb	2.7 ppb
DI	0.9 ppb	-0.3 ppb	-4.1 ppb	-2.5 ppb	0.1 ppb	-1.2 ppb	-0.6 ppb	-0.1 ppb	0.0 ppb	1.2 ppb	0.0 ppb	0.0 ppb	0.0 ppb
DI	0.8 ppb	-0.3 ppb	-4.4 ppb	-3.0 ppb	0.0 ppb	-1.3 ppb	-1.0 ppb	-0.1 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	-0.1 ppb

Label	66Zn (STD)	75As (STD)	88Sr (STD)	107Ag (STD)	111Cd (STD)	115In (STD)	137Ba (STD)	202Hg (KED)	208Pb (STD)	209Bi (STD)
DI	-0.7 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
DI	-0.4 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
Blank	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb
STD1	3.4 ppb	1.9 ppb	1.9 ppb	3.7 ppb	1.9 ppb	0.2 ppb	3.0 ppb	1.0 ppb	1.8 ppb	1.0 ppb
STD2	34.9 ppb	28.4 ppb	33.6 ppb	26.8 ppb	33.1 ppb	30.4 ppb	34.2 ppb	17.2 ppb	33.1 ppb	13.2 ppb
STD3	45.7 ppb	44.6 ppb	46.1 ppb	45.8 ppb	46.4 ppb	45.7 ppb	45.5 ppb	41.7 ppb	47.9 ppb	41.1 ppb
STD4	99.6 ppb	101.5 ppb	99.8 ppb	100.3 ppb	99.7 ppb	100.8 ppb	99.9 ppb	106.1 ppb	99.0 ppb	107.4 ppb
DI	-0.3 ppb	0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	1.8 ppb	0.0 ppb	0.1 ppb
DI	0.1 ppb	0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	0.0 ppb	1.1 ppb	0.0 ppb	0.1 ppb
DI	-0.3 ppb	0.1 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb
DI	-0.1 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb
Raw	2520.4 ppb	0.4 ppb	167.7 ppb	4.8 ppb	1.9 ppb	4.6 ppb	15.1 ppb	2.3 ppb	521.0 ppb	1.4 ppb
Double	15320.8 ppb	0.0 ppb	0.8 ppb	0.0 ppb	0.9 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	9.0 ppb	0.0 ppb
Jaja	15320.8 ppb	0.0 ppb	0.8 ppb	0.0 ppb	0.3 ppb	0.0 ppb	0.2 ppb	0.1 ppb	2.8 ppb	0.0 ppb
CYB	15320.8 ppb	0.4 ppb	0.8 ppb	0.0 ppb	0.3 ppb	0.0 ppb	0.2 ppb	0.4 ppb	2.0 ppb	0.0 ppb
PYB	15320.8 ppb	1.9 ppb	2.2 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.2 ppb	-0.3 ppb	0.1 ppb	0.0 ppb
DI	610.6 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb
DI	232.6 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb	0.0 ppb	-0.1 ppb	0.0 ppb	0.0 ppb

ANNEXURE 07: ACUTE TOXICITY STUDY REPORTS

BIOCHEMICAL AND HEMATOLOGY REPORTS



Phone No: (0831) 2461 355, 2467 495 , Email: jrdbgm@gmail.com

JEEVAN REGIONAL DIAGNOSTIC

3361, OPP.LINGARAJ COLLEGE. COLLEGE ROAD BELAGAVI-590001

Patient's Name : THESIS OF DR.GEERA G GADAD Age: Sex:

Referred by : SELF

Ref No. : TR-97-24 Received On : 27/06/2024

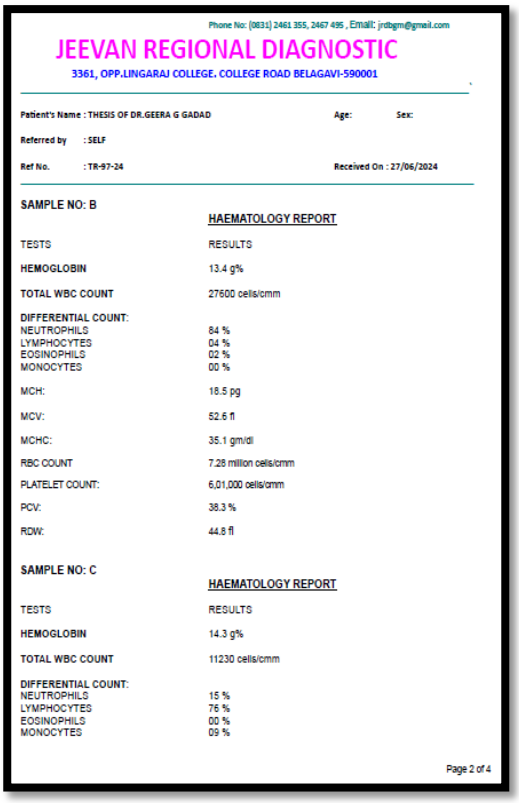
SAMPLE NO:	A	B	C	D	E
TOTAL BILRUBIN (mgs%):	0.4	0.4	0.6	0.4	0.6
DIRECT BILIRUBIN (mgs%):	0.1	0.1	0.1	0.1	0.1
SGOT (U/L):	44	29	48	26	34
SGPT (U/L):	37	41	34	42	43
ALKALINE PHOSPHTASE (U/L):	140	60	66	32	110
TOTAL PROTEIN (gm%):	8.6	8.1	8.6	8.0	7.6
ALBUMINE (gm%):	4.6	4.0	4.6	4.1	3.7
UREA (mgs%)	29	42	38	30	35
CREATININE (mgs%)	0.4	0.6	0.4	0.8	0.6

SAMPLE NO: A

HAEMATOLOGY REPORT

TESTS	RESULTS
HEMOGLOBIN	14.3 g%
TOTAL WBC COUNT	15220 cells/cmm
DIFFERENTIAL COUNT:	
NEUTROPHILS	20 %
LYMPHOCYTES	71 %
EOSINOPHILS	01 %
MONOCYTES	08 %
MCH:	18.0 pg
MCV:	53.3 fl
MCHC:	33.8 gm/dl
RBC COUNT	7.93 million cells/cmm
PLATELET COUNT:	5,24,000 cells/cmm
PCV:	42.3 %
RDW:	50.9 fl

Page 1 of 4



Phone No: (0831) 2461 355, 2467 495 , Email: jrdbgm@gmail.com

JEEVAN REGIONAL DIAGNOSTIC

3361, OPP.LINGARAJ COLLEGE. COLLEGE ROAD BELAGAVI-590001

Patient's Name : THESIS OF DR.GEERA G GADAD Age: Sex:

Referred by : SELF

Ref No. : TR-97-24 Received On : 27/06/2024

SAMPLE NO: B

HAEMATOLOGY REPORT

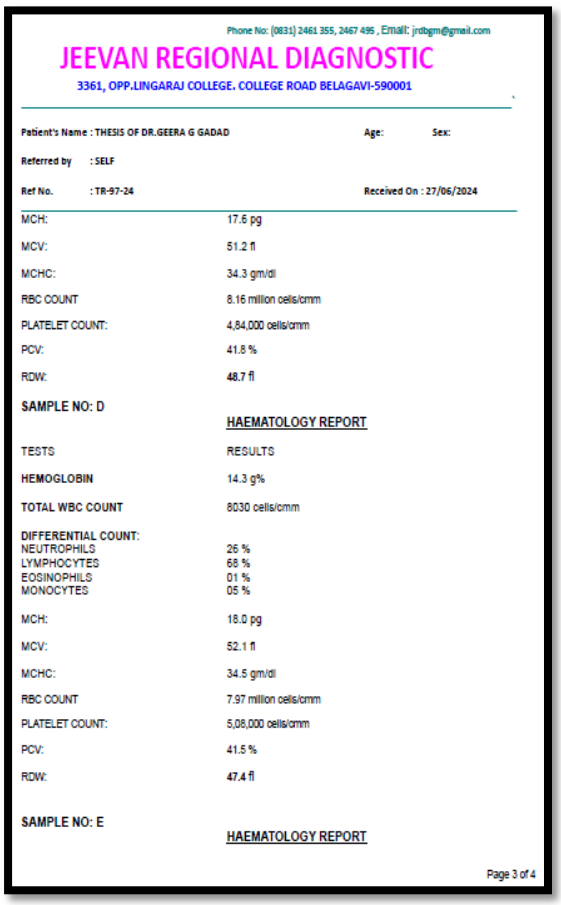
TESTS	RESULTS
HEMOGLOBIN	13.4 g%
TOTAL WBC COUNT	27600 cells/cmm
DIFFERENTIAL COUNT:	
NEUTROPHILS	84 %
LYMPHOCYTES	04 %
EOSINOPHILS	02 %
MONOCYTES	00 %
MCH:	18.5 pg
MCV:	52.6 fl
MCHC:	35.1 gm/dl
RBC COUNT	7.28 million cells/cmm
PLATELET COUNT:	6,01,000 cells/cmm
PCV:	38.3 %
RDW:	44.8 fl

SAMPLE NO: C

HAEMATOLOGY REPORT

TESTS	RESULTS
HEMOGLOBIN	14.3 g%
TOTAL WBC COUNT	11230 cells/cmm
DIFFERENTIAL COUNT:	
NEUTROPHILS	15 %
LYMPHOCYTES	76 %
EOSINOPHILS	00 %
MONOCYTES	09 %

Page 2 of 4



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JEEVAN REGIONAL DIAGNOSTIC

3361, OPP.LINGARAJ COLLEGE. COLLEGE ROAD BELAGAVI-590001

Patient's Name : THESIS OF DR.GEERA G GADAD Age: Sex:

Referred by : SELF

Ref No. : TR-97-24 Received On : 27/06/2024

MCH:	17.6 pg
MCV:	51.2 fl
MCHC:	34.3 gm/dl
RBC COUNT	8.16 million cells/cmm
PLATELET COUNT:	4,84,000 cells/cmm
PCV:	41.8 %
RDW:	48.7 fl

SAMPLE NO: D

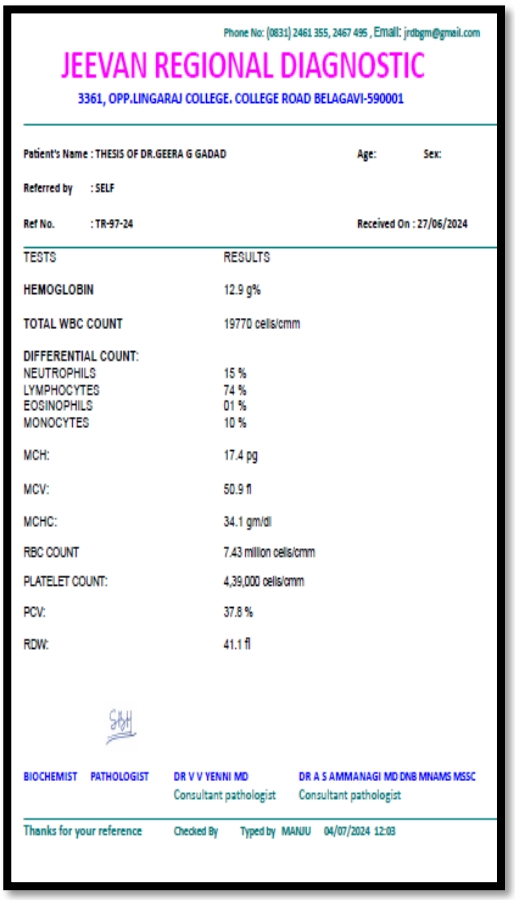
HAEMATOLOGY REPORT

TESTS	RESULTS
HEMOGLOBIN	14.3 g%
TOTAL WBC COUNT	8030 cells/cmm
DIFFERENTIAL COUNT:	
NEUTROPHILS	26 %
LYMPHOCYTES	68 %
EOSINOPHILS	01 %
MONOCYTES	05 %
MCH:	18.0 pg
MCV:	52.1 fl
MCHC:	34.5 gm/dl
RBC COUNT	7.97 million cells/cmm
PLATELET COUNT:	5,08,000 cells/cmm
PCV:	41.5 %
RDW:	47.4 fl

SAMPLE NO: E

HAEMATOLOGY REPORT

Page 3 of 4



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JEEVAN REGIONAL DIAGNOSTIC

3361, OPP.LINGARAJ COLLEGE. COLLEGE ROAD BELAGAVI-590001

Patient's Name : THESIS OF DR.GEERA G GADAD Age: Sex:

Referred by : SELF

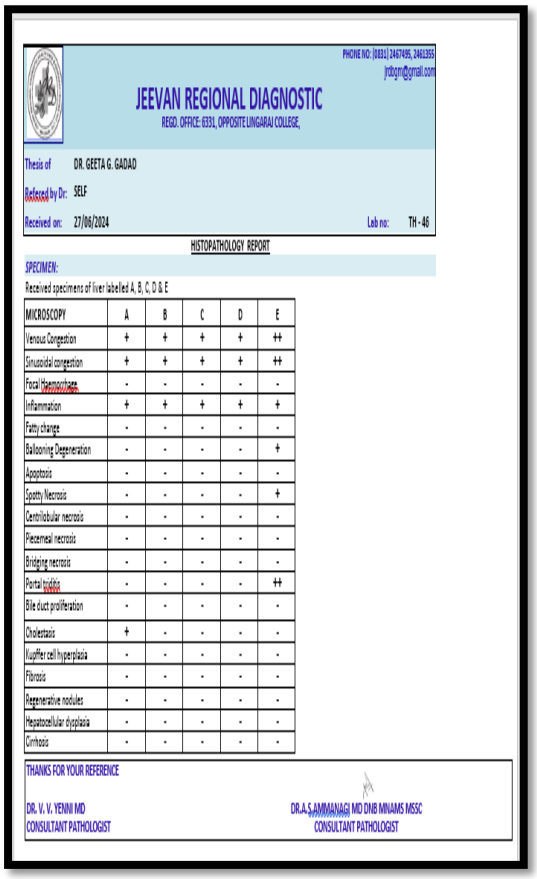
Ref No. : TR-97-24 Received On : 27/06/2024

TESTS	RESULTS
HEMOGLOBIN	12.9 g%
TOTAL WBC COUNT	19770 cells/cmm
DIFFERENTIAL COUNT:	
NEUTROPHILS	15 %
LYMPHOCYTES	74 %
EOSINOPHILS	01 %
MONOCYTES	10 %
MCH:	17.4 pg
MCV:	50.9 fl
MCHC:	34.1 gm/dl
RBC COUNT	7.43 million cells/cmm
PLATELET COUNT:	4,39,000 cells/cmm
PCV:	37.8 %
RDW:	41.1 fl

BIOCHEMIST PATHOLOGIST DR V V YENNI MD Consultant pathologist DR A S AMMANAGI MD DNB MNAMS MSSC Consultant pathologist

Thanks for your reference Checked By Typed by MANJU 04/07/2024 12:03

MAJOR ORGAN TISSUE HPR REPORTS



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jrdcgm@gmail.com

JEEVAN REGIONAL DIAGNOSTIC
REGD. OFFICE: 6331, OPPOSITE LINGARAJ COLLEGE,

Thesis of DR. GEETA G. GADAD
Refered by Dr: SELF
Received on: 27/06/2024 Lab no: TH - 46

HISTOPATHOLOGY REPORT

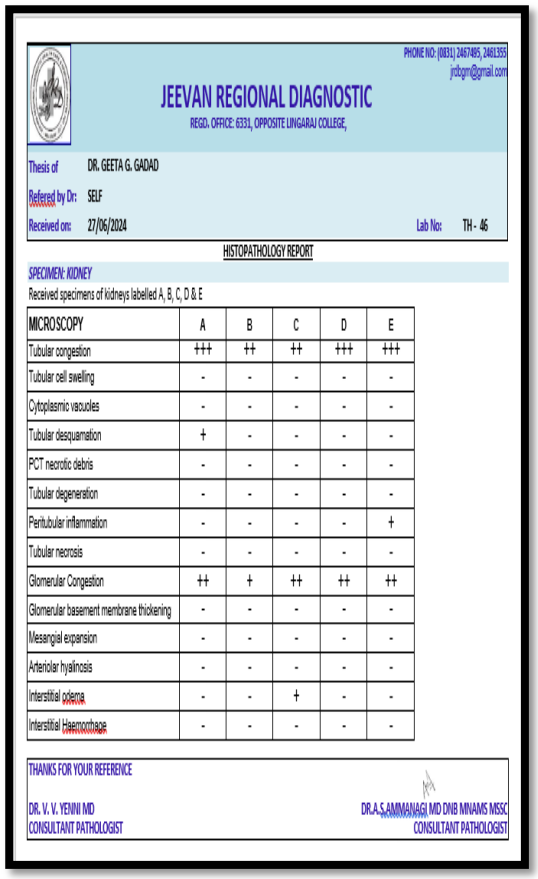
SPECIMEN:
Received specimens of liver labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
Venous Congestion	+	+	+	+	++
Sinusoidal congestion	+	+	+	+	++
Focal Haemorrhage	-	-	-	-	-
Inflammation	+	+	+	+	+
Fatty change	-	-	-	-	-
Ballooning Degeneration	-	-	-	-	+
Apoptosis	-	-	-	-	-
Spotty Necrosis	-	-	-	-	+
Centrilobular necrosis	-	-	-	-	-
Piecemeal necrosis	-	-	-	-	-
Bridging necrosis	-	-	-	-	-
Portal triditis	-	-	-	-	++
Bile duct proliferation	-	-	-	-	-
Cholestasis	+	-	-	-	-
Kupffer cell hyperplasia	-	-	-	-	-
Fibrosis	-	-	-	-	-
Regenerative nodules	-	-	-	-	-
Hepatocellular dysplasia	-	-	-	-	-
Cirrhosis	-	-	-	-	-

THANKS FOR YOUR REFERENCE

DR. V. V. YENNI MD
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HISTOPATHOLOGY REPORT

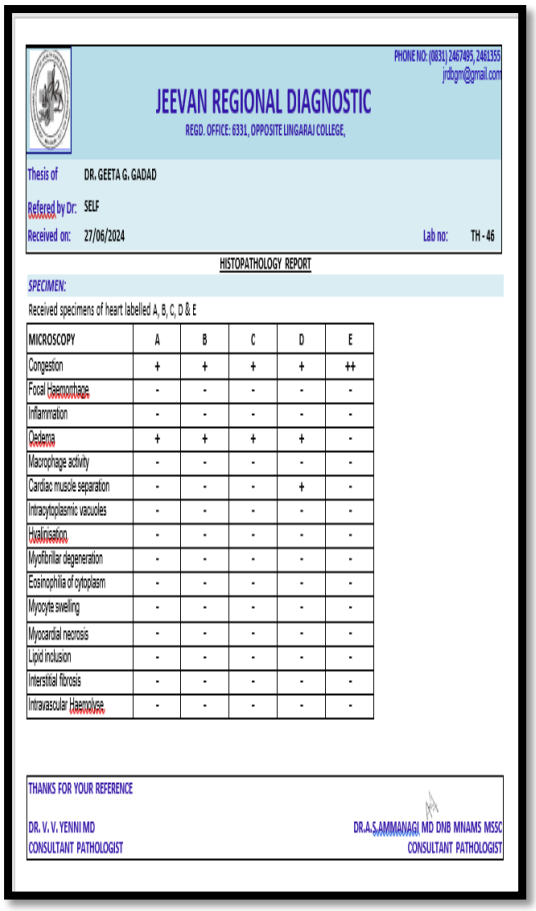
SPECIMEN: KIDNEY
Received specimens of kidneys labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
Tubular congestion	+++	++	++	+++	+++
Tubular cell swelling	-	-	-	-	-
Cytoplasmic vacuoles	-	-	-	-	-
Tubular desquamation	+	-	-	-	-
PCT necrotic debris	-	-	-	-	-
Tubular degeneration	-	-	-	-	-
Peritubular inflammation	-	-	-	-	+
Tubular necrosis	-	-	-	-	-
Glomerular Congestion	++	+	++	++	++
Glomerular basement membrane thickening	-	-	-	-	-
Mesangial expansion	-	-	-	-	-
Arteriolar hyalinosis	-	-	-	-	-
Interstitial oedema	-	-	+	-	-
Interstitial Haemorrhage	-	-	-	-	-

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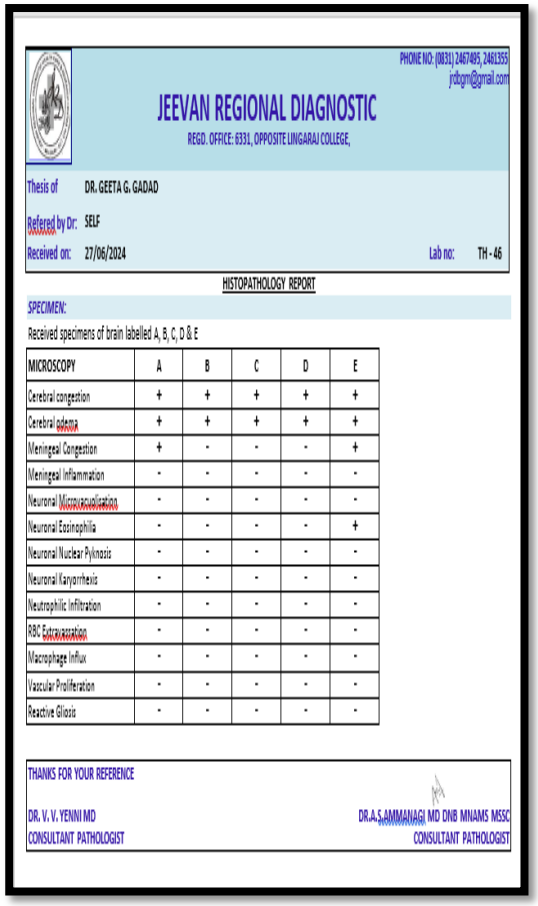
SPECIMEN:
Received specimens of heart labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
Congestion	+	+	+	+	++
Focal Haemorrhage	-	-	-	-	-
Inflammation	-	-	-	-	-
Oedema	+	+	+	+	-
Macrophage activity	-	-	-	-	-
Cardiac muscle separation	-	-	-	+	-
Intracytoplasmic vacuoles	-	-	-	-	-
Hyalinisation	-	-	-	-	-
Myofibrillar degeneration	-	-	-	-	-
Eosinophilia of cytoplasm	-	-	-	-	-
Myocyte swelling	-	-	-	-	-
Myocardial necrosis	-	-	-	-	-
Lipid inclusion	-	-	-	-	-
Interstitial fibrosis	-	-	-	-	-
Intravascular Haemolyse	-	-	-	-	-

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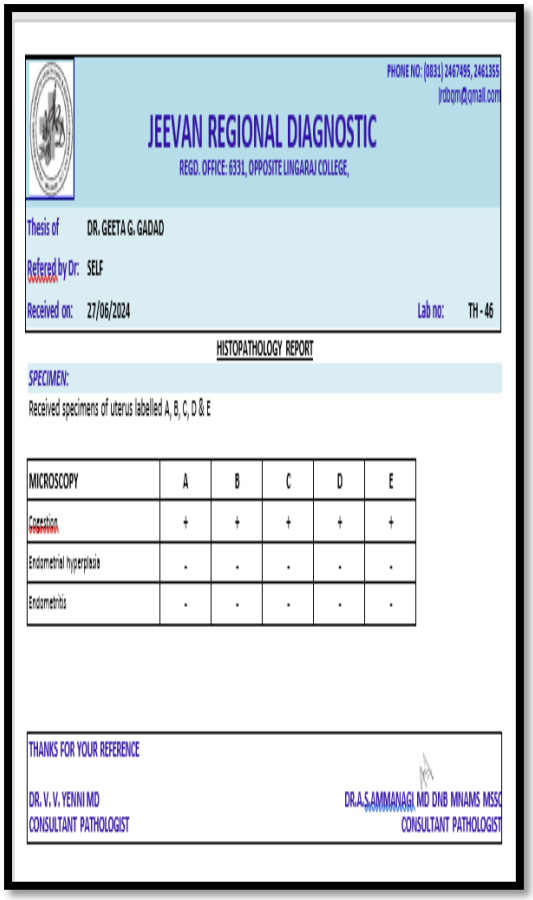
SPECIMEN:
Received specimens of brain labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
Cerebral congestion	+	+	+	+	+
Cerebral oedema	+	+	+	+	+
Meningeal Congestion	+	-	-	-	+
Meningeal Inflammation	-	-	-	-	-
Neuronal Microvacuolation	-	-	-	-	-
Neuronal Eosinophilia	-	-	-	-	+
Neuronal Nuclear Pyknosis	-	-	-	-	-
Neuronal Karyorrhexis	-	-	-	-	-
Neutrophilic infiltration	-	-	-	-	-
RBC Extravasation	-	-	-	-	-
Macrophage Influx	-	-	-	-	-
Vascular Proliferation	-	-	-	-	-
Reactive Gliosis	-	-	-	-	-

THANKS FOR YOUR REFERENCE

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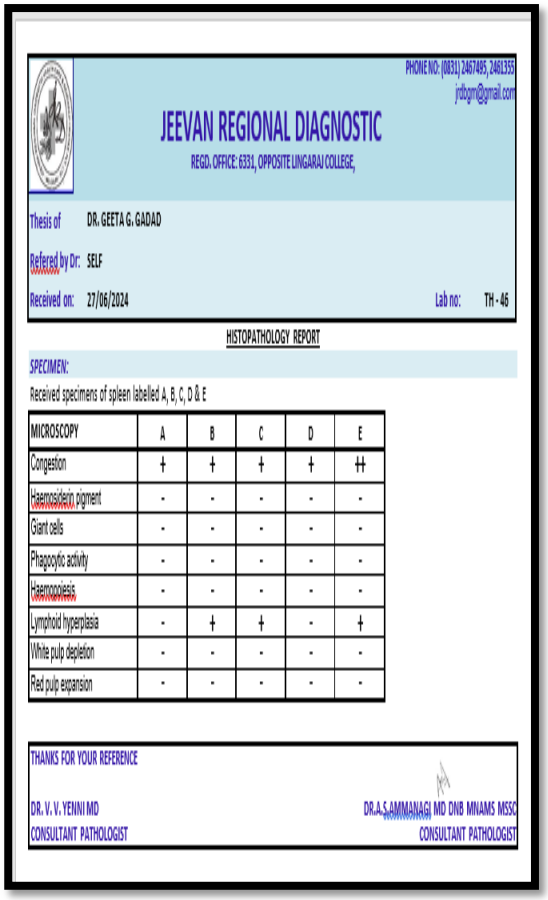
SPECIMEN:
Received specimens of uterus labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
Congestion	+	+	+	+	+
Endometrial hyperplasia	-	-	-	-	-
Endometritis	-	-	-	-	-

THANKS FOR YOUR REFERENCE

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HISTOPATHOLOGY REPORT

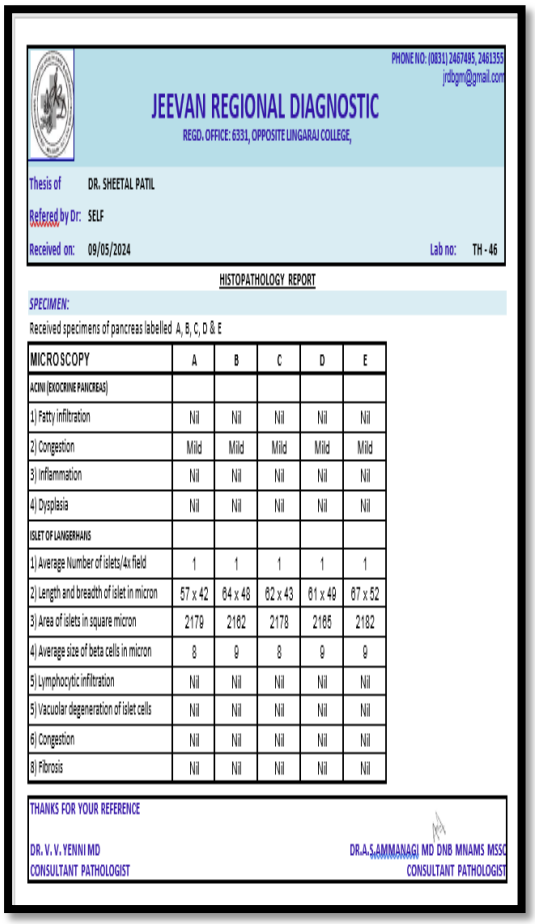
SPECIMEN:
Received specimens of spleen labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
Congestion	+	+	+	+	++
Haemosiderin pigment	-	-	-	-	-
Giant cells	-	-	-	-	-
Phagocytic activity	-	-	-	-	-
Haemopoiesis	-	-	-	-	-
Lymphoid hyperplasia	-	+	+	-	+
White pulp depletion	-	-	-	-	-
Red pulp expansion	-	-	-	-	-

THANKS FOR YOUR REFERENCE

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Thesis of DR. SHEETAL PATIL
Refered by Dr: SELF
Received on: 09/05/2024 Lab no: TH - 46

HISTOPATHOLOGY REPORT

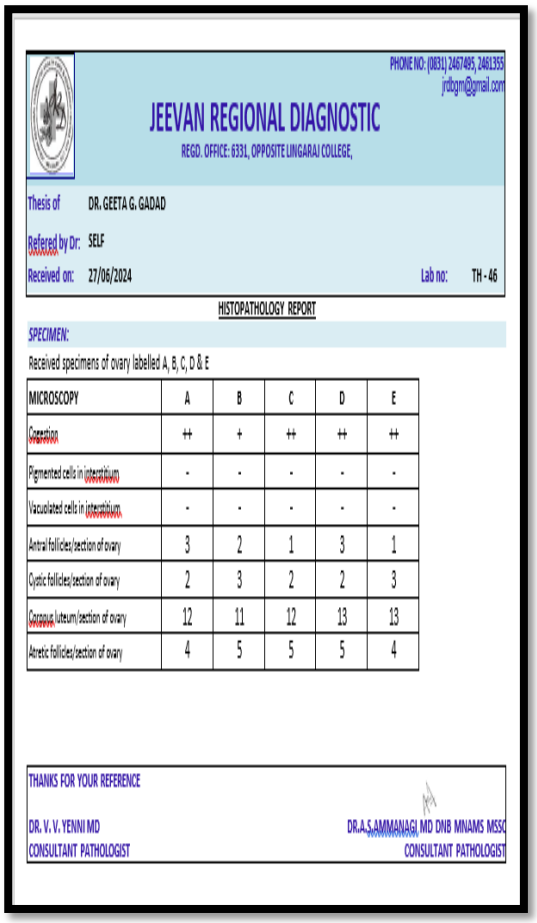
SPECIMEN:
Received specimens of pancreas labelled A, B, C, D & E

MICROSCOPY	A	B	C	D	E
ACINI (EXOCRINE PANCREAS)					
1) Fatty infiltration	Nil	Nil	Nil	Nil	Nil
2) Congestion	Mild	Mild	Mild	Mild	Mild
3) Inflammation	Nil	Nil	Nil	Nil	Nil
4) Dysplasia	Nil	Nil	Nil	Nil	Nil
ISLET OF LANGERHANS					
1) Average Number of islets/4x field	1	1	1	1	1
2) Length and breadth of islet in micron	57 x 42	64 x 48	62 x 43	61 x 49	67 x 52
3) Area of islets in square micron	2179	2162	2178	2165	2182
4) Average size of beta cells in micron	8	9	8	9	9
5) Lymphocytic infiltration	Nil	Nil	Nil	Nil	Nil
5) Vacuolar degeneration of islet cells	Nil	Nil	Nil	Nil	Nil
6) Congestion	Nil	Nil	Nil	Nil	Nil
8) Fibrosis	Nil	Nil	Nil	Nil	Nil

THANKS FOR YOUR REFERENCE

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Refered by Dr: SELF
Received on: 27/06/2024 Lab no: TH - 46

HISTOPATHOLOGY REPORT

SPECIMEN:
Received specimens of ovary labelled A, B, C, D & E

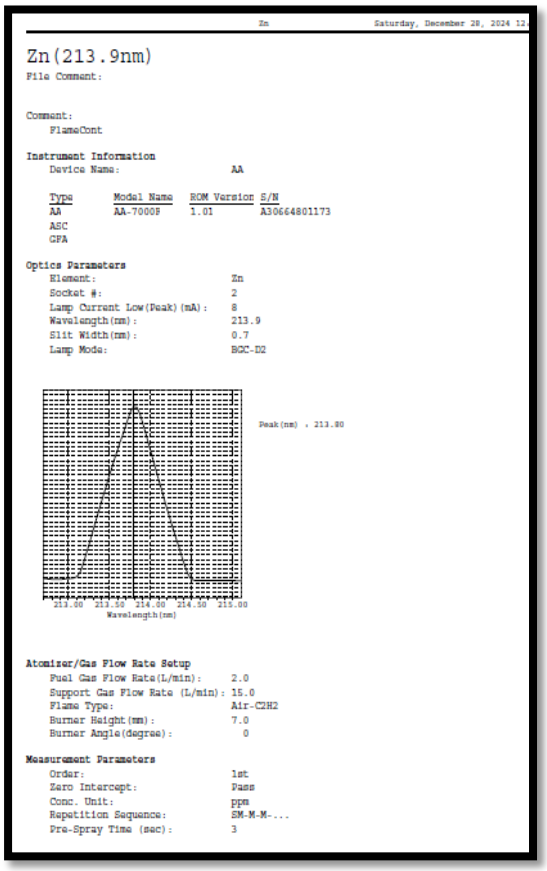
MICROSCOPY	A	B	C	D	E
Congestion	++	+	++	++	++
Pigmented cells in interstitium	-	-	-	-	-
Vacuolated cells in interstitium	-	-	-	-	-
Antral follicles/section of ovary	3	2	1	3	1
Cystic follicles/section of ovary	2	3	2	2	3
Corpus luteum/section of ovary	12	11	12	13	13
Atretic follicles/section of ovary	4	5	5	5	4

THANKS FOR YOUR REFERENCE

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ANNEXURE 08: BIOAVAILABILITYSTUDY AAS RESULTS



Zn Saturday, December 28, 2024 12:

Zn(213.9nm)

File Comment:

Comment:
FlameCont

Instrument Information

Device Name: AA

Type	Model Name	ROM Version	S/N
AA	AA-7000F	1.01	A30664801173
ASC			
GFA			

Optics Parameters

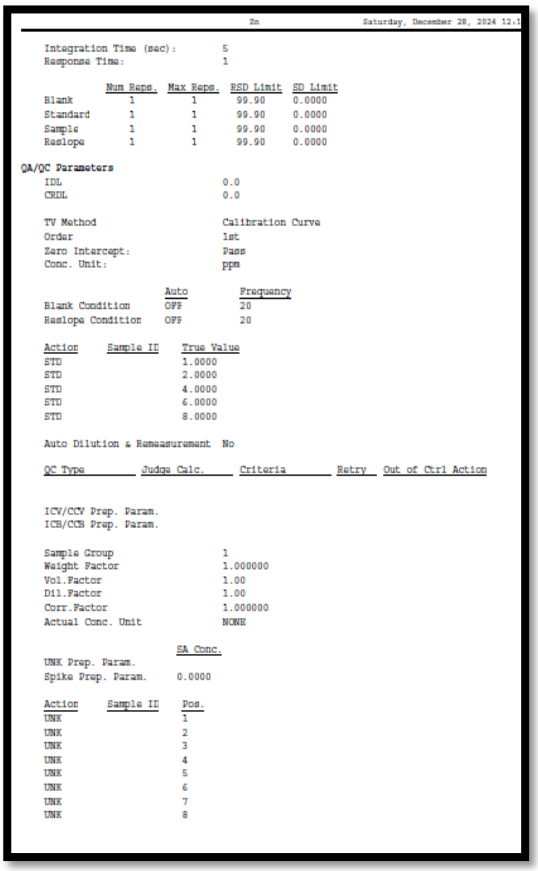
Element:	Zn
Socket #:	2
Lamp Current Low(Peak)(mA):	8
Wavelength(nm):	213.9
Slit Width(nm):	0.7
Lamp Mode:	BGC-D2

Atomizer/Gas Flow Rate Setup

Fuel Gas Flow Rate(L/min):	2.0
Support Gas Flow Rate (L/min):	15.0
Flame Type:	Air-C2H2
Burner Height(mm):	7.0
Burner Angle(degree):	0

Measurement Parameters

Order:	1st
Zero Intercept:	Pass
Conc. Unit:	ppm
Repetition Sequence:	SM-M-M-...
Pre-Spray Time (sec):	3



Zn Saturday, December 28, 2024 12:1

Integration Time (sec):	5
Response Time:	1

	Num Reps.	Max Reps.	RSD Limit	SD Limit
Blank	1	1	99.90	0.0000
Standard	1	1	99.90	0.0000
Sample	1	1	99.90	0.0000
Reslope	1	1	99.90	0.0000

QA/QC Parameters

IDL	0.0
CRDL	0.0
TV Method	Calibration Curve
Order	1st
Zero Intercept:	Pass
Conc. Unit:	ppm

	Auto	Frequency
Blank Condition	OFF	20
Reslope Condition	OFF	20

Action	Sample ID	True Value
STD		1.0000
STD		2.0000
STD		4.0000
STD		6.0000
STD		8.0000

Auto Dilution & Remeasurement No

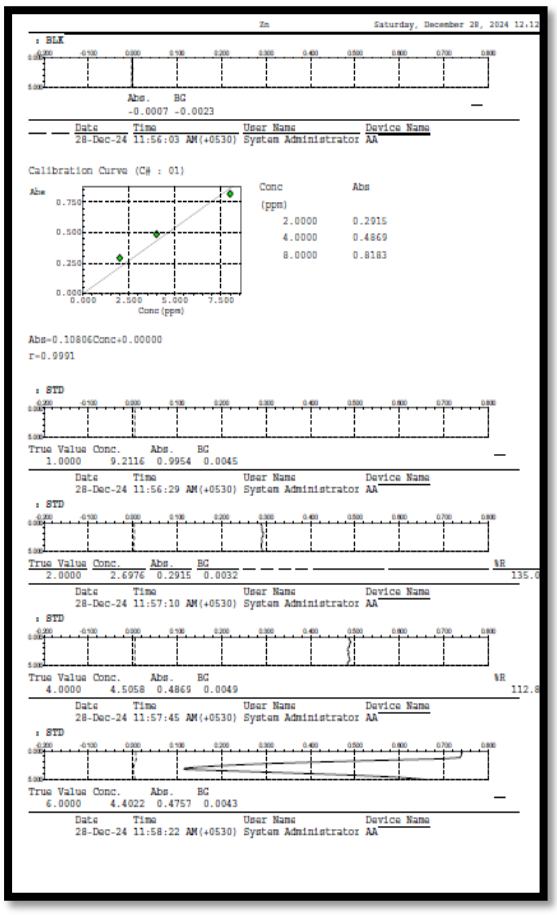
QC Type	Judge Calc.	Criteria	Retry	Out of Ctrl Action

ICV/CCV Prep. Param.
ICB/CCB Prep. Param.

Sample Group	1
Weight Factor	1.000000
Vol.Factor	1.00
Dil.Factor	1.00
Corr.Factor	1.000000
Actual Conc. Unit	NONE

	SA Conc.
UNK Prep. Param.	
Spike Prep. Param.	0.0000

Action	Sample ID	Pos.
UNK		1
UNK		2
UNK		3
UNK		4
UNK		5
UNK		6
UNK		7
UNK		8



Zn Saturday, December 28, 2024 12:12:

: BLK

Abs. BC
-0.0007 -0.0023

Date	Time	User Name	Device Name
28-Dec-24	11:56:03 AM(+0530)	System Administrator	AA

Calibration Curve (C# : 01)

Conc (ppm)	Abs
2.0000	0.2915
4.0000	0.4869
8.0000	0.8183

Abs=0.10806Conc+0.00000
r=0.9991

: STD

True Value	Conc.	Abs.	BC
1.0000	9.2116	0.9954	0.0045

Date	Time	User Name	Device Name
28-Dec-24	11:56:29 AM(+0530)	System Administrator	AA

: STD

True Value	Conc.	Abs.	BC	%R
2.0000	2.6976	0.2915	0.0032	135.0

Date	Time	User Name	Device Name
28-Dec-24	11:57:10 AM(+0530)	System Administrator	AA

: STD

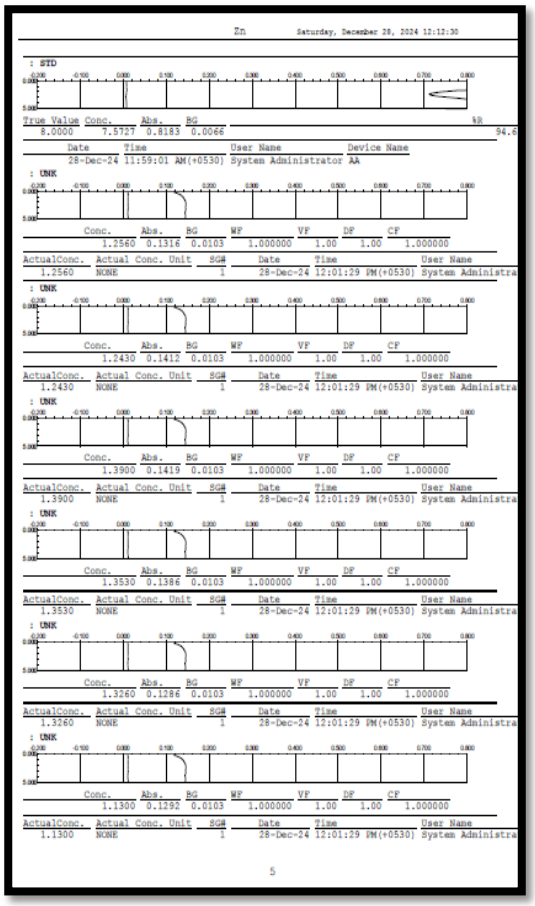
True Value	Conc.	Abs.	BC	%R
4.0000	4.5058	0.4869	0.0049	112.6

Date	Time	User Name	Device Name
28-Dec-24	11:57:45 AM(+0530)	System Administrator	AA

: STD

True Value	Conc.	Abs.	BC
6.0000	4.4022	0.4757	0.0043

Date	Time	User Name	Device Name
28-Dec-24	11:58:22 AM(+0530)	System Administrator	AA



Zn Saturday, December 28, 2024 12:12:30

: STD

True Value	Conc.	Abs.	BC	%R
8.0000	7.5727	0.8183	0.0066	94.6

Date	Time	User Name	Device Name
28-Dec-24	11:59:01 AM(+0530)	System Administrator	AA

: UNK

Conc.	Abs.	BC	WF	VF	DF	CF
1.2560	0.1316	0.0103	1.000000	1.00	1.00	1.000000

ActualConc.	Actual Conc. Unit	SG#	Date	Time	User Name
1.2560	NONE	1	28-Dec-24	12:01:29 PM(+0530)	System Administra

: UNK

Conc.	Abs.	BC	WF	VF	DF	CF
1.2430	0.1412	0.0103	1.000000	1.00	1.00	1.000000

ActualConc.	Actual Conc. Unit	SG#	Date	Time	User Name
1.2430	NONE	1	28-Dec-24	12:01:29 PM(+0530)	System Administra

: UNK

Conc.	Abs.	BC	WF	VF	DF	CF
1.3900	0.1419	0.0103	1.000000	1.00	1.00	1.000000

ActualConc.	Actual Conc. Unit	SG#	Date	Time	User Name
1.3900	NONE	1	28-Dec-24	12:01:29 PM(+0530)	System Administra

: UNK

Conc.	Abs.	BC	WF	VF	DF	CF
1.3530	0.1386	0.0103	1.000000	1.00	1.00	1.000000

ActualConc.	Actual Conc. Unit	SG#	Date	Time	User Name
1.3530	NONE	1	28-Dec-24	12:01:29 PM(+0530)	System Administra

: UNK

Conc.	Abs.	BC	WF	VF	DF	CF
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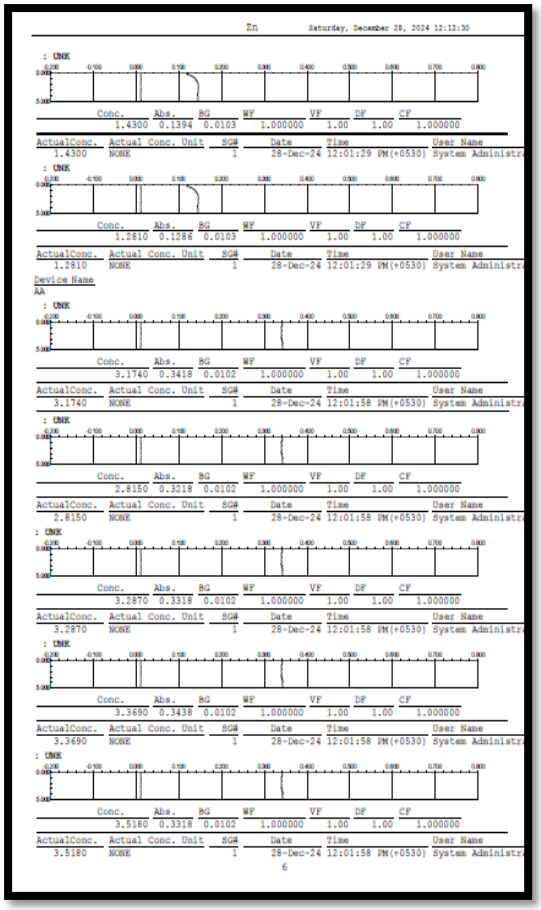
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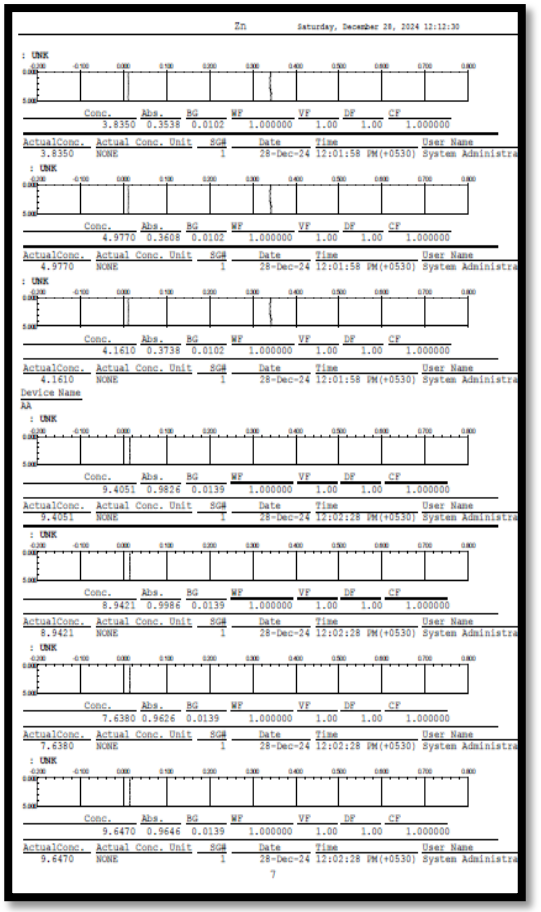
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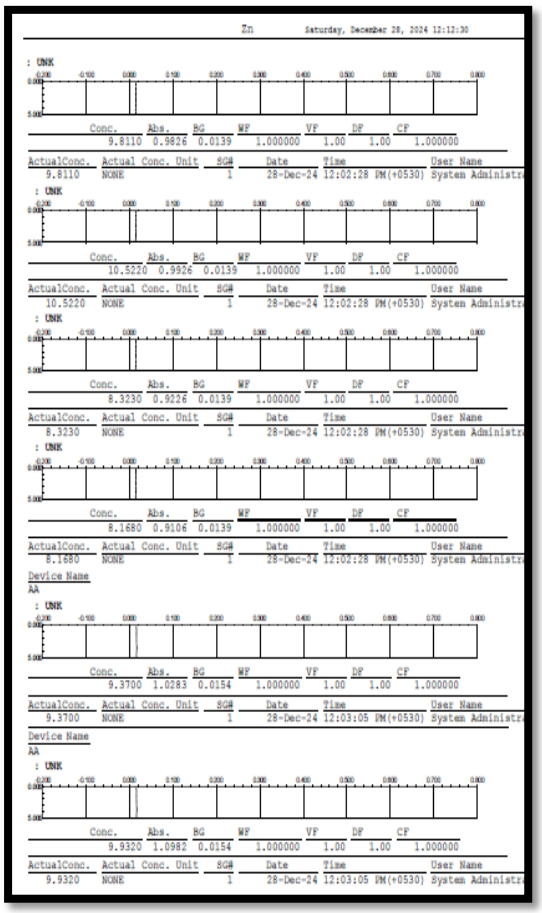
Conc.	Abs.	BC	WF	VF	DF	CF
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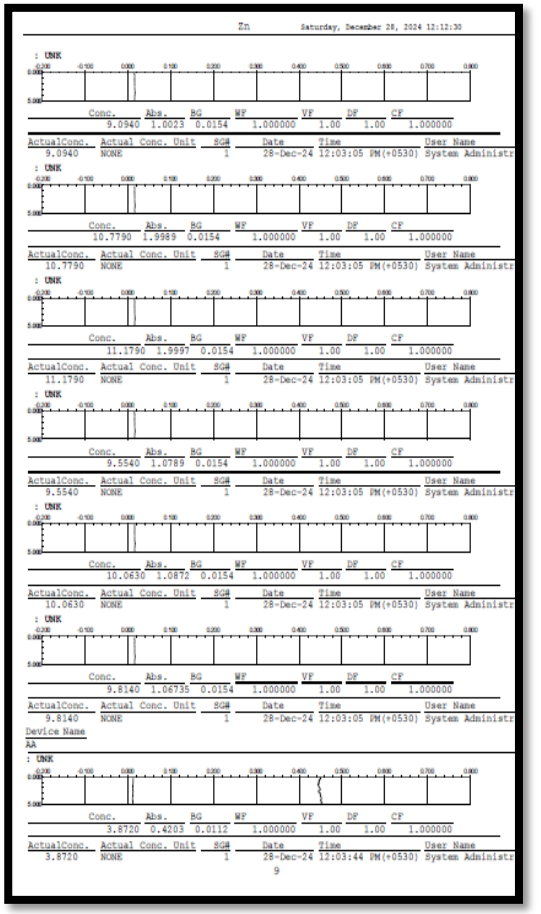
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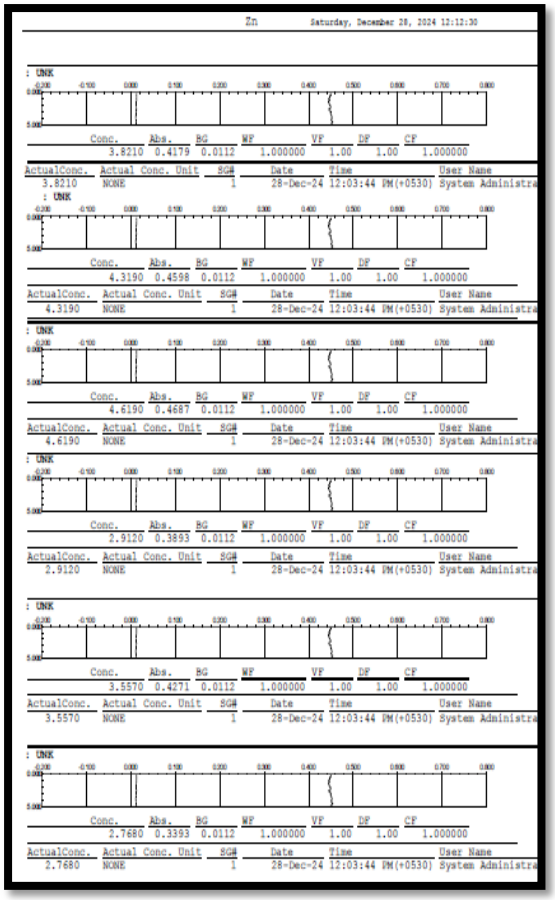
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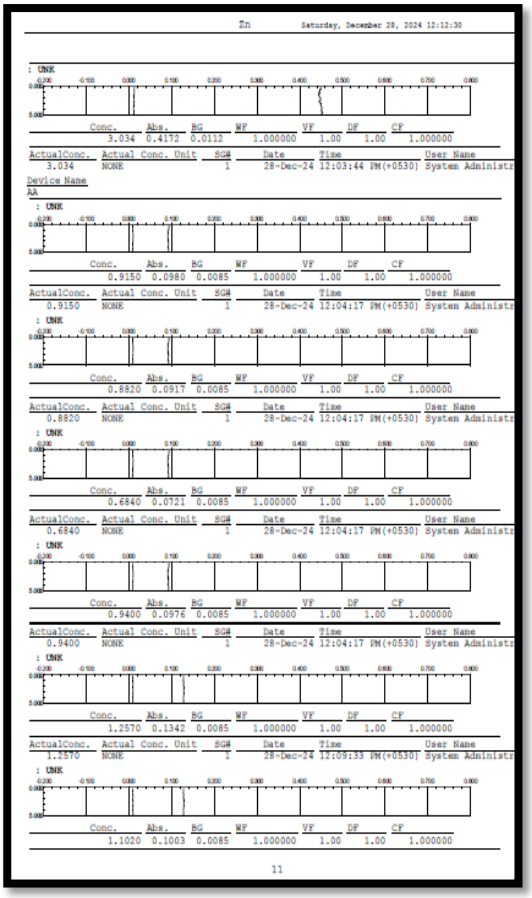

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Saturday, December 28, 2024 12:12:30
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ActualConc. Actual Conc. Unit SG# Date Time User Name
1.4300 NONE 1 28-Dec-24 12:01:29 PM(+0530) System Administr
: UNK
Conc. Abs. BG WF VF DF CF
1.2810 0.1286 0.0103 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
1.2810 NONE 1 28-Dec-24 12:01:29 PM(+0530) System Administr
Device Name
AA
: UNK
Conc. Abs. BG WF VF DF CF
3.1740 0.3418 0.0102 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
3.1740 NONE 1 28-Dec-24 12:01:58 PM(+0530) System Administr
: UNK
Conc. Abs. BG WF VF DF CF
2.8150 0.3218 0.0102 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
2.8150 NONE 1 28-Dec-24 12:01:58 PM(+0530) System Administr
: UNK
Conc. Abs. BG WF VF DF CF
3.2870 0.3318 0.0102 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
3.2870 NONE 1 28-Dec-24 12:01:58 PM(+0530) System Administr
: UNK
Conc. Abs. BG WF VF DF CF
3.3690 0.3438 0.0102 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
3.3690 NONE 1 28-Dec-24 12:01:58 PM(+0530) System Administr
: UNK
Conc. Abs. BG WF VF DF CF
3.5180 0.3318 0.0102 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
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6

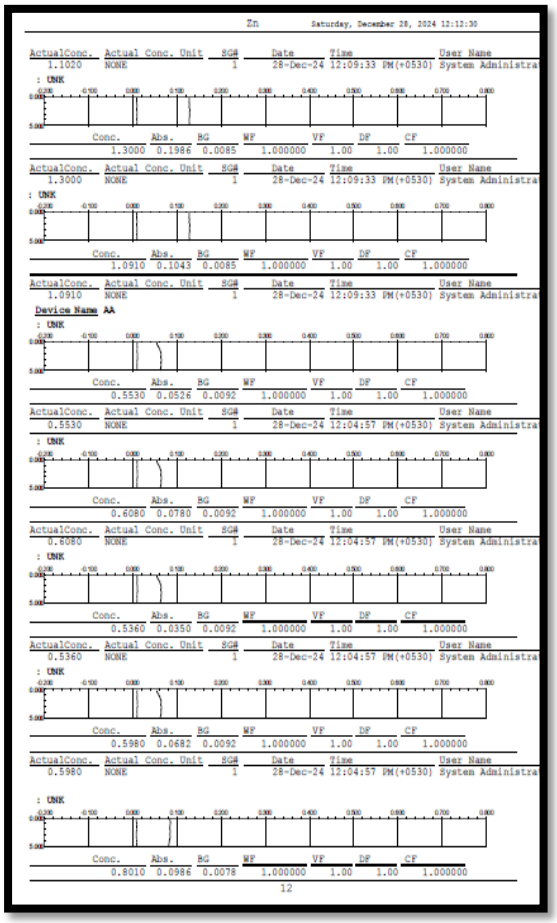

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: UNK
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ActualConc. Actual Conc. Unit SG# Date Time User Name
4.9770 NONE 1 28-Dec-24 12:01:58 PM(+0530) System Administra
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4.1610 NONE 1 28-Dec-24 12:01:58 PM(+0530) System Administra
Device Name
AA
: UNK
Conc. Abs. BG WF VF DF CF
9.4051 0.9826 0.0139 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
9.4051 NONE 1 28-Dec-24 12:02:28 PM(+0530) System Administra
: UNK
Conc. Abs. BG WF VF DF CF
8.9421 0.9986 0.0139 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
8.9421 NONE 1 28-Dec-24 12:02:28 PM(+0530) System Administra
: UNK
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7.6380 0.9626 0.0139 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
7.6380 NONE 1 28-Dec-24 12:02:28 PM(+0530) System Administra
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Conc. Abs. BG WF VF DF CF
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7

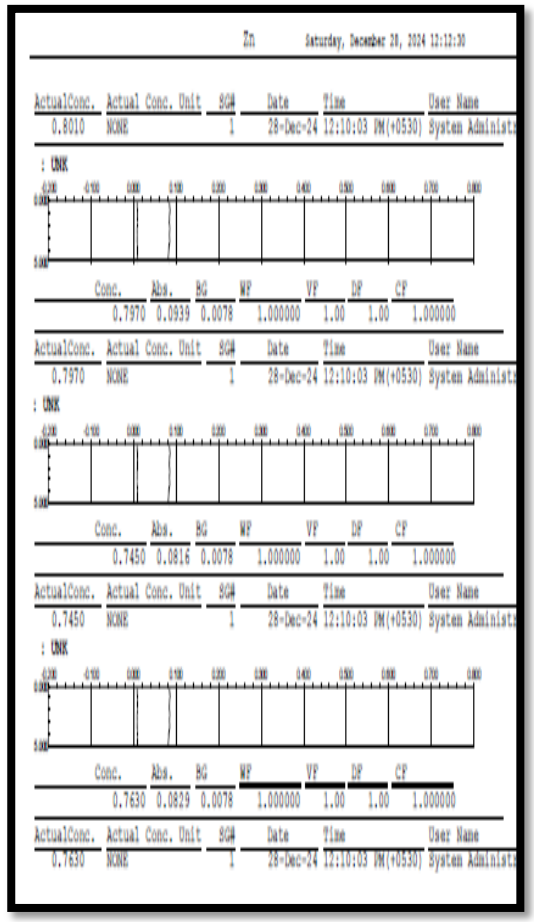

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: UNK
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Device Name
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9.9320 NONE 1 28-Dec-24 12:03:05 PM(+0530) System Administr


Zn
Saturday, December 28, 2024 12:12:30
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ActualConc. Actual Conc. Unit SG# Date Time User Name
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Device Name
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3.8720 0.4203 0.0112 1.000000 1.00 1.00 1.000000
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9


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Saturday, December 28, 2024 12:12:30
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2.9120 NONE 1 28-Dec-24 12:03:44 PM(+0530) System Administra
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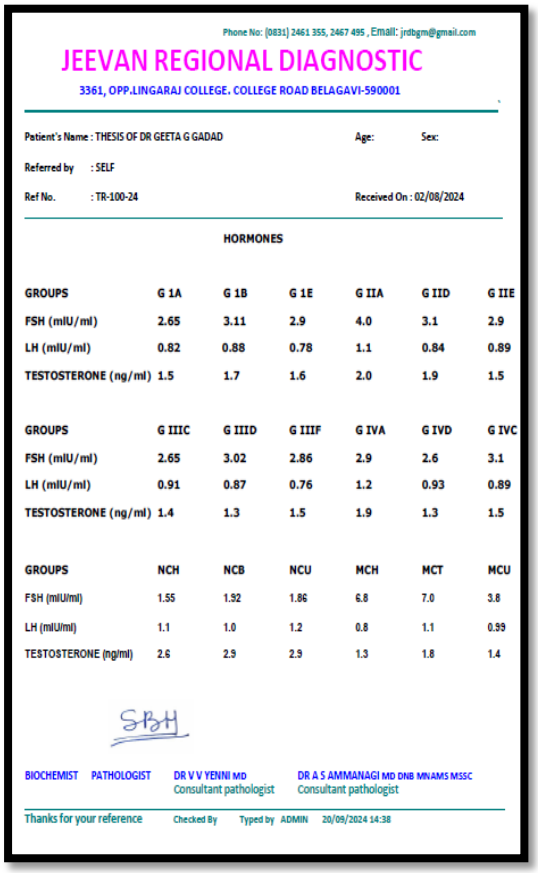

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Device Name
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0.9150 NONE 1 28-Dec-24 12:04:17 PM(+0530) System Administr
: UNK
Conc. Abs. BG WF VF DF CF
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0.8820 NONE 1 28-Dec-24 12:04:17 PM(+0530) System Administr
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11


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Device Name AA
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: UNK
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0.6080 0.0780 0.0092 1.000000 1.00 1.00 1.000000
ActualConc. Actual Conc. Unit SG# Date Time User Name
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: UNK
Conc. Abs. BG WF VF DF CF
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ActualConc. Actual Conc. Unit SG# Date Time User Name
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: UNK
Conc. Abs. BG WF VF DF CF
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0.5980 NONE 1 28-Dec-24 12:04:57 PM(+0530) System Administra
: UNK
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12


Zn
Saturday, December 28, 2024 12:12:30
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0.7450 NONE 1 28-Dec-24 12:10:03 PM(+0530) System Administ
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ActualConc. Actual Conc. Unit SG# Date Time User Name
0.7630 NONE 1 28-Dec-24 12:10:03 PM(+0530) System Administ

ANNEXURE 09: SPERMATOGENIC ACTIVITY STUDY REPORTS

BIOCHEMICAL PARAMETER REPORTS



Phone No: (0831) 2461 355, 2467 495 , Email: jrdbgm@gmail.com

JEEVAN REGIONAL DIAGNOSTIC

3361, OPP.LINGARAJ COLLEGE. COLLEGE ROAD BELAGAVI-590001

Patient's Name : THESIS OF DR GEETA G GADAD Age: Sex:

Referred by : SELF

Ref No. : TR-100-24 Received On : 02/08/2024

HORMONES

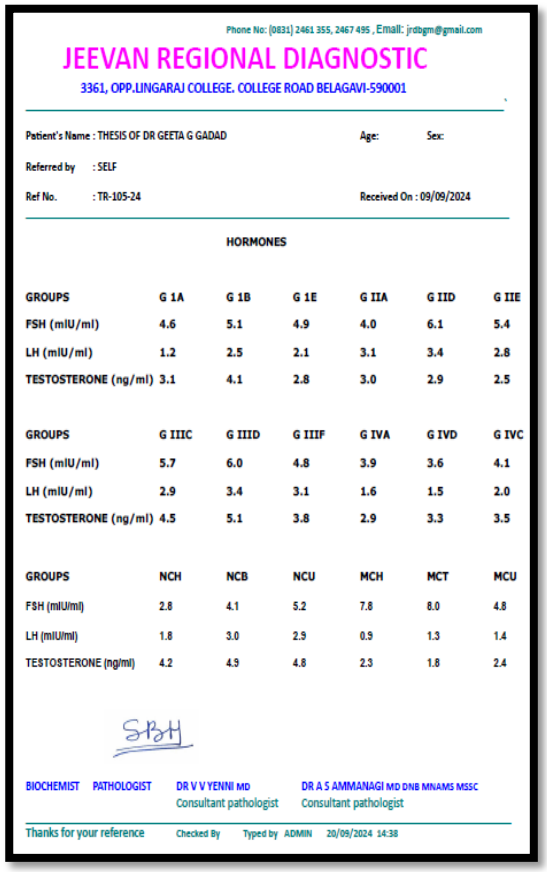
GROUPS	G 1A	G 1B	G 1E	G IIA	G IID	G IIE
FSH (mIU/ml)	2.65	3.11	2.9	4.0	3.1	2.9
LH (mIU/ml)	0.82	0.88	0.78	1.1	0.84	0.89
TESTOSTERONE (ng/ml)	1.5	1.7	1.6	2.0	1.9	1.5

GROUPS	G IIIC	G IIID	G IIIF	G IVA	G IVD	G IVC
FSH (mIU/ml)	2.65	3.02	2.86	2.9	2.6	3.1
LH (mIU/ml)	0.91	0.87	0.76	1.2	0.93	0.89
TESTOSTERONE (ng/ml)	1.4	1.3	1.5	1.9	1.3	1.5

GROUPS	NCH	NCB	NCU	MCH	MCT	MCU
FSH (mIU/ml)	1.55	1.92	1.86	6.8	7.0	3.8
LH (mIU/ml)	1.1	1.0	1.2	0.8	1.1	0.99
TESTOSTERONE (ng/ml)	2.6	2.9	2.9	1.3	1.8	1.4

BIOCHEMIST PATHOLOGIST DR V V YENNI MD Consultant pathologist DR A S AMMANAGI MD DNB MNAMS MSSC Consultant pathologist

Thanks for your reference Checked By Typed by ADMIN 20/09/2024 14:38



Phone No: (0831) 2461 355, 2467 495 , Email: jrdbgm@gmail.com

JEEVAN REGIONAL DIAGNOSTIC

3361, OPP.LINGARAJ COLLEGE. COLLEGE ROAD BELAGAVI-590001

Patient's Name : THESIS OF DR GEETA G GADAD Age: Sex:

Referred by : SELF

Ref No. : TR-105-24 Received On : 09/09/2024

HORMONES

GROUPS	G 1A	G 1B	G 1E	G IIA	G IID	G IIE
FSH (mIU/ml)	4.6	5.1	4.9	4.0	6.1	5.4
LH (mIU/ml)	1.2	2.5	2.1	3.1	3.4	2.8
TESTOSTERONE (ng/ml)	3.1	4.1	2.8	3.0	2.9	2.5

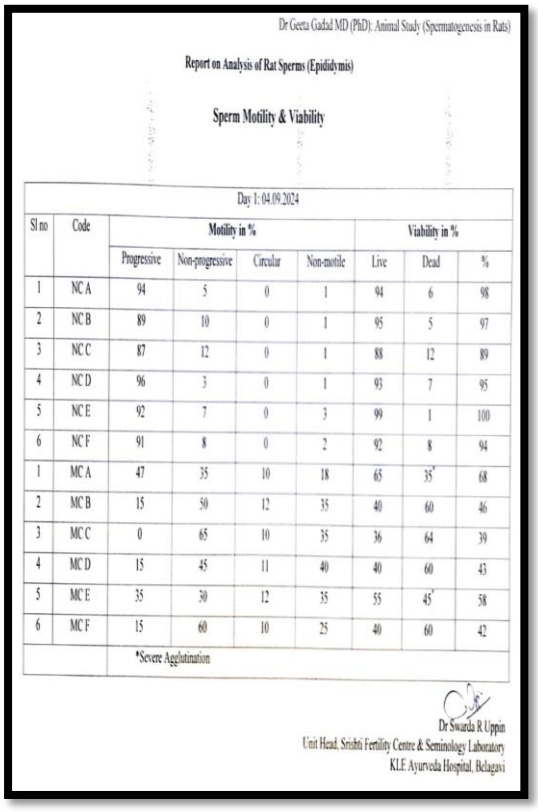
GROUPS	G IIIC	G IIID	G IIIF	G IVA	G IVD	G IVC
FSH (mIU/ml)	5.7	6.0	4.8	3.9	3.6	4.1
LH (mIU/ml)	2.9	3.4	3.1	1.6	1.5	2.0
TESTOSTERONE (ng/ml)	4.5	5.1	3.8	2.9	3.3	3.5

GROUPS	NCH	NCB	NCU	MCH	MCT	MCU
FSH (mIU/ml)	2.8	4.1	5.2	7.8	8.0	4.8
LH (mIU/ml)	1.8	3.0	2.9	0.9	1.3	1.4
TESTOSTERONE (ng/ml)	4.2	4.9	4.8	2.3	1.8	2.4

BIOCHEMIST PATHOLOGIST DR V V YENNI MD Consultant pathologist DR A S AMMANAGI MD DNB MNAMS MSSC Consultant pathologist

Thanks for your reference Checked By Typed by ADMIN 20/09/2024 14:38

SPERM PARAMETERS REPORT



Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

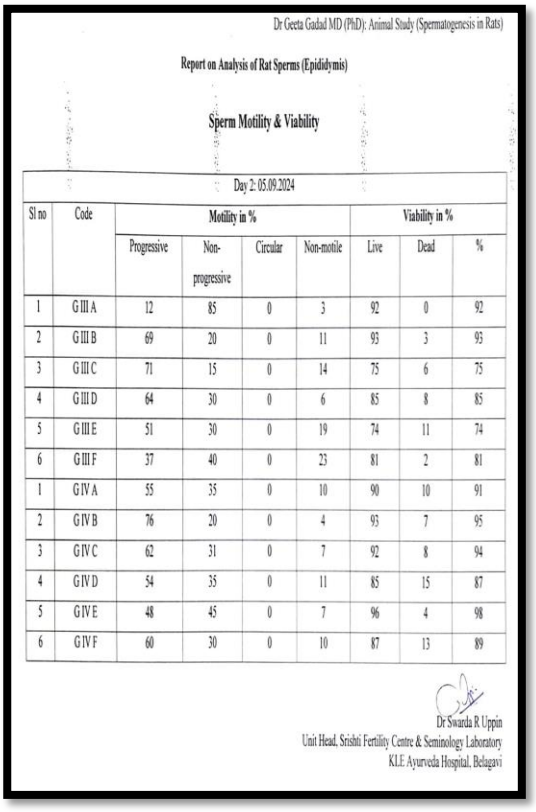
Report on Analysis of Rat Sperms (Epididymis)

Sperm Motility & Viability

Day 1: 04.09.2024

Sl no	Code	Motility in %				Viability in %		
		Progressive	Non-progressive	Circular	Non-motile	Live	Dead	%
1	NC A	94	5	0	1	94	6	98
2	NC B	89	10	0	1	95	5	97
3	NC C	87	12	0	1	88	12	89
4	NC D	96	3	0	1	93	7	95
5	NC E	92	7	0	3	99	1	100
6	NC F	91	8	0	2	92	8	94
1	MC A	47	35	10	18	65	35*	68
2	MC B	15	50	12	35	40	60	46
3	MC C	0	65	10	35	36	64	39
4	MC D	15	45	11	40	40	60	43
5	MC E	35	30	12	35	55	45*	58
6	MC F	15	60	10	25	40	60	42
		*Severe Agglutination						

Dr Swarda R Uppin
Unit Head, Srishti Fertility Centre & Seminology Laboratory
KLE Ayurveda Hospital, Belagavi



Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

Report on Analysis of Rat Sperms (Epididymis)

Sperm Motility & Viability

Day 2: 05.09.2024

Sl no	Code	Motility in %				Viability in %		
		Progressive	Non-progressive	Circular	Non-motile	Live	Dead	%
1	G III A	12	85	0	3	92	0	92
2	G III B	69	20	0	11	93	3	93
3	G III C	71	15	0	14	75	6	75
4	G III D	64	30	0	6	85	8	85
5	G III E	51	30	0	19	74	11	74
6	G III F	37	40	0	23	81	2	81
1	G IV A	55	35	0	10	90	10	91
2	G IV B	76	20	0	4	93	7	95
3	G IV C	62	31	0	7	92	8	94
4	G IV D	54	35	0	11	85	15	87
5	G IV E	48	45	0	7	96	4	98
6	G IV F	60	30	0	10	87	13	89

Dr Swarda R Uppin
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Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

Report on Analysis of Rat Sperms (Epididymis)

Sperm Motility & Viability

Day 3: 06.09.2024

Sl no	Code	Motility in %				Viability in %		
		Progressive	Non-progressive	Circular	Non-motile	Live	Dead	%
1	GI A	15	75	0	10	84	16	88
2	GI B	24	60	0	16	93	7	96
3	GI C	39	47	0	14	97	3	100
4	GI D	56	28	0	16	95	5	98
5	GI E	40	50	0	10	70	30	76
6	GI F	46	40	0	14	93	7	96
1	GII A	2	70	6	22	72	28	76
2	GII B	1	80	2	17	21	79	26
3	GII C	2	55	6	37	85	15	89
4	GII D	33	50	4	13	74	26	78
5	GII E	38	45	4	13	85	15	88
6	GII F	36	48	4	12	58	42	62

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Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

Report on Analysis of Rat Sperms (Epididymis)

Sperm Morphology

Day 1: 04.09.2024

Sl no	Code	Morphology in %						
		Normal	Bent tail	Double head	Bent neck	Flattened head	Headless	Tailness
1	NC A	92	3	0	2	2	1	0
2	NC B	90	2	1	3	3	1	0
3	NC C	93	2	0	2	2	1	0
4	NC D	91	3	0	3	1	2	0
5	NC E	89	3	0	4	2	2	0
6	NC F	92	2	0	2	3	1	0
1	MC A	35	10	1	8	10	15	21
2	MC B	40	12	0	7	12	17	12
3	MC C	42	11	0	10	15	14	8
4	MC D	45	8	1	11	10	18	7
5	MC E	38	10	0	9	13	20	10
6	MC F	40	12	0	10	12	20	6

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Report on Analysis of Rat Sperms (Epididymis)

Sperm Morphology

Day 2: 05.09.2024

Sl no	Code	Morphology in %						
		Normal	Bent tail	Double head	Bent neck	Flattened head	Headless	Tailness
1	GIII A	75	6	0	6	8	3	2
2	GIII B	80	4	0	5	7	2	2
3	GIII C	72	6	0	7	9	4	2
4	GIII D	70	7	0	8	7	5	3
5	GIII E	78	5	0	5	8	2	2
6	GIII F	73	5	0	6	10	3	3
1	GIV A	83	5	0	4	5	2	1
2	GIV B	85	4	0	3	5	2	1
3	GIV C	81	6	0	4	5	3	1
4	GIV D	80	5	0	5	4	3	1
5	GIV E	84	4	0	3	4	2	1
6	GIV F	79	6	0	5	6	2	2

Dr Swarda R Uppin
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KLE Ayurveda Hospital, Belagavi

Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

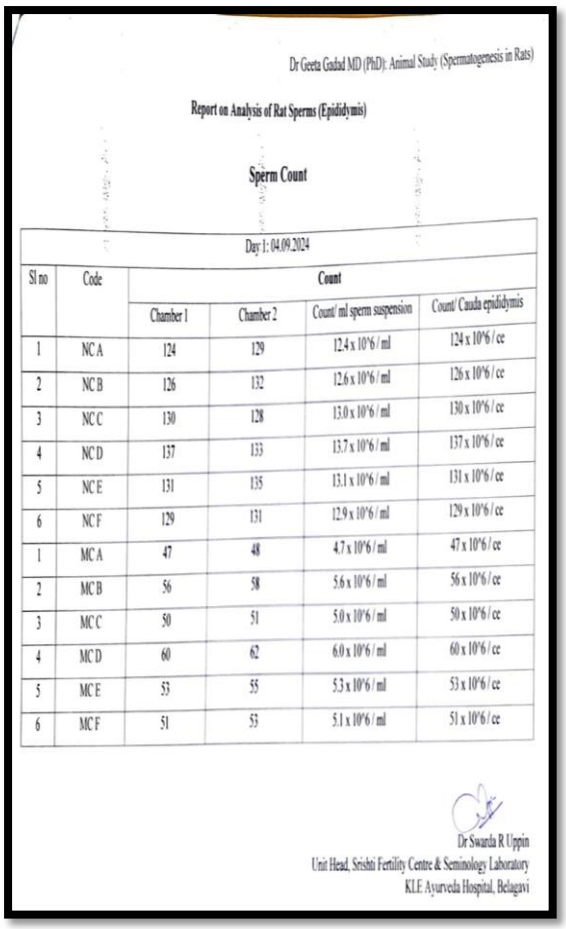
Report on Analysis of Rat Sperms (Epididymis)

Sperm Morphology

Day 3: 06.09.2024

Sl no	Code	Morphology in %						
		Normal	Bent tail	Double head	Bent neck	Flattened head	Headless	Tailness
1	GI A	46	10	2	8	18	12	4
2	GI B	73	6	0	5	10	5	1
3	GI C	76	5	0	6	8	4	1
4	GI D	78	7	1	4	6	3	1
5	GI E	72	6	0	8	9	4	1
6	GI F	82	6	0	3	7	1	1
1	GII A	55	8	1	6	10	12	8
2	GII B	50	9	0	7	11	10	7
3	GII C	58	7	0	5	12	9	9
4	GII D	52	8	0	6	9	10	7
5	GII E	60	7	0	5	11	10	7
6	GII F	53	8	1	6	12	10	10

Dr Swarda R Uppin
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KLE Ayurveda Hospital, Belagavi



Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

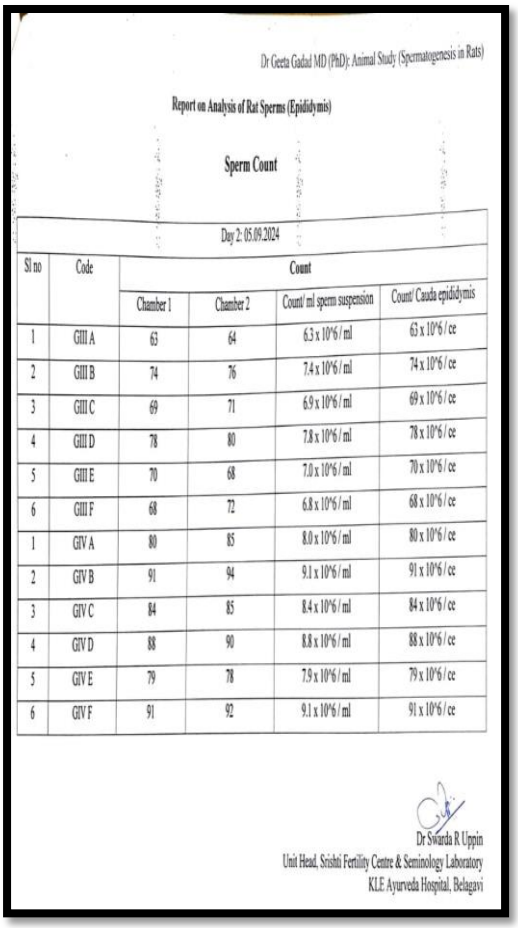
Report on Analysis of Rat Sperms (Epididymis)

Sperm Count

Day 1: 04.09.2024

Sl no	Code	Count			
		Chamber 1	Chamber 2	Count/ ml sperm suspension	Count/ Cauda epididymis
1	NC A	124	129	12.4 x 10^6 / ml	124 x 10^6 / ce
2	NC B	126	132	12.6 x 10^6 / ml	126 x 10^6 / ce
3	NC C	130	128	13.0 x 10^6 / ml	130 x 10^6 / ce
4	NC D	137	133	13.7 x 10^6 / ml	137 x 10^6 / ce
5	NC E	131	135	13.1 x 10^6 / ml	131 x 10^6 / ce
6	NC F	129	131	12.9 x 10^6 / ml	129 x 10^6 / ce
1	MC A	47	48	4.7 x 10^6 / ml	47 x 10^6 / ce
2	MC B	56	58	5.6 x 10^6 / ml	56 x 10^6 / ce
3	MC C	50	51	5.0 x 10^6 / ml	50 x 10^6 / ce
4	MC D	60	62	6.0 x 10^6 / ml	60 x 10^6 / ce
5	MC E	53	55	5.3 x 10^6 / ml	53 x 10^6 / ce
6	MC F	51	53	5.1 x 10^6 / ml	51 x 10^6 / ce

Dr Swarda R Uppin
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KLE Ayurveda Hospital, Belagavi



Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

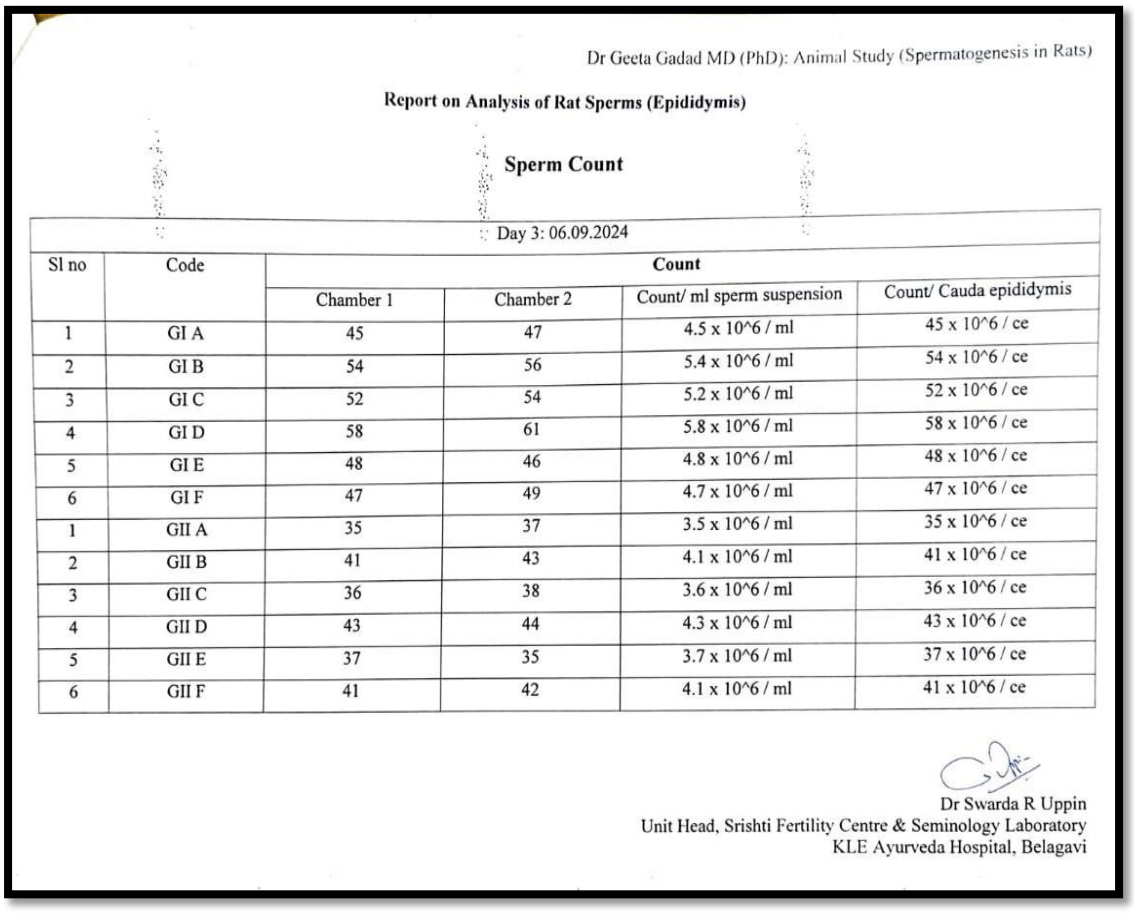
Report on Analysis of Rat Sperms (Epididymis)

Sperm Count

Day 2: 05.09.2024

Sl no	Code	Count			
		Chamber 1	Chamber 2	Count/ ml sperm suspension	Count/ Cauda epididymis
1	GIII A	63	64	6.3 x 10^6 / ml	63 x 10^6 / ce
2	GIII B	74	76	7.4 x 10^6 / ml	74 x 10^6 / ce
3	GIII C	69	71	6.9 x 10^6 / ml	69 x 10^6 / ce
4	GIII D	78	80	7.8 x 10^6 / ml	78 x 10^6 / ce
5	GIII E	70	68	7.0 x 10^6 / ml	70 x 10^6 / ce
6	GIII F	68	72	6.8 x 10^6 / ml	68 x 10^6 / ce
1	GIV A	80	85	8.0 x 10^6 / ml	80 x 10^6 / ce
2	GIV B	91	94	9.1 x 10^6 / ml	91 x 10^6 / ce
3	GIV C	84	85	8.4 x 10^6 / ml	84 x 10^6 / ce
4	GIV D	88	90	8.8 x 10^6 / ml	88 x 10^6 / ce
5	GIV E	79	78	7.9 x 10^6 / ml	79 x 10^6 / ce
6	GIV F	91	92	9.1 x 10^6 / ml	91 x 10^6 / ce

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Dr Geeta Gadad MD (PhD): Animal Study (Spermatogenesis in Rats)

Report on Analysis of Rat Sperms (Epididymis)

Sperm Count

Day 3: 06.09.2024

Sl no	Code	Count			
		Chamber 1	Chamber 2	Count/ ml sperm suspension	Count/ Cauda epididymis
1	GI A	45	47	4.5 x 10^6 / ml	45 x 10^6 / ce
2	GI B	54	56	5.4 x 10^6 / ml	54 x 10^6 / ce
3	GI C	52	54	5.2 x 10^6 / ml	52 x 10^6 / ce
4	GI D	58	61	5.8 x 10^6 / ml	58 x 10^6 / ce
5	GI E	48	46	4.8 x 10^6 / ml	48 x 10^6 / ce
6	GI F	47	49	4.7 x 10^6 / ml	47 x 10^6 / ce
1	GII A	35	37	3.5 x 10^6 / ml	35 x 10^6 / ce
2	GII B	41	43	4.1 x 10^6 / ml	41 x 10^6 / ce
3	GII C	36	38	3.6 x 10^6 / ml	36 x 10^6 / ce
4	GII D	43	44	4.3 x 10^6 / ml	43 x 10^6 / ce
5	GII E	37	35	3.7 x 10^6 / ml	37 x 10^6 / ce
6	GII F	41	42	4.1 x 10^6 / ml	41 x 10^6 / ce

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TESTICULAR HPR REPORT

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jrdbgm@gmail.com

JEEVAN REGIONAL DIAGNOSTIC

REGD. OFFICE: 6331, OPPOSITE LINGARAJ COLLEGE,
COLLEGE ROAD, BELGAUM-02.

Thesis of DR GEETA G GADAD

Refered by Dr: SELF

Received on: 06/09/2024 Lab no: TH - 64

HISTOPATHOLOGY REPORT

SPECIMEN:

Received specimen of testes labelled as 1, 2, 3, 4, 5, 6, 7, 8, 9, 19, 11, 12, 13, 14, 15, 16, 17 and 18

MICROSCOPY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Average diameter of seminiferous tubules in micron	259	232	286	270	279	281	306	300	312	307	300	314	148	145	151	342	335	349
Height of seminiferous tubule epithelium in micron	91	70	86	104	101	107	71	72	70	120	118	122	56	54	58	102	100	104
Johnsen's mean testicular biopsy score in 10 tubules	9.8	9.7	9.8	9.8	9.5	9.7	8.5	9.0	8.0	9.3	9.0	9.8	5.5	5.3	5.7	9.9	9.7	9.9
Number of Leydig cells per 20 intertubular regions	18	17	18	19	20	18	24	20	28	19	18	20	42	40	44	20	19	21
Distortion of architecture of testis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Distortion of architecture of germinal epithelium	-	-	-	-	+	+	-	-	-	-	-	-	++	-	++	-	-	-
Multinucleated giant cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Detachment of germ cells	-	-	-	-	-	+	-	-	-	-	-	-	++	+	++	-	-	-
Germ cell death	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-

THANKS FOR YOUR REFERENCE

DR. V. V. YENNI MD
CONSULTANT PATHOLOGIST

DR.A.S.AMMANAGI MD DNB MNAMS MSSC
CONSULTANT PATHOLOGIST

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JNMC Campus, Nehru Nagar, Belagavi-590 010, Karnataka State, India
☎: 0831-2444444 FAX: 0831-2493777 Web: http://www.kledeemeduniversity.edu.in E-mail: info@kledeemeduniversity.edu.in

Ref. No. KAHER/AA/24-25/D-4370 23/9/2024

Ph.D. PROGRAM

Evaluation of Six Monthly Reports submitted by the Candidate

Name of the Ph. D Scholar:	Dr. Geeta G Gadad
Name of the Guide:	Dr. Prashant G Jadar
Batch / Faculty/Category	2020-21 / Ayurveda/Part-time
Registration No.:	DO1220024

Topic: Evaluation of Potentiated of Yashada Bhasma for safety, bioavailability and spermatogenic activity

Report	Excellent	Good	Satisfactory	Poor
I			✓	
II			✓	
III			✓	
IV		✓		
V		✓		
VI			✓	
VII			✓	
VIII			✓	

DIRECTOR ACADEMIC AFFAIRS Deemed-to-be University

(Dr. Jyoti M Nagamoti)
Director, Academic Affairs

Cc to :
Dr. Prashant G Jadar , Prof. of Rasashastra and Bhaishajya Kalpana, KLE Shri. B M K Ayurveda Mahavidyalaya, Belagavi-Guide

NB: Research scholars are hereby informed in future to strictly enclose the source of documents to review half yearly reports

ANNEXURE 10: PUBLICATIONS

IJBPAS, December, 2024, 13(12): 6603-6615

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ACUTE ORAL TOXICITY EVALUATION OF *POTENTIATED YASHADA BHASMA* (ZINC OXIDE NANO-PARTICLES)

GADAD GG, JADAR PG[2*] AND PATIL SF[3]

1: Ph.D Scholar, Dept. of Rasashastra and Bhaishajya Kalpana, KAHER'S Shri B M Kankanawadi Ayurveda Mahavidhyalaya Belagavi, Karnataka, India

2: Professor, Dept. of Rasashastra and Bhaishajya Kalpana, KAHER'S Shri B M Kankanawadi Ayurveda Mahavidhyalaya Belagavi, Karnataka, India

3: Associate Professor, Department of Agada Tantra, KAHER'S Shri B M Kankanawadi Ayurveda Mahavidhyalaya Belagavi, Karnataka, India

***Corresponding Author: Dr. P. G. Jadar: E Mail: drjadar@yahoo.co.in**

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https://doi.org/10.31032/IJBPAS/2024/13.12.8557

ABSTRACT

Ayurvedic pharmaceutics deals with preparation and processing of metal and mineral based medicines. *Bhasmikarana* (incineration) is one such method to make the drug particles in nano scale aiming for better assimilation and bio-availability. Added on with such classical processing techniques, curating *bhasmikarana* along with mechanical size reduction techniques raises the question of greater bio-availability in lesser doses than recommended. Hence this paper attempted to evaluate the acute toxic effects of one such curated formula - Potentiated *Yashada Bhasma* (PYB) as a primary step in exploring the drug's safety and efficacy following OECD guidelines 420 with five female wistar rats. After confirmation of nil effects in sighting study (2000 mg PYB/kg) on one animal, rest four animals were also given the same dose and observed for adverse effects at different time points up to 14 days. On 15th day blood was collected for haematological and biochemical evaluation and later all the animals were euthanized. Major organs were dissected and sent for histopathological examination. The results revealed slight alterations in the total WBC count, neutrophils, SGOT and total bilirubin levels. Histopathological findings showed mild to moderate congestion in all major organs along with slight inflammatory changes especially in kidney, brain

and liver while symptomatically all the animals appeared completely normal at 2000mg/kg, which is almost ten times of therapeutic dose of *Yashada bhasma*. Thus, it can be concluded that PYB at 2000 mg/kg is found symptomatically safe in-spite of mild varied values with blood and histopathological investigations. However clinically this higher dose is very unlikely to occur, thus PYB can be considered safe.

Keywords: Zinc oxide nano particles, *yashada bhasma*, safety, adverse effects, metallic medicines

INTRODUCTION:

Rasashastra - the alchemy branch of Ayurveda, deals with metallic and mineral preparations to convert them into most potent, safe and assimilable forms for better therapeutic effects. The processes of drugs in *Rasashastra* are focused primarily to minimize the dose, to make the medicine completely imperceptible to taste and to produce instantaneous effects invariably **[1]**. The fact, that classically synthesized ayurvedic *bhasma* (nanoparticles) are capable enough of rendering large bio-availability in no time upon administration, is unquestionable. One of the key factors responsible for exhibition of such property is the size reduction during various processing techniques like *shodhana* (purification), *marana* (incineration), *jarana* (roasting) etc., that ultimately promotes the particles' fineness leading to increased absorption systemically **[2]**.

Yashada (zinc) in Ayurvedic system of medicine is a wonder drug in treating and managing respiratory illness, parkinsonism, diabetes etc., **[3]** Elmorsy EH *et al*. (2024) conducted an experimental study investigating the impact of high-fat diet (HFD) on spermatogenesis and testicular function in rats, with a focus on zinc's potential protective effects. The 12-week study revealed significant HFD-induced testicular dysfunction, oxidative stress, and inflammation, which were mitigated by zinc supplementation **[4]**. Seetha Chandran *et al*. (2019) assessed the anticancer efficacy of *Yashada Bhasma*, an Ayurvedic zinc-based preparation, against pancreatic cancer. Two formulations, *Parada Marita yashada Bhasma* (PMY) and *Vanaspati Jarita Marita Yashada Bhasma* (JMY), exhibited significant dose-dependent growth inhibition in human pancreatic cancer cells (MIA PaCa-2) in vitro. These findings suggest *Yashada Bhasma*'s potential as a bio-active nano-sized therapeutic agent for cancer therapy as well **[2]**.

With existing classical techniques for processing metals and minerals, additionally applying intermediate size reduction to raw mineral and metal drugs beyond already specified pharmaceutical techniques, presents an opportunity for enhanced efficacy. This

process potentially allows for lower doses, resulting in finer particles that promote swift absorption and optimal therapeutic outcomes. Alongside such creative horizons with Ayurvedic preparations, safety and adverse check is yet another important aspect to be analysed at every step made before introducing into clinical use.

Building on existing processing methods, this study investigates the acute toxicity of Potentiated *Yashada Bhasma* (PYB), a curated formulation prepared by incorporating additional size reduction techniques into the traditional manufacturing process of classical *Yashada Bhasma*. The study adheres to the guidelines outlined in OECD 420 (Organization for Economic Cooperation and Development), ensuring rigorous evaluation of PYB's safety profile.

MATERIALS AND METHODS:

PHARMACEUTICAL PART:

The test drug PYB was prepared in the laboratory of *Rasashastra* and *Bhaishajya kalpana* of KAHER's Shri BMK Ayurveda Mahavidylaya, Belagavi. For the same, raw sample of *Yashada* was procured from Shri Dhootpapeshwar Ltd (batch no. 1PRML00278/Jun/2021) with certificate of analysis having purity of 99.62% w/w. After authentication of *Yashada* (certificate no. CRF/Auth 42/2021) by the experts of the AYUSH Approved Drug Testing Laboratory at Shri BMK Ayurveda Mahavidyalaya, *shodhana* of *parada* (mercury), *gandhaka* (sulphur) and *yashada* were carried out following the classical methods specified in the text *Rasatarangini* **[5]**. *Samanya shodhana* (general purification method) and *vishesha shodhana* (specific purification method) were done only to *yashada* followed by *Jarana* (roasting) **[1]**. As a first step of potentiation, the *jarita yashada* (*yashada* that has undergone *jarana* process), was subjected to additional size reduction with the aid of a ball mill for 24 hrs thus converting granular form of *jarita yashada* into a finer one. Following the reference of another classical text *Rasachandamshu* **[6]**, the above obtained ball-milled *jarita yashada* was further subjected to thorough grinding in wet grinder for palettization with the fresh juice of aloe vera and lemon juice later was processed in Electric Muffle Furnace (EMF) for *marana* (incineration) in *gajaputa* (950°C). The same way four such consecutive *marana* were carried out in EMF in-spite of attainment of all *bhasma siddhi lakshanas* (classical characteristic tests for nano sized particles) by the end of second *marana*. This was done to aid further size reduction after the second incineration. The next step followed is subjecting the obtained sample from the above

step for instrumental screening to confirm the desired particle size and compound formation and thus arrived at the end point of preparation of PYB.

EXPERIMENTAL PART:

Animal experimentation was carried out at CPCSEA approved Animal House Facility (License no. 1017/PO/Re/S/06/CPCSEA) of KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka. Upon obtainment of ethical clearance (Approval no. BMK/IAEC/Res.No.-21/2021-11) from the Institutional Animal Ethical Committee, acute toxicity study of PYB was done following the guideline number 420 of OECD **[7]**. For the same, five female wistar rats (aged eight to 12 weeks) that weighed about 150 - 250 g were bought from licensed animal breeder at Pune. All the animals were acclimatized for five days before commencement of the experiment under specified ambient temperature (25 °C± 3°C) and humidity maintained at 40 - 60%. Rat food (Scientist's Choice rat pellets feed supplied by VRK Nutritional Solutions, Pune India) and tap water was provided for all the animals throughout the experiment.

Proper identification marks were made on each animal and one out the five animals was randomly chosen for sighting study to decide the dose for acute toxicity. Selected animal was kept under 12 hrs fasting (only water was fed) prior to the administration of sighting dose. 2000 mg/kg of PYB was administered orally (344 mg of PYB for 172 g weighed animal) along with 2 ml of 0.5% sodium CMC (carboxy-methyl cellulose) solution. Three hours of fasting was maintained post administration of sighting dose. Observations on signs of toxicity, changes in skin/fur, changes in eyes, mucus membrane, somatomotor activity, behavior pattern, tremor, convulsions, salivation, diarrhea, lethargy, sleep and coma were monitored after 2, 4, 6, 8, 12, 24 and 48 hours of sighting dose administration. After confirming the absence of any such sign in the animal, the same dose (2000 mg/kg) was decided as the dose for acute toxicity study.

For the same, the remaining four animals were made to fast for 12 hours before commencement and later administered with 2000 mg/kg of PYB orally with 0.5% sodium CMC solution. Observations regarding signs of toxicity, changes in skin/fur, changes in eyes, mucus membrane, somatomotor activity, behavior pattern, tremor, convulsions, salivation, diarrhea, lethargy, sleep, coma were monitored at 2, 4, 6, 8, 12, 24 and 48 hours post administration of toxic dose. Observations regarding signs of toxicity, clinical signs and mortality were

monitored at least once daily for 14 days for all the animals including sighting study animal. Any signs of abnormal behavior or toxicity were scored and recorded on scale 0 to 5. Body weight was recorded on days 1, 7, 14 & 15 after dosing. On 15th day blood was withdrawn from retro-orbital vein of all the five animals and sent for assessing haematological and biochemical parameters after storing in EDTA non EDTA bulbs. Later all the animals were euthanized by inhalation of di-ethyl ether and all the major organs (ovaries, uterus, fallopian tubes, kidney, spleen, pancreas, heart, liver and brain) were dissected by taking mid-line incision in the abdomen, later weighed and then sent for examination of histopathological changes.

RESULTS:

Results of the sighting study indicated no evidence of adverse toxicity in the test animal. This finding was corroborated by the acute toxicity study, where no subjects exhibited adverse effects at the same PYB dose. Behavioral assessments revealed no alterations throughout the observation period. Moreover, all animals exhibited gradual weight gain by day 15 as summarized in **Table 1**. Additional results included onset of increased food consumption within 48 hrs of dosing and faecal colour change to light yellow with moderate softening that lasted up to one day.

Results of both biochemical as well as haematological parameters are shown in **Tables 2 and 3** respectively. It can be seen that none of the parameters are found to be alarmingly increased in all the test animals in-spite of gradual weight gain. Even when the values are to be compared between individual animals no any significant change seem to be present. There was not much of notable change in the weight of major organs as well **(Table 4)**.

The histopathological examination revealed a range of changes across major organs. Congestion was a common finding, affecting the ovaries, brain, heart, kidney and spleen **(Figure 01: (a) to (h))**. The brain also displayed mild oedema, inflammation, and mild neuronal eosinophilia. The heart showed cardiac muscle separation, while the kidneys suffered tubular desquamation. Liver damage included inflammation, ballooning degeneration, spotty necrosis, and cholestasis. Additionally, the spleen exhibited mild to moderate congestion and lymphoid hyperplasia.

Table 1: Weight of the animals during the study

Sl. No.	Animals	Weight of the animals in gram			
		Day 1	Day 7	Day 14	Day 15
1.	Tail - A	172	176	184	185
2.	Head - B	166	175	190	192
3.	Left arm- C	165	172	180	185
4.	Right arm- D	155	167	182	186
5.	Unmarked - E	153	156	169	173

Table 2: Effect of PYB on biochemical parameters of all animals

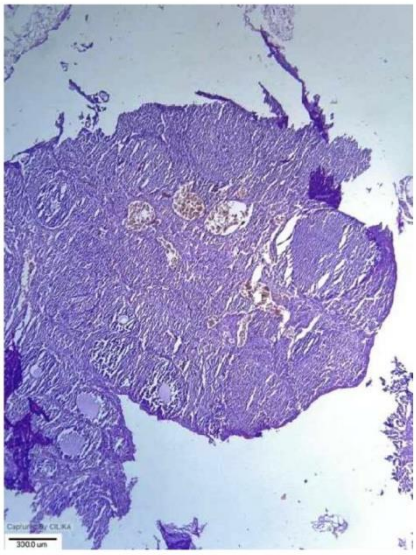
Parameter	A	B	C	D	E
Total bilirubin (mgs%)	0.4	0.4	0.5	0.4	0.5
Direct bilirubin (mgs%)	0.1	0.1	0.1	0.1	0.1
SGOT (U/L)	44	29	46	25	34
SGPT (U/L)	37	41	34	42	43
Alkaline phosphatase (U/L)	140	60	55	32	110
Total protein (gm%)	8.6	8.1	8.5	8	7.5
Albumin (gm%)	4.6	4	4.5	4.1	3.7
Urea (mgs%)	29	42	36	30	35
Creatinine (mgs%)	0.4	0.5	0.4	0.6	0.5

Table 3: Effect of PYB on haematological parameters of all animals

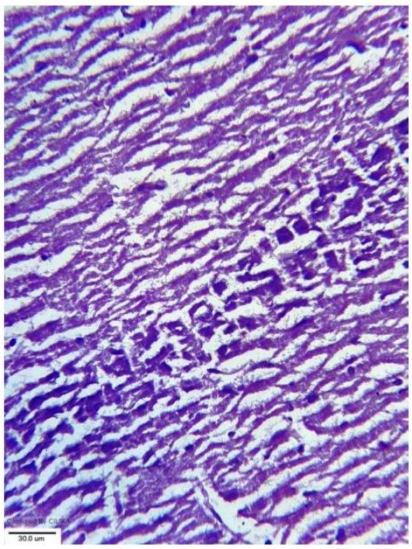
Parameter	A	B	C	D	E
Hemoglobin (gm%)	14.3	13.4	14.3	14.3	12.9
Total WBC (10^3 cells/cu.mm)	15.22	27.60	11.23	8.03	19.77
Neutrophils (%)	20	84	15	26	15
Lymphocytes (%)	71	40	76	68	74
Eosinophils (%)	01	02	00	01	01
Monocytes (%)	08	00	09	05	10
MCH (pg)	18	18.5	17.6	18	17.4
MCV (fl)	53.3	52.6	51.2	52.1	50.9
MCHC (gm/l)	33.8	35.1	34.3	34.5	34.1
RBC (million cells/cu.mm)	7.93	7.28	8.16	7.97	7.43
Platelets (10^5 cells/cu.mm)	5.24	6.01	4.84	5.08	4.39
PCV (%)	42.3	38.3	41.8	41.5	37.8
RDW (fl)	50.9	44.8	48.7	47.4	41.1

Table 4: Weight of major organs of all the animals

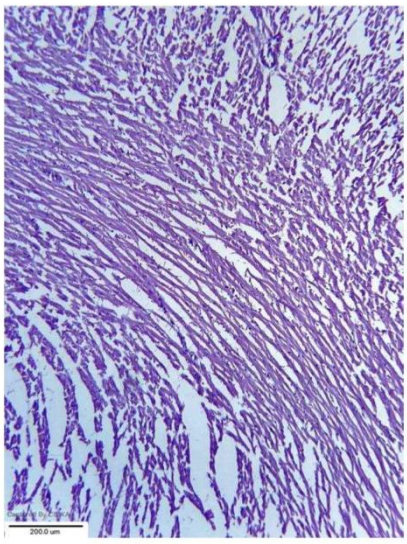
Organs	A	B	C	D	E
Ovaries, Uterus and Tubes	0.59 gm	1.16 gm	0.97 gm	0.75 gm	0.72 gm
Kidney	1.36 gm	1.18 gm	1.38 gm	1.24 gm	1.44 gm
Spleen	0.77 gm	0.59 gm	0.61 gm	0.59 gm	0.91 gm
Pancreas	0.69 gm	0.51 gm	0.48 gm	0.58 gm	0.46 gm
Heart	0.71 gm	1.00 gm	0.67 gm	0.89 gm	0.82 gm
Liver	7.19 gm	7.53 gm	8.18 gm	8.24 gm	7.64 gm
Brain	1.51 gm	1.47 gm	1.60 gm	1.52 gm	1.18 gm



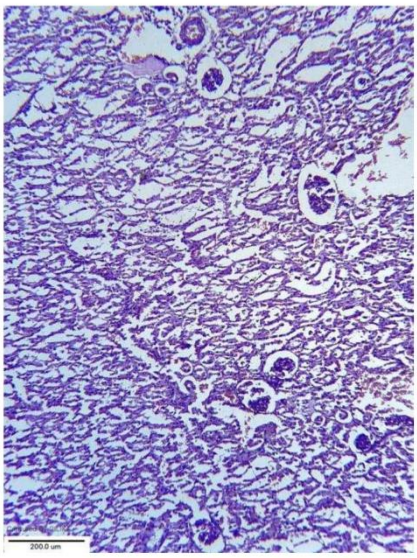
(a)



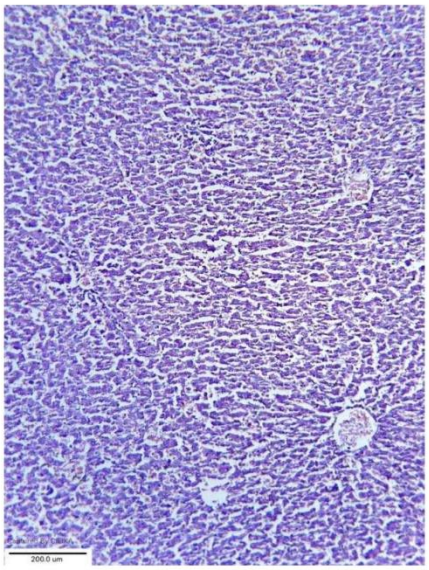
(b)



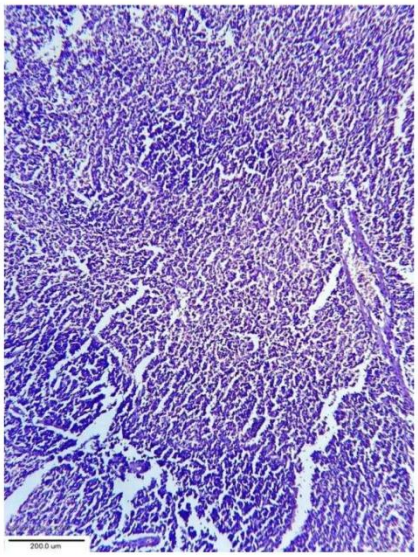
(c)



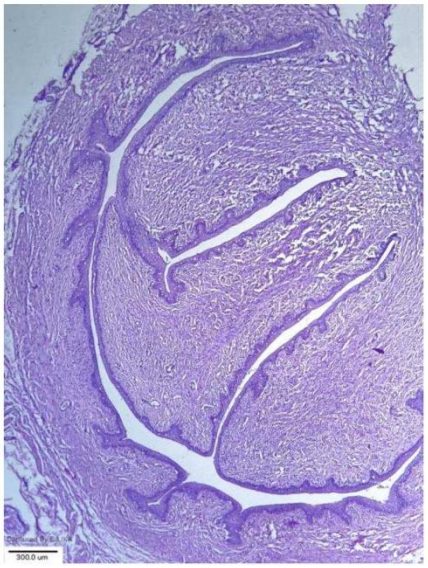
(d)



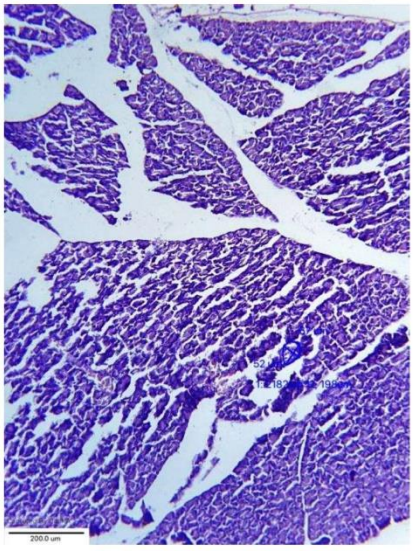
(e)



(f)



(g)



(h)

Figure 1: Images of histopathological examination of major organs. (a) Ovary (b) Brain (c) Heart (d) Kidney (e) Liver (f) Spleen (g) Uterus (h)

DISCUSSION:

There has always been a concern of heavy metal toxicity for metallic and mineral based preparations. To overcome such fear and also to gain clarity with respect to careful dose fixation in clinical practice, assessing the toxic potential of any formulation is invariably essential. In the present study, toxicity of PYB was assessed as there was additional size reduction hours compared classical *bhasma* preparation. The OECD Guidelines 420 is a globally recognized standard protocol for acute oral toxicity testing, ensuring scientific validity, animal welfare, and regulatory compliance. It provides a standardized, step-by-step approach to evaluate chemical toxicity, minimizing animal numbers and distress while promoting reliable and reproducible results. In rodent toxicity testing, rats are the species of choice, and females are commonly used. Research has shown that while sex-related differences in sensitivity are generally minimal, females are slightly more sensitive than males in instances where differences are observed **[7]**. Due to this reason the present study specifically employed female species to test acute toxicity. During the ethical permission approval total of 18 animals were sanctioned for any mortality during the sighting study for step down procedure as per OECD 420 guidelines. As no mortality was observed, so the study was completed with initial five animals only.

Ashish Arora *et al*., (2023) in their study explored the toxic effects of *yashada bhasma* and *yashada pushpa bhasma* in swiss albino mices at doses each 15.6 mg/kg and 78 mg/kg in comparison with control group. It was reported that *yashada bhasma* at both the doses did not show any signs of toxicity justified by insignificant variation in body weight, hematological and biochemical parameters **[8]**. The same was even found supporting with the findings of Rinku D Umrani *et al*., (2013), where traditionally prepared *yashada bhasma* was found to be non-toxic at the dose of 300 mg/kg **[9]**. Considering the data from above works, the dose for sighting study in the present attempt was arrived at 2000 mg/kg which is almost ten times the therapeutic dose (125 - 250 mg) **[10]**. Body weight fluctuations serve as a sensitive indicator of animal health. Specifically, a weight loss exceeding 10% is a reliable marker of toxicity, even in the absence of other observable adverse effects **[11]**. Present study did not show derangement in the body weight, instead has shown a steady increase. The probable reason for this weight gain can be attributed towards *yashada bhasma* effect in improvement of appetite and

use of sodium CMC for dissolving the test trug PYB in dosing. Findings made by Alper Baran *et al*., (2020) says that Sodium carboxymethyl cellulose (CMC) was found to be non-toxic in zebrafish embryos, but its increased lipid accumulation in a dose-dependent manner and altered gene expression related to obesity-linked lipid metabolism **[12]**. The second reason due to CMC can be denied to normal weights and HPC reports of liver after the dissection of animals.

The physiological range of hematological parameters - hemoglobin (mgs%), total WBC count (10^3 cells cu.mm), neutrophils (%), lymphocytes (%), eosinophils (%), monocytes (%), MCH (pg), MCV (fl), MCHC (gm/l) and RBC (million cells/cu.mm), in female wistar rats aged about eight to 12 weeks is found between the ranges 13±1 to 15±1, 7±2 to 10±3, 14±65 to 45±22, 81±6 to 43±23, 1±1 to 1±2, 2±1 to 1±1, 20±1 to 18±1, 57±4 to 60±2, 20±1 to 18±1 and 6±1 to 8±1 respectively **[13]**. Based upon the results fetched from present study we can say that almost all of the animals had normal ranges in their haematological parameters. Only the second animal's total WBC count and neutrophil is observed to be higher than the normal values. On the other hand, the normal limits of total bilirubin of female wistar rats is 0.08 - 0.24 mg/dl as reported by Suresh Patel *et al*., (2024). Normal range of total bilirubin, SGOT (U/L), SGPT (U/L), alkaline phosphatase (U/L), total protein (gm%), albumin (gm%), urea (mgs%) and creatinine (mgs%) are reported as 0.1 - 0.2, 72.94 - 204.13, 16.53 - 37.95, 36.47 - 108.52, 5.2 - 8.2, 3.7 - 5.6, 27.35 - 60.74 and 0.35 - 0.87 respectively **[14]**. Although some biochemical parameters - total bilirubin, exhibited slightly abnormal values in the present study, these variations did not translate to clinically significant adverse events or symptomatic manifestations. Prolonged stool discoloration beyond 24 hours may indicate a risk of jaundice made evident by increased total bilirubin levels. Conversely, increased food and water consumption suggests the absence of clinically manifested pathology, despite altered haematological and biochemical parameters.

With the changes found in histopathological examination of the tissues from major organs, it can be witnessed that the overall toxic effect of PYB at 2000 mg/kg is found at the range of mild to moderate severity. The increased leukocyte and neutrophils count from the hematological parameter can be substantiated with the inflammatory and congestive changes in the

brain tissues **[15]**. The neurotoxic effects of zinc at higher concentrations might have contributed to such changes histopathologically **[16]**. Toxicity effects at a low grade witnessed in the heart tissues is substantiated with previous findings of zinc causing untoward effects in the high-density lipoprotein levels and also causing elevated risks of suffering cardio-vascular diseases **[17]**. There are various researches that state the beneficial effects of zinc in managing chronic kidney disease. But owing to the caustic nature, certain zinc compounds are found suggestive of causing injuries to the renal structures in the form of interstitial nephritis and tubular necrosis **[18]**. Structural changes in the liver tissue is directly attributed towards the exposure of increased levels of zinc leading to jaundice, cholestatis and hepatic failure **[19]**. This again corroborates the elevated total bilirubin levels in all the animals. The spleen's lymphoid hyperplasia suggested immune system activation justifiable with leukocytosis observed in the second animal. The euthanizing factor also has to be considered with respect to the changes found at the tissue levels of all the major organs. When euthanasia is done with di-ethyl ether, the chemical inhalation is prone to cause tissue necrosis at brain and damages liver and kidney as well **[20]**. Hence in the present work, the range of damage seen in histopathology might have contributed by euthanasia also.

The current study focused on the acute toxicity potential of PYB. Testing of blood parameters for all the animals at baseline could have fetched comparable data in the present work. Future research directions include conducting chronic toxicity studies on PYB or other potentiated metallic and mineral drugs to generate clinically relevant data, enhancing the safe therapeutic application of such metals.

CONCLUSION:

The present work has successfully established acute toxicity profile of PYB that was curated based on the ideology of size reduction to potentially increase bio-availability. From the obtained results it can be concluded that at the dose of 2000 mg/kg, PYB is found to be completely safe symptomatically in-spite of certain alterations in hematological, biochemical and histopathological observations. It is also to be reminded that the dose handled in present study is ten times the therapeutic dose of *yashada bhasma* and hence the scope of PYB to produce adverse toxic effects very much unlikely. Findings of the present study can be interpreted at clinical level to comply the

safety of such metal-based formulations of Ayurveda.

CONFLICTS OF INTEREST: Nil

SOURCES OF FUNDING: The work did not receive any sort of financial assistance from any body or organization.

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Comparative Pharmaceutico-Analytical Study of Classical, Yashada Bhasma and Potentiated Yashada Bhasma

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Abstract:
Bhasma in Ayurveda are nano-sized particles prepared using unique methods of incineration that are reported for increased bio-availability and therapeutic outcome, when such methods are aided with techniques to further initiate size-reduction it may give rise to therapeutically modified and potent *bhasma* than the ones prepared as per classical lines. Hence the present study attempts to curate *Potentiated Yashada Bhasma* (PYB) by altering few processing methods to the existing incineration methods of *Yashada Bhasma* (CYB) mentioned in classics. Following general and specific purification techniques, PYB was prepared by subjection of ball milling and wet grinding for specified duration as additional steps to the existing method of preparation of CYB. Both the samples prepared passed all classical tests of *bhasma*. The physico-chemical changes revealed changes in total ash, acid insoluble ash and loss on drying. Instrumental techniques using DLS detected particle size of PYB (261.1nm) much lower than CYB (696.3nm). The stability of PYB in Zeta Potential (-28mV) was found satisfactory, thus indicating proper preparation of PYB. The study suggests the effectiveness of potentiation techniques that could be adopted logically to the existing classical methods to considerably reduce operational costs and achieve better therapeutic outcomes in lower doses than required.

Key words: *Yashada, Bhasmeekarana,* Nano-particles, Zinc oxide, Potentiation**,** DLS

INTRODUCTION:

Ayurvedic Pharmaceutics deals with conversion of various raw materials like minerals, metals into bioassimiable therapeutically active compounds called as *bhasmas*.[1] These have been applied as stable formulations ranging in submicronic to nano dimension, exhibiting high bioavailability at lower dose for therapeutic treatments of various diseases.[2] The pharmaceutical processes involved in synthesis of *bhasma* are different for different metals or minerals. Variability of these procedures depends completely upon physical properties, chemical properties of metals and minerals and their targeted therapeutic action.

Nanotechnology is one among the novel area of research in modern material science.[3] Nanoparticles are frequently synthesized by either top–down or bottom–up approaches. Top– down approach is based on the mechanical methods of size reduction by breaking down the bulk materials gradually to nanosized structures. Bottom–up approaches are based on the assembly of atoms or molecules to molecular structure in nanoscale range.[4] Using these approaches a number of processes are available for the biosynthesis of nanoparticles for, e.g., reduction in solution, radiation aspired,

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electrochemical, and microwave-assisted process and recently via green chemistry route.[5] Though the similarity in approach of synthesis, all the engineered nanoparticles are not meant for human use the way *bhasma* preparations were meant due to their safety and stability issues.[6]

Yashada bhasma (zinc calyx) is one such preparation consists of various elements which are essential micronutrients responsible for various bodily functions. *Yashada bhasma* (Zinc calyx) is prepared by a series of pharmaceutical processing[7] of metallic *yashada* into submicronic to nano size range zinc oxide, suitable for therapeutic administration.[8] This emphasizes the classical concept of size reduction and potentiation. Based on this *yashada bhasma* can be further potentiated by additional size reduction methods in existing pharmaceutical processing steps in presence of herbal extracts by grinding and milling for its improved therapeutic effect. A classical method of *bhasma* preparation by *bhasmikarana* (incineration) process, which is in practice, is proved safer and efficacious. In *puti lohas* (low melting point metals) like *yashada*, *jarana* (roasting) is an additional procedure between *shodhana* (purification) and *marana* (incineration), for easy *bhasmikarana*. This can be further fine-tuned with additional dry milling to attain lesser particle size and that sample can be further taken to classical method of *bhasmikarana* by grinding and incineration. These additional size reduction methods based on classical concept are better methods to potentiate them further by characterizing them in nano range. It is well known that size reduction of particles increases solubility and hence bioavailability. Therefore, thorough *bhasmikaran*a with added size reduction procedures are expected to reduce the size of metal oxide particles enhancing their bioavailability and bioactivity. Interestingly, in modern science, several researchers have demonstrated enhanced bioavailability of nanoparticles as compared to their bulk form. For example, Ishihara et al. reported higher bioavailability of micronized zinc oxide as compared to standard zinc oxide.[9] In another report, poor water-soluble iron compounds when formulated as nanoparticles displayed oral bioavailability similar to soluble salts.[10]

Thus, it is safe to assume that *bhasma* (calyx) contain nanoparticles that lead to enhanced bioactivity. Thus, the study is aimed to potentiate *yashada bhasma* (zinc calyx) by adopting further size reduction techniques addition to classical methods and comparatively evaluate their particle size and various physico chemical properties using advanced instrumental techniques.

MATERIALS AND METHODS:

Materials:

The raw material *Yashada* (raw zinc) has been sourced from Dhootapapeshwar Pvt. Ltd (batch no. 1PRML00278/Jun/2021) with certificate of analysis having purity of 99.62% w/w. Additional ingredients, including *Parada* (Mercury), *Gandhaka* (Sulphur), *Tilataila* (Gingelly oil), *Kanji* (sour gruel), *Kulattha* (Horse gram) seeds, *Sudha Churna* (Limestone), and *Apamarga* (*Achyranthes aspera* Linn.) Panchanga *Churna*, were obtained from the GMP-certified KLE Ayurveda Pharmacy in Khasbag. *Takra* (Buttermilk) was prepared using milk purchased from a local cowshed and *gomutra* (cow's urine) was also collected from a local cowshed. *Kumari* (Aloe vera) was procured from the natural habitat in Belagavi. All the drugs were authentified by the experts and physico chemical analysis of raw drugs was done in the AYUSH Approved Drug Testing Laboratory at Shri BMK Ayurveda Mahavidyalaya. The instrumental characterizations were done in KLE college of Pharmacy, Nanowatts Bangalore and IIT Dharwad and IIT Bombay.

Methods:

Pharmaceutical part: This has been initiated with *samanya shodhana* (general purification) of 1kg of raw *yashada* (zinc). The raw *yashada* was placed in a *loha darvi* (iron ladle) and melted completely. The molten zinc was poured into *kanji* (sour gruel) using *pithara yantra* (Special instrument). After this, the zinc was removed, washed in warm water, dried, weighed, and pH of media was checked. This process was repeated two more times according to the traditional texts. The same purification method was followed with other media, such as, *takra* (buttermilk), *kulattha kwatha* (horse gram decoction) *gomutra* (cow urine) and *tilataila* (sesame oil) in each three times. Following this, *visesha shodhana* (special purification) was carried out using *churnodaka* (limewater) as the medium, repeating the process seven times in the same manner as for *samanya shodhana*.[7]

Jarana (Roasting) was then performed on the purified *yashada*. This was done by adding one-fourth the weight of *apamarga panchanga churna* (*Achyranthes aspera* Linn) to the purified *yashada* in a wide-mouthed iron vessel. The *churna* was gradually added to molten state of zinc and the mixture was triturated using an iron ladle. The mixture when

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turns to complete powder form, it was then made a heap in the center of iron vessel and covered with a *sharava* (earthen plate) and intense heat was given until at the edges of *sharava* look *jwalaangara nibham* (red hot). After this stage was reached, the fire was extinguished, and the vessel was allowed to cool naturally. The next day, after self-cooling, the contents were removed from the iron vessel and sieved to obtain a fine powder of *jarita yashada*. This *jarita yashada* was mixed with sufficient quantity of water and washed repeatedly to remove excess alkalinity by checking pH each time and it was dried to powder and used for further processing.[7] After this *jarana,* the procedure was divided into two steps as: preparation of classical *yashada bhasma* and preparation of potentiated *yashada Bhasma*

Preparation of Classical *yashada bhasma* (CYB):

To prepare *Classical yashada bhasma*, the *jarita yashada* was then weighed and placed in the center of the *khalva yantra* (grinding apparatus), where it was triturated with 1/4th quantity of *shuddha parada* (purified mercury). *shuddha gandhaka* (purified sulfur) to prepare *kajjali* (black sulfide mixture). After obtaining *kajjali siddha laxanas*, *bhavana* (wet grinding) was carried out with *kumari swarasa* (Aloe vera juice) and *nimbu swarasa* (lemon juice) until the mixture reached the proper consistency for forming *chakrikas* (small discs). These *chakrikas* approximately resembling the size of *kuppilu beeja* (3 to 5 grams), were shaped and left to dry. Once, completely dried, the *chakrikas* were arranged between two *sharavas* (earthen plates), sealed with seven layers of *kapad mitti* (cloth coated with mud), and dried in sunlight. After thorough drying, the *samputa* was subjected to *puta* (incineration) in an electric muffle furnace at 950°C by maintaining peak temperature for 15 mins. After this furnace was made off and left for self-cooling. This procedure was repeated one more times, adhering to the guidelines of *bhasma siddhi lakshanas*[11] Observations done during the preparation are tabulated in Table 1-3.

Preparation of Potentiated *Yashada bhasma* (PYB):

The same method was followed in the preparation of Potentiated *yashada bhasma*, with additional size reduction in processing with modern instruments (Ball mill and Wet grinder). The potentiated *yashada bhasma* was prepared by additional size reduction at three different steps of classical *bhasmikarana* and increased number of *puta* (Total 4) even after attainment of *bhasma siddha laxanas* after second *puta*. Observations are tabulated in Table 4-6.The interventional steps are: dry milling of *jarita yashada* using ball mill for 24 hours, dry milling of PYB *kajjali* for additional 48 hours and thorough and continuous wet grinding in wet grinder for 24 hours in each *puta* before palettization.

Analytical Part: Both the samples were subjected to classical and modern instrumental analysis.

***Bhasma pareeksha* (Classical parameters):** The classical evaluation parameters like *varna* (color), *rekhapurna* (fineness), *varitara/unama* (lightness), *nischandra* (lusterless), *niruttha* and *apunarbhava* (absence of free metal) helps to judge the *bhasma siddha laxana* (completion of calcination process).

Physico chemical analysis: To ascertain the quality of *bhasma*, based on performed procedure of incineration and check any contamination including moisture during and after the preparation of *bhasma*, basic physical tests like loss on drying, ash value, acid insoluble ash were carried out.

Instrumental Characterization: To evaluate the end products (CYB, PYB) with special reference to particle size, distribution, stability, morphology, compound formed, shape and elemental composition various instrumental analysis were done like ICPAES, DLS- Zeta potential, XRD and SEM.

OBSERVATIONS:

Table 01. Ingredients and their quantity in each *puta* of CYB

Sl. No.	*Puta*	Ingredients	Quantity
1.	1st	*Jarita yashada*	200 gm
	Puta	*Shuddha Parada*	50 gm

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		Shuddha Gandhaka	50 gm
2.	2nd *Puta*	1st *Puta Bhasma*	150 gm
		Shuddha Parada	37.5 gm
		Shuddha Gandhaka	37.5 gm

Table 02. *Bhavana dravya* quantity and weight of *chakrika* at each incineration of CYB

Sl. No	Date	Quantity of *bhavana dravya*		Hours of *bhavana*			Weight of *chakrika* in *bhasmikarana*	
		Kumari	*Nimbu*	*Kumari*	*Nimbu*	Total	Before	After
1.	1st *puta*	150 ml	150 ml	8 hrs	6 hrs	15 hrs	330 gm	150 gm
2.	2nd *puta*	100 ml	70 ml	3 hrs	3 hrs	6 hrs	270 gm	160 gm

Table 03. Observations during each incineration of CYB

Sl. No	Puta	Observations & Results
1.	**1st** *puta*	All the *chakrikas* completely baked and turned off white colour. The *chakrikas* were soft to touch and were breaking with slight difficulty. After powedring of *chakrikas*, the *bhasma* was smooth in texture. *Varitara* was not passed completely but partially passed. *Rekhapurnata* was appreciated.
2.	2nd *puta*	All the *chakrikas* were completely baked and turned into light yellow colour. The *chakrikas* softer than the 1st *puta* and can be easily broken. The texture of the *chakrikas* was soft like butter while touching. *Varitara was* completely passed. *Niruttha* and *apunarbhava* test were also passed.

Table 04. Ingredients and their quantity in each incineration of PYB

Sl. No.	*Puta*	Ingredients	Quantity
1.	1st *Puta*	*Jarita yashada*	200gm
		Shuddha Parada	50gm
		Shuddha Gandhaka	50gm
2.	2nd *Puta*	1st *Puta Bhasma*	140 gm
		Shuddha Parada	35 gm
		Shuddha Gandhaka	35 gm
3.	3rd *Puta*	2nd *Puta Bhasma*	60 gm
		Shuddha Parada	15 gm
		Shuddha Gandhaka	15 gm
4.	4th *Puta*	3rd *Puta Bhasma*	37 gm
		Shuddha Parada	9.3 gm
		Shuddha Gandhaka	9.3 gm

***Note:** Quantity of *bhasma* obtained at each *puta* was not fully taken for next *puta*, decided as per the quantity of *Sh. Parada*.

Table 05. *Bhavana dravya* quantity and weight of *chakrika* at each PYB *puta*

Sl. No	Date	Quantity of *Bhavana dravya*		Hours of *bhavana*			Weight of *Chakrika* in *Bhasmikarana*	
		Kumari	*Nimbu*	*Kumari*	*Nimbu*	Total	Before	After

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3.	1st *puta*	800ml	250ml	15hrs	9hrs	24hrs	323gm	196gm
4.	2nd *puta*	600 ml	200 ml	15hrs	9hrs	24hrs	208 gm	116 gm
5.	3rd *puta*	500 ml	170 ml	15hrs	9hrs	24hrs	87 gm	43 gm
6.	4th *puta*	200 ml	80 ml	15hrs	9hrs	24hrs	53 gm	25 gm

Table 06. Observations during each incineration

Sl. No.	*Puta*	Observations & Results
1.	**1st *Puta***	All the *chakrikas* completely baked and turned off white colour. The *chakrikas* were soft to touch and were breaking with slight difficulty. After powedring of *chakrikas*, the *bhasma* was smooth in texture. *Varitara* was not passed completely but partially passed. *Rekhapurnata* was appreciated.
2.	**2nd** *puta*	All the *chakrikas* completely baked and turned into yellow colour. The *chakrikas* were soft to touch and were breaking with slight difficulty. After powedring of *chakrikas*, the *bhasma* was smooth in texture. The colour of the *bhasma* faded when compared to the 1st *puta.* *Varitara was* passed. *Rekhapurnata* was appreciated.
3.	**3rd** *puta*	All the *chakrikas* were completely baked and turned into yellow colour. The *chakrikas* softer than the 2nd puta and can be easily broken. The texture of the *chakrikas* was very soft like butter while touching. The colour of the *bhasma* still more faded when compared to the 2nd *puta* *Varitara* completely passed. Except *niruttha* and *apunarbhava* rest all *bhasma pariksha* were done.
4.	**4th** puta	All the *chakrikas* completely baked and turned into yellow colour. The *chakrikas* softer than the 3rd *puta*. The texture of the chakrikas was softer like butter while touching when compared to 3rd *puta*. The colour of the *bhasma* still more faded when compared to the 3rd *puta* *apunarbhava* and *niruttha* were also passed.

RESULTS:

Bhasma Pareeksha

Classical *yashada bhasma* took 2 *puta* whereas Potentiated *yashada bhasma* was given 4 *puta* even after attaining *bhasma siddha laxanas* after 2nd *puta* to get additional size reduction. The observational results of classical *bhasma* parameters and organoleptic characters after each successive *puta* of both the samples are enlisted in Table 7.

Table 07. Organoleptic characters and *bhasma pareeksha*

Parameters	CYB		PYB			
	1st *puta*	2nd *puta*	1st *puta*	2nd *puta*	3rd *puta*	4th *puta*
Colour	Off white	Light yellow	Off white	Light yellow	Light yellow	Pale yellow
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless
Touch	Soft	Softer	Soft	Softer	Very soft	Very soft
Appearance	Powder	Fine powder	Powder	Fine powder	Amorphous	Amorphous
Rekhapurnatva	Positive	Positive	Positive	Positive	Positive	Positive
Varitara	Partially positive	Positive	Partially positive	Partially positive	Positive	Positive
Unama	Partially positive	Positive	Partially positive	Positive	Positive	Positive
Nishchandrata	Positive	Positive	Positive	Positive	Positive	Positive
Niruttha	Not done	Positive	Not done	Not done	Not done	Positive

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Apunarbhava	Not done	Positive	Not done	Not done	Not done	Positive

Physical Characterization

Physical parameters of both the sample are comparable with slight differences in total ash, acid insoluble ash and loss on drying. Results shows increased organic matter in PYB sample as depicted in Table 8.

Table 08. Physical parameters

Sl.No.	Physicochemical analysis	CYB	PYB
1.	Total ash	98.7%	96.2%
2.	Acid insoluble ash	52.331 %	55.24%
3.	Loss on drying	0.09 %	0.29%

Instrumental characterization

ICPAES: Elemental Analysis of *Yashada* at different steps of processing was done to analyze the percentage of major element zinc and results are depicted in Table 9. Results show that there is subsequent decrease in zinc percentage from raw *yashada* to potentiated *yashada bhasma*.

Table 09. ICPAES Results of raw, intermediate and final samples

Sl. No	***Yashada* samples**	**% of zinc**
1.	Raw *yashada*	99.8%
2.	*Shodita yashada*	92.9%
3.	*Jarita yashada*	82.2%
4.	Classical *yashada bhasma*	77.13%
5.	Potentiated *yashada bhasma*	70.29%

Particle size

Particle size analysis of CYB and PYB samples done using dynamic light scattering (DLS) method by particle size analyser (Malvern Zetasizer) are as shown in Table 10 and Fig 1 & Fig 2. Particle size findings suggest that particle size of PYB was reduced two and half time than CYB. Polydispersibility index of PYB is 0.412 which suggest uniform particle size distribution contributing to more homogeneity compared to CYB with PDI 1 which shows slightly greater heterogeneity.

Zeta potential

In present study both the samples exhibited negative zeta potentials as shown in Table 10 and Fig 3 & Fig 4, indicating that both samples are negatively charged and show good colloidal stability. The difference is very minimal. Both the samples are expected to remain well dispersed and demonstrate strong electrostatic repulsion, preventing aggregation on suspension.

Table 10. Particle size and Zeta potential Analysis

Samples	**Particle Size(nm)**	**PDI**	**Zeta potential (mV)**
CYB	696.3	1	-28.88
PYB	261.1	0.412	-28.20

***PDI- Polydispersibility index**

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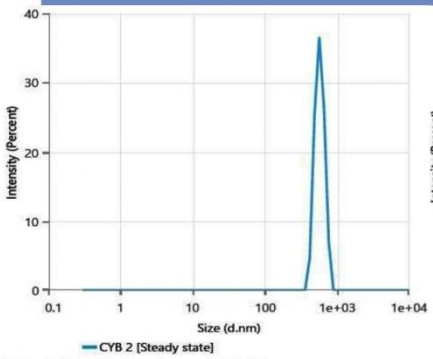


Fig.1 Particle size of CYB

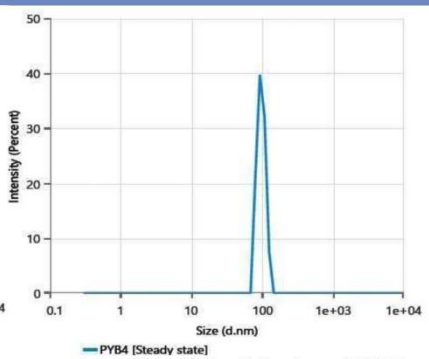


Fig. 2 Particle size of PYB

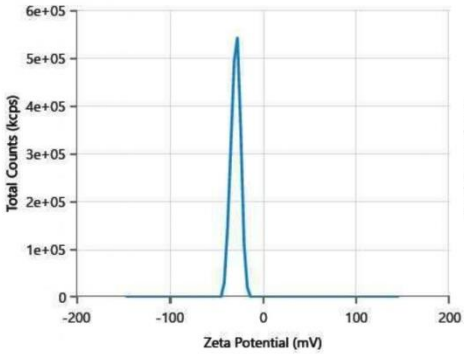


Fig. 3. Zeta potential of CYB

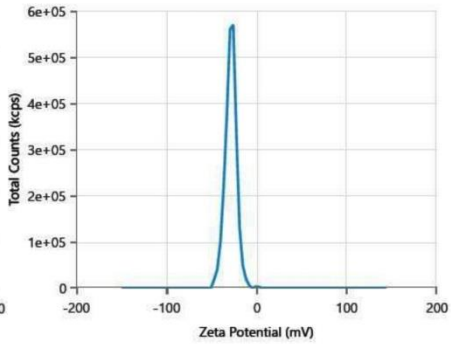


Fig. 4. Zeta potential of PYB

X-Ray Diffraction

X-ray diffraction study (XRD) of both samples done using Rigaku smart Lab S 200 confirms that they contain ZnO with wurtzite phase and the entire diffraction peaks are in agreement with the standard JCPDS data (card No. 36-1451) as shown in Fig. 5 & 6.

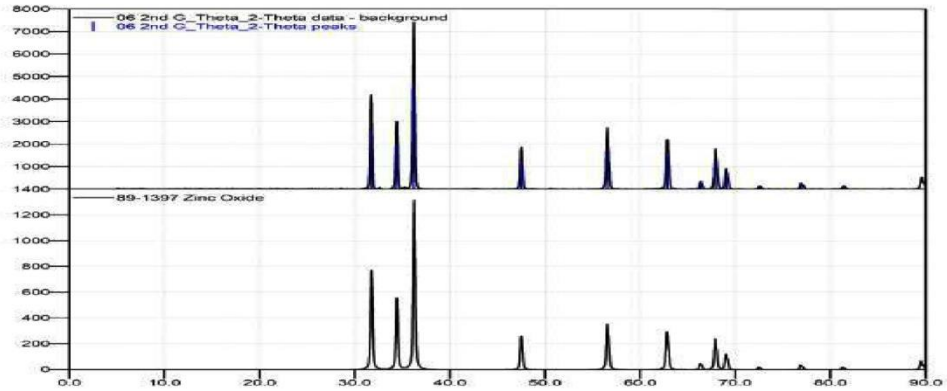


Fig 5. XRD of CYB

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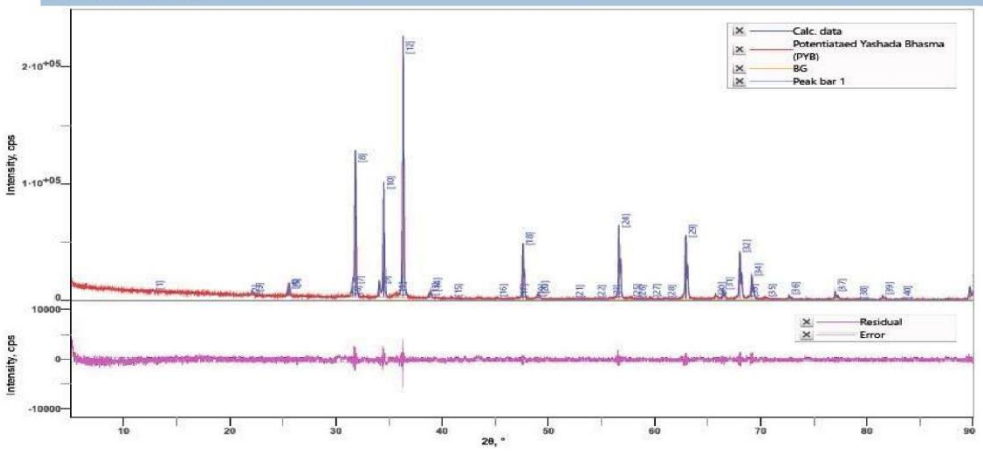


Fig 6. XRD of PYB

SEM analysis:

SEM analysis revealed that the shapes of particles of *bhasma* of both samples were majorly rectangle, spherical and hexagonal shapes. Compared to CYB in PYB the hexagonal wuzurite structure can be well appreciated. The particles in CYB sample are heterogeneous and agglomerated ranged between 200 to 702 nm and in PYB, particles are homogenous and well dispersed ranged between 20nm – 263nm as depicted in Fig.7 & 8.

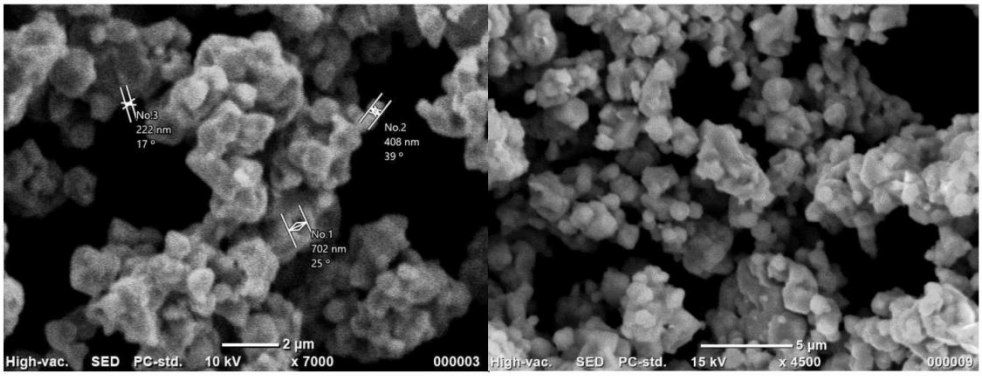


Fig. 7. SEM micrograph of CYB

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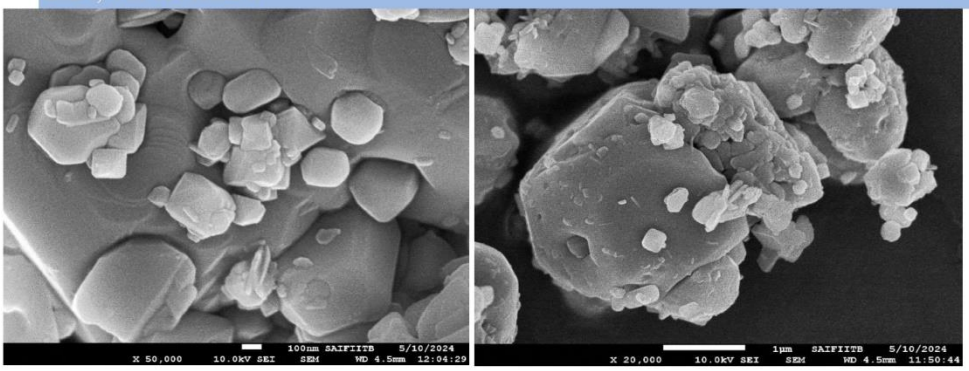


Fig. 8. SEM micrograph of PYB

DISCUSSION:

Ayurvedic method of *bhasmikarana* (process of making nano-sized particles) is a well-known and validated procedure to obtain metallic and mineral based nano-particles. The ideology of size reduction being the most basic principle of *Rasashastra* (Ayurvedic alchemy), application of the same to already nano-sized particles raises the question of possibility to get increased bioavailability owing to even more small particle size and invariably better therapeutic outcome in lesser doses than recommended. Hence the present study aimed at accelerating further size reduction to classically prepared yashadha bhasma and analyze it through instrumental techniques appropriate.

Here potentiation was done at three different time points as detailed in the materials and methods section. Ball milling of *jarita yashada* and PYB *kajjali* respectively for 24 and 48 hrs was preferred here as the additional step of potentiation. This can be validated with the fact that ball milling has been extensively adopted to modify the supramolecular structure of any active pharmaceutical ingredient (API) into its polymorph, co-amorphous or cocrystal structures leading to increased solubility and absorption.[12] As most of the *bhasma* in *Ayurveda* are insoluble in water, currently used potentiating method with ball mill logically aids the efficacy by creating more space for the solubility of API. The second method of potentiation used in current study is wet grinding of PYB in a wet grinder with the juice of Aloe vera for 24 hrs, every time before incineration in EMF. Wet grinding techniques are reported to be significantly more efficient and effective than dry grinding, offering substantial benefits including lower energy consumption, reduced agglomeration, and finer particle sizes, ultimately leading to improved process outcomes and reduced operational costs.[13] The above described two potentiating methods are not generally followed as adopted in potentiation part of this study anywhere in classical *bhasma* preparation. Also, even while the *bhasma siddhi lakshanas* were observed at the 2nd incineration of PYB, but it was subjected to two more times of incineration in EMF with a motive to further bring down the particle size. Optimizing particle size reduction of PYB, intended for oral delivery, can substantially augment its absorption potential by facilitating seamless diffusion across physiological barriers, including the gut mucin layer and enterocyte membranes.[14]

While comparing the observations of preparation methods of both CYB and PYB, it can be seen that the quantity of *bhavana dravya* (both juices of Aloe vera and lemon) has been used extremely higher during 1st incineration of PYB than that of CYB with the only difference until the point being ball milling of *jarita yashada* in PYB method. This is highly indicative of surface area being more created in the particles due to ball milling of *jarita yashada* and this has probably led to increased quantity of liquids consumed in wet grinding before incineration. Due to addition of comparatively more amount of liquids in PYB, the time taken for *bhavana* (trituration) has also remarkably increased in PYB method thus allowing more contact time for the particles in between the grinding surfaces and liquids. Also, in PYB preparation the time of grinding was fixed to 24 hrrs, which has led to increased consumption of *bhavana dravya* compared to CYB. Classically the colour of *yashada bhasma* is found as *indu-sannibha* (colour of moon), i.e., white to pale yellow coloured. It can be noted that pale yellow colour was observed in PYB than CYB. Also, the soft feel to touch which was observed after 2nd incineration in both the samples became very soft to touch by the end of third and fourth incineration in PYB indicating successful further size reduction of particles. The same effect probably has resulted in the amorphous from of PYB while CYB was noted as only fine powder.

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Total ash value of PYB is noted lesser than CYB. This reflects more incorporation of inorganic content in PYB.[15] The difference in total ash value and acid insoluble ash values can be corroborated with the methodology adopted. Present study followed the SOP outlined as per Ayurvedic Pharmacopoeia of India which specified heating of 2-3 g sample in silica or platinum crucible at a temperature not more than 450℃ until the sample is free from carbon. But the finding from Keshsun Liu (2019) is suggestive of ashing 1-4 g of samples overnight at 600℃ especially for biomass.[16] Since PYB's preparation involved more contact time with juices of Aloe vera and lemon there could be more incorporation of organic matter into PYB. ICPAES detected step wise reduction in percentage concentration of Zinc in raw *yashada, shodita yashada*, *jarita yashada*, CYB and PYB. With each procedure of *shodhana*, *jarana*, *marana* and *marana* of PYB being carried out at every step, these special techniques have only brought about such a change at the level of zinc concentration thus indicating the importance of significance of such techniques. This is also suggestive of conversion of elemental zinc into its possible oxides and sulphides upon incineration in the presence of oxygen and sulphur (from *kajjali*) at high temperatures in all the steps.

Zeta potential of both CYB and PYB are found with almost similar values. Zeta potential is a critical parameter evaluating delivery system stability by measuring particle surface charge. A threshold of 30 mV distinguishes between stable, repulsive particles and unstable, aggregating ones.[17] In present case, both the zeta potential of both CYB and PYB are of values nearing to -30 mv which is probably suggesting the stability of the drugs during its delivery.

XRD results of PYB furnished peaks that exactly correspond to the peaks of CYB which is Zinc oxide. This also coincides with the findings made by Avani Pareek et al., where *Yashada bhasma* prepared following traditional technique also yielded ZnO hexagonal structures validated by XRD.[18] This justifies the proper formation of metal oxides in the preparation method adopted for PYB.

The effectiveness of potentiation method adopted for the preparation of PYB has reflected remarkably in DLS analysis. Particle size of CYB through DLS was recorded as 696.3 nm whereas for PYB it was found as 261.1 nm (size reduction of two and half times than CYB). This ensures a greater bio-availability for PYB than CYB into the systems facilitating grater therapeutic action in lesser dosses than recommended. SEM analysis of both CYB and PYB did not show much of change except for the hexagonal wuzurite arrangement in PYB. This clearly demonstrates the ability of the method of potentiation adopted is able to alter the structural integrity of compounds formed. With such changes, more than CYB, PYB may exhibit higher therapeutic benefits with lesser doses than recommended.

CONCLUSION:

Present study has effectively come up with a newer idea of potentiation of CYB with ball milling and wet grinding logically to augment better therapeutic outcome. Basic physio-chemcical assessment and analysis with higher end instruments like SEM, Zeta Potential etc., has shown characteristic changes that have occurred in the due course of potentiation. Thus, PYB prepared using the described method of potentiation aids in further size reduction with incorporation of more quantities of organic content as justified by the findings.

SCOPE FOR FURTHER STUDY:

However present study has logically adopted various methods of potentiation, it is recommended for such modified *bhasma* preparations to screen for their safety and toxicity studies. Further animal model studies to decide the therapeutic dose of PYB in comparison to CYB for any condition specified will bring about better dose fixation to PYB.

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CONFLICTS OF INTEREST: All the authors do not have any competing interest with any parties in regards to the

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work carried out in the present study.

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