"COMPARATIVE EVALUATION OF ANTI-CANDIDAL PROPERTY OF ACRYLIC RESIN REINFORCED WITH MAGNESIUM OXIDE AND SILVER NANOPARTICLES AND THEIR EFFECT ON CYTOTOXIC LEVELS": AN IN-VITRO STUDY"

BY

Dr. MEEKHA PETER

REG. NO- IM0218004

Dissertation

Submitted to
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In

PROSTHODONTICS AND CROWN & BRIDGE
(BRANCH – I)

Under the guidance of

Dr. MAHANTESH BEMBALAGI M.D.S.
Professor

DEPARTMENT OF PROSTHODONTICS

AND CROWN & BRIDGE

KAHER V.K. INSTITUTE OF DENTAL SCIENCES,

BELAGAVI, KARNATAKA.

2018 – 2021

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KLE V.K. INSTITUTE OF DENTAL SCIENCES,
BELAGAVI, KARNATAKA

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Date: **Dr. MEEKHA PETER**

Place: Belagavi. **REG. NO- IM0218004**

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BELAGAVI, KARNATAKA

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Date:
Place:

GUIDE
Dr. MAHANTESH BEMBALAGI M.D.S.
Professor,
Department of Prosthodontics and
Crown & Bridge,
KAHER Vishwanath Katti Institute
Of Dental Sciences, Belagavi.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
KLE V.K. INSTITUTE OF DENTAL SCIENCES,
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Date:

Place:

CO-GUIDE

Dr. SANJAY MISHRA
Scientist-Grade:2, KAHER’S
BSRC associate professor,
College of Pharmacy,
Belagavi-590010.

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Head of Department

Dr. ANANDKUMAR G. PATIL M.D.S.
Professor & Head,
Department of Prosthodontics
and Crown & Bridge,
KAHER Vishwanath Katti Institute
of Dental Sciences, Belagavi.
Date:
Place: Belagavi

Principal

Dr. ALKA D. KALE M.D.S.
Principal,
KAHER Vishwanath Katti Institute of
Dental Sciences,
Belagavi.

Date:
Place: Belagavi

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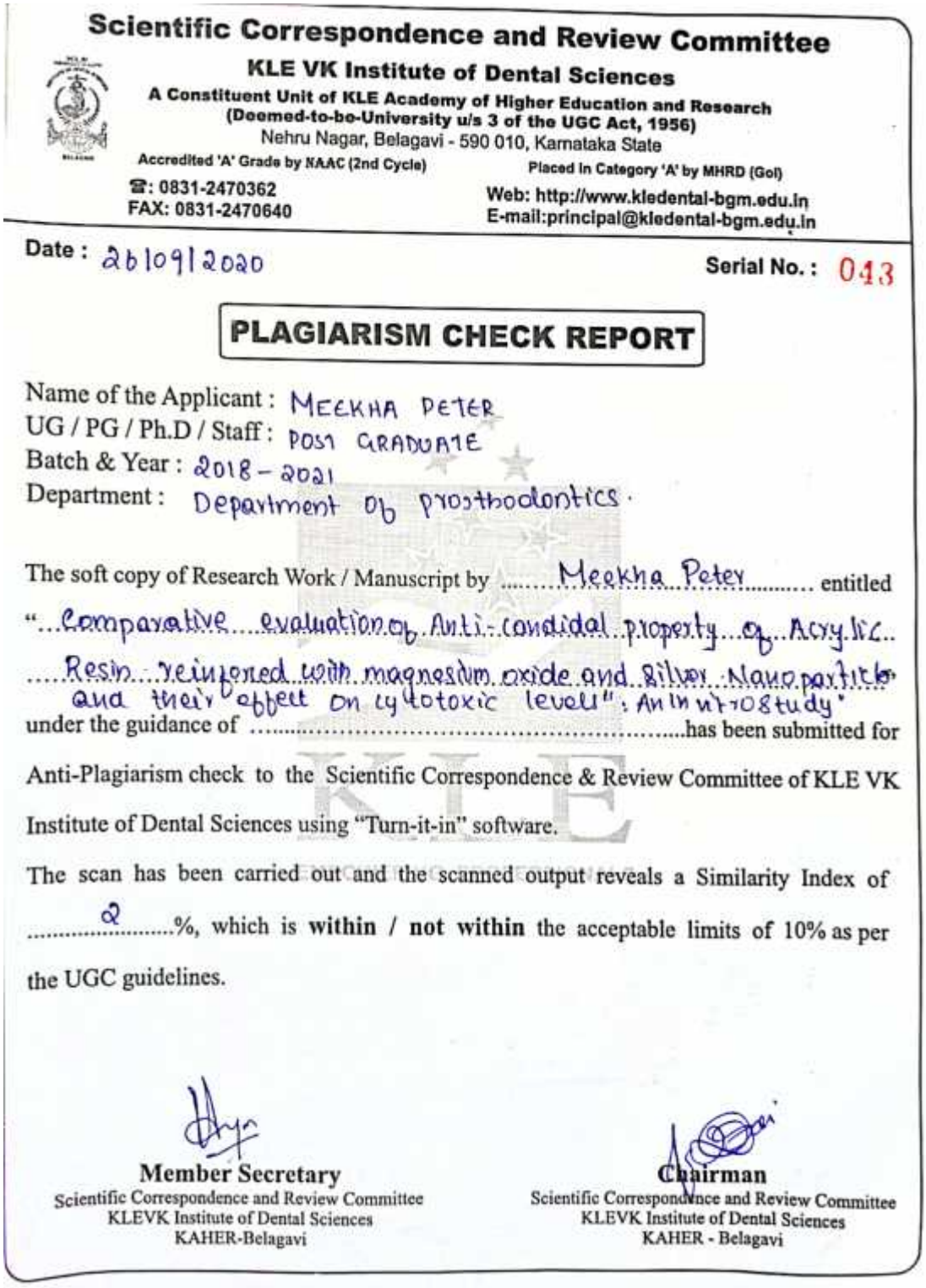

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☎: 0831-2470362
FAX: 0831-2470640

Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

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UG / PG / Ph.D / Staff : POST GRADUATE
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KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (3rd Cycle) & Placed in Category 'A' by MHRD (GoI)
☎ 0831-2470362
FAX: 0831-2479640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

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This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Dr. Meekha Peter** entitled **COMPARATIVE EVALUATION OF ANTI-CANDIDAL PROPERTY OF ACRYLIC RESIN REINFORCED WITH MAGNESIUM OXIDE AND SILVER NANOPARTICLES AND THEIR EFFECT ON CYTOTOXIC LEVELS: AN IN-VITRO STUDY** has been done under my guidance and considered satisfactory.

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ACKNOWLEDGEMENT

"Feeling gratitude and not expressing it is like wrapping a present and not giving it".

A sense of triumph is very much justified at this stage of completion of my dissertation, even more so is a sense of gratitude to all my peers, mentors and well-wishers.

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"The task of the excellent teacher is to stimulate "apparently ordinary" people to unusual effort. The tough problem is not in identifying winners: it is in making winners out of ordinary people."

I feel honored to be a student of my respected teacher and guide ***Dr. MAHANTESH BEMBALAGI$_{MDS}$,*** *Professor and HOD, Department of Prosthodontics and Crown and Bridge, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi, without whose everlasting inspiration, incessant encouragement, constructive criticism, and with valuable suggestions for improvement, the completion of this study would not have been possible. His unlimited patience, meticulous supervision at every step and everlasting zeal for perfection has not only enabled me to complete the dissertation, but has also helped me tremendously during the postgraduate programme.*

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Dr. MEEKHA PETER

LIST OF ABBREVIATIONS USED IN THE STUDY

PMMA	-	Polymethyl Methacrylate
mm	-	Millimeter
DIZ	-	Diameter of inhibition zone
Ag NPs	-	Silver Nanoparticles
MgO NPs	-	Magnesium oxide nanoparticles
%	-	Percentage
Fig	-	Figure
hrs	-	Hours
SPSS	-	Statistical package for social science
Mins	-	Minutes
^{0}C	-	Degree Centigrade
µg	-	Microgram
µl	-	Microlitre

ABSTRACT

STATEMENT OF PROBLEM: – Denture stomatitis is a common pathologic condition affecting 65% of denture wearers commonly associated with Candida species especially *Candida albicans*. Various measures like mechanical and chemical

denture cleansing as well as use of local and systemic antibiotics have been tried to overcome this condition. Due to increase in resistance exhibited by the microorganisms to the existing treatment modalities, there is a need for development

of polymethyl methacrylate (PMM) material having inherent activity especially against *Candida albicans*.

Purpose: - To evaluate and compare the effect of the anti-candidal property of acrylic resin by incorporating *silver nanoparticles* and *Magnesium oxide nanoparticles and* also assessed its toxic cellular effects of different concentrations of nanoparticles on mice fibroblasts.

MATERIALS AND METHOD: - The silver and magnesium oxide nanoparticles obtained commercially and separately weighed to three different concentrations (2%, 4% and 6%). Samples were fabricated of 12 control samples (without any nanoparticles incorporated), 36 samples of silver nanoparticles and 36 samples of magnesium oxide nanoparticles. A total of 84 samples (12 in each group) were fabricated to evaluate the anti-candidal effect through disk diffusion method. Three concentrations of samples fabricated and candida albicans was streaked and then incubated for 24 hours. Cytotoxicity of different concentrations of nanoparticles was checked by MTT assay.

Statistical analysis was carried out using Two-way ANOVA for comparison of two groups with both mean score at different concentrations. Pair wise comparison of two groups with both mean scores at different concentrations done by Tukey's multiple posthoc procedure.

RESULT: - Anti-candidal activity was evaluated for Silver and Magnesium oxide nanoparticles group for three different concentration 2%, 4% and 6% after 24 hours. Mean, standard deviation and standard error were calculated. The mean values (DIZ) for Silver nanoparticles at 2%, 4% and 6% after 24 hours were 13.71(±2.07), 18.58 (±1.62) and 27.96 (±1.76) respectively. The mean values (DIZ) for Magnesium oxide nanoparticles at 2%, 4% and 6% after 24 hours were 11.63 (±1.35), 14.38 (±1.63) and 18.25 (±1.39) respectively. Thus Silver nanoparticles exhibited more anti-candidal activity as compared to the Magnesium oxide nanoparticle group and control group had complete growth of Candida. However, magnesium oxide nanoparticles showed higher Cytotoxicity and cannot be considered for clinical applications.

CONCLUSIONS: - According to the obtained results we can recommend the use of silver nanoparticles when incorporated at 2%, 4% and 6% into the denture base resins proved to be beneficial to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity.

KEY WORDS: Nanoparticles, Acrylic resin, Antifungal activity, Cytotoxicity

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INTRODUCTION

Edentulism is observed as a paramount complication globally especially affecting the geriatric population and is characterized by loss of all natural teeth. Prevalence of edentulism accounts for about 16.3% of the Indian population. [1] Over these years, 75% of population who wears prosthesis has been discovered to be the carriers of Candida strains and those strains were responsible for Candida associated Denture stomatitis. *Candida albicans* has been the foremost strain found inside the oral cavity and exhibited inflammatory pathologies in complete denture wearers. [2]

Candida albicans is opportunistic yeast which exists in two different forms and becomes virulent at different host conditions and leads to oral diseases known as Oral Candidiasis. [2] Focus on *Candida albicans* is necessary as it is considered as the chief causative agent of Candidosis, which ranges from external mucosal lesions, to, a life-threatening systemic state. Candida Glabrata and Candida Tropicalis are the Candida species usually isolated from oral candidosis. [3] These potential pathogen adheres to the dental prosthesis such as removable partial dentures, fixed prosthesis and dental implants, and it serves as a source of infection if not maintained properly. A rise in demand for prosthodontic treatment, mainly complete dentures among the elderly edentulous individuals, was noted. As per the Epidemiological studies denture stomatitis prevails to 70% in denture wearers. Composition of biomaterials and surface texture of acrylic resins and liners plays a major role in adhesion of micro organisms and resulting in denture sore mouth and candidiasis. [5]

Complete denture have been the usual treatment option for teeth loss.[6, 4] Various materials, such as metallic and non- metallic denture bases (Poly methyl methacrylate) are employed for the making of these dentures and the commonest is poly methyl methacrylate (PMMA).[7, 4] Since 1937, PMMA has been opted because of its superiority in esthetics, robustness, easy to repair, ease of processing and its cost effectiveness. However, they are vulnerable to microbial population in the oral habitat.[8, 9]

Factors such as surface roughness due to the presence of cracks and grooves, poor dental hygiene, less salivary flow leads to plaque accumulation causing Denture Stomatitis.[9, 10]

Denture- induced Stomatitis is a reflection of an inflammation in the underlying denture bearing tissues usually seen in palatal and gingival mucosa due to prolonged use of ill fitting dentures.[11, 12] It is also known as denture sore mouth or prosthetic stomatitis or denture related erythematous stomatitis.[11] Multiple causative factors such as diabetes, immune-suppression, poor oral hygiene, fungal infections and continuous use of denture is accountable for this condition.[12, 13]

Over these decades, many methods have been used to treat Denture-induced Stomatitis which includes systemic as well as local application of antifungal agents such as Amphotericin B, Ketoconazole, Clotrimazole and Nystatin, which further developes resistance to yeasts.[14] However, the success rates of topical application of these antifungal drugs is relatively less due to the prolonged use of dentures even during medication, unpleasant taste and poor maintenance of effective concentration at the infected site. Hence, the combination of antifungal agents with tissue conditioners and acrylic resins seems to be effective and provides a therapeutic

outcome by extending the action of drugs followed by recovery of inflamed tissues.[15, 16] Thus, the need to curb denture stomatitis is initiated by combination of antifungal and antimicrobial substitutes into the denture base materials.[17, 18]

Even today, a vast number of dentures are made up of heat activated polymethyl methacrylate (PMMA) and copolymers because of its biocompatibility with oral surfaces. Recurrent cleaning and soaking of dentures in chemical cleansers is essential to ensure elimination of colonization of C.albicans.[19] But, it has some limitations like unused monomer hypersensitivity, very low mechanical strength, and low hardness. Thus, to get the better of these problems, resins have been improved with an aggregate of different materials.[20]

Nanotechnology is a very recent introduction which involves the study of materials on nano scale which ranges from 1 and 100 nm respectively. So, the materials used in Prosthodontics have been reinforced with nanoparticles since most of the denture wearers are geriatric patients and it is cumbersome for the patient to cleanse the denture properly because of poor manual dexterity and memory loss.[21]Thus, with the arrival of nano technology, silver nanoparticles have been synthesized and it was found to possess antimicrobial properties with low filler content.

Silver ions are potent antimicrobial agents broadly exerted in medical field such as dressings for lesion and prostheses and it has got several ascendancy which includes non poisonous profile and biologically compatible with human cells. These smaller size ions results in a significant surface area to mass ratio which amplifies its physical, biological along with chemical properties.[22]

Silver nanoparticles (Ag NPs) are capable enough to perforate the cell membranes and thereby increase the antimicrobial activity against some microorganisms in biofilm which are considered more defiant to antimicrobial agents than the planktonic pathogens. It is stated in the literatures that silver ions react with peptidogycan cell wall and bacterial proteins which give rise to loss of cellular content and eventually, cell death. Also, it interacts with DNA chain and prevents cell reproduction. [23] The sustained liberation of these ions provides long term antibacterial activity and suggested its use with polymers such as acrylic resins and tissue conditioners.[22]

Similarly, some other metal oxide nanoparticles are seen to be bactericidal such as ZnO, CuO, MgO and TiO. Antifungal activity is found against C.albicans and current studies disclosed a growing attention in its application on denture induced stomatitis.[24] Nano magnesium oxide exhibited antibacterial effect in aqueous conditions due to the development of super oxides and it is also used as a catalyst for organic reactions.[25]

Toxicity and biocompatibility of these nanoparticles is another characteristic to be considered. Elemental release occurs with its contact with adjacent tissues resulting in distribution of ions all over the body through blood vessels, which increases the probability of causing unfavorable biologic impacts such as Cytotoxicity, mutagenicity and allergic reactions.[26]

Therefore focus of this study is to assess the anti-candidal effect of acrylic resins when incorporated with *Silver nanoparticles* and *Magnesium oxide nanoparticles*. Also to determine the cytotoxicity of acrylic resins incorporated with *Silver nanoparticles* and *Magnesium oxide nanoparticles*.

NEED FOR THE STUDY

One third of overall population exhibits an oral inflammatory manifestation known as denture stomatitis. The Principal etiological factor in the pathogenesis is the presence of candida species, generally *c. albicans*, on intaglio facet of the prosthesis and its adherence to denture base resins.[27] Commonly perceived signs and symptoms are pain, burning sensation, mucosal bleeding, oral malodor, xerostomia and clinical appearance of inflamed mucosa.[12] Thus we have to ensure satisfaction of the patient by averting this condition.

Denture base materials are more vulnerable to microbial colonization in oral cavity and it can be prevented by oral hygiene maintenance and denture cleansing.[9] Denture plaque is the main element for causing denture stomatitis in denture wearers, so denture cleansing and removal of plaque is considered vital to retain a good oral hygiene.[28] Denture cleansing can be done by chemical method, mechanical method or a combination of both. Some of the chemical methods are soaking in household solution, use of denture cleansers, microwave radiation and airdrying.[6] Mechanical methods such as brushing the dentures after each meal with water or chemical agents efficiently removed the plaque and formed biofilms.[2] However, it is not possible with the elderly, who lacks physical proficiency or mental ability.[29]

Antibacterial denture creams and denture cleansers were prescribed to denture wearers to minimize the formation of biofilm, nevertheless because of insufficient surface disinfection and rapid recolonization of micro organisms specially candida albicans recommended that denture treatment is troublesome.[9]

Amphotericin B and nystatin is used to treat denture stomatitis and it is observed as a substitute drug for intravenous therapy of potential candidal infections. However, utilization of these drugs has also become challenging due to constant flow of saliva and regular swallowing which decreases the drug to mucosa contact and also leads to gastrointestinal side effects like vomiting, diarrhea and nausea. Also, patient's adherence to treatment regimen has also been a challenging determinant, thus making the outcome, questionable.[15, 17]

Systemic and local antibiotic agents such as fluconazole have been adviced for denture stomatitis and candidal infections but its unrestrained use led to antimicrobial resistance.[30] But, it is noted that candida genus exists in biofilm exhibits limited susceptibility to antimycotic medication than planktonic microbes.[22]

Therefore, denture- induced stomatitis is a matter of concern in the domain of dentistry and therefore it is necessary to prevent its occurrence by developing a denture base resin with inherent anti candidal property. Thus, acrylic resins are integrated with antimicrobial activity with biocides namely nanosilver, nanotitanium dioxide and organic compounds such as quaternary ammonium salts.[30]

Over these years nanotechnology has become an exceptional merit in the field of dentistry. Nanoparticles of different variety are incorporated to the materials used in dentistry including PMMA to improve its properties which incorporate metal ions and metal oxides. And these metal ions such as silver, zinc and copper and zinc displayed bactericidal action.[31]

Nanosilver shows a sturdy antibacterial property against the broad spectrum of bacteria which comprises of antibiotic resistant strains as well. Silver exhibits a well

permitted tissue retaliation and low destructive profile.[29] The biomechanics of antimicrobial property of silver supported compound is not scientifically proven and it is a controversial topic. It is called as an oligodynamic action of silver owing to the catalytic activity of silver and further structural damage of bacteria.[32]

Similarly, another metal oxide used in the current study is magnesium oxide nanoparticle and all these nanoparticles possess great surface area to mass ratio that ensures healthier infiltration to cells and tissues.[24] Bacterial cell wall is the prime intention of metal oxide nanoparticles. These metal oxides are extremely active chemically and physically because of its large surface area. A wide range of metal oxide nanoparticles demonstrated improved temperature stability in contrast to other molecules and killed bacteria through numerous molecular mechanisms which includes direct binding succeeded by destruction of the organism's membrane generating reactive oxygen species (ROS).[33]

However literatures reported that silver nanoparticles have increased toxicity than silver microparticles as silver ions are perceived to be biologically active and may have side effects on human cells.[33] Antifungal effect of PMMA nanoparticle compound is due to the constant release of incorporated ions into the physiologic environment. Thus, it is vital to evaluate this material to recognize whether it is causing Cytotoxicity or genotoxicity in culture cells.[26]

Therefore the rationale of the current study is to evaluate the anti candidal activity of acrylic resins integrated with three concentrations of silver and magnesium oxide nanoparticles and to assess the Cytotoxicity of acrylic resins reinforced with silver nanoparticles and magnesium oxide nanoparticles.

HYPOTHESIS

Null Hypothesis-:

There is no difference in the anti-candidal property, of acrylic resin incorporated with *Silver nanoparticles* and *Magnesium oxide nanoparticles*.

Alternative Hypothesis-

There is a difference in the anti-candidal property, of acrylic resin incorporated with *Silver nanoparticles* and *Magnesium oxide nanoparticles*.

AIMS AND OBJECTIVES

Aim of the study:

To evaluate and compare the effect of the anti-candidal property of acrylic resin by incorporating *silver nanoparticles* and *Magnesium oxide nanoparticles.*

Objectives:

- To evaluate the anti-candidal property of the acrylic resin incorporated with *Silver nanoparticles.*
- To evaluate the anti-candidal property of the acrylic resin incorporated with *magnesium oxide nanoparticles*.
- To evaluate the possible toxic cellular effects of different concentrations of *silver nanoparticles* and magnesium oxide nanoparticles on mouse fibroblasts.
- To compare the anti-candidal property of acrylic resin incorporated with *silver nanoparticles* and *magnesium oxide nanoparticles*.

REVIEW OF LITERATURE

D.R. Radford et al in 1998 conducted an in-vitro study to investigate adhesion of *Candida albicans* to denture base materials with altered roughness of the surface, also to record the reaction on candidal adhesion of different salivary pellicle to these surfaces. They concluded that greater adhesion of *Candida albicans* are seen with uneven rather than even surfaces. However, saliva decreases adherence of *C. albicans* and consequently reduces result of surface coarseness and free surface energy variation among materials.[34]

Luciana Assirati Casemiro et al in 2007 conducted an in vitro study to assess the disinfectant capability of acrylic resins with various percentages of zinc zeolites and silver, to determine whether the inclusion of zeolites changes the flexural strength and durability of the denture bases. The result of the study was incorporation of Irgaguard B5000 2.5% to the resin developed an antimicrobial effect in opposition to all strains. Flexural strength and durability reduced drastically with incorporation 2.5% and 5.0% of zeolites. So they concluded that silver–zinc zeolite incorporation to acrylic resins produces disinfectant activity, but based on zeolite percentage it hampers the mechanical properties. [35]

Kim, Keuk-Jun et al in 2008 manufactured spherical silver nanoparticles and evaluated their antifungal effects on fungal pathogens of the skin and their effects on polymorphism of candida albicans. Silver nanoparticles exhibited increased activity in opposition to clinical isolates and ATCC strains of Trichophytonmentagrophytes and Candida species. The activity of silver nanoparticles was similar to amphotericin B, but more than that of fluconazole. The effects revealed nano-Ag exerted activity on

the mycelia. Therefore, in the ongoing study they indicated that silver nanoparticles have considerable antifungal activity, and needed further inspection for clinical use.[36]

A. Nasrollahi et al in 2011 conducted a study to examine the antifungal outcome of silver nanoparticles on *Candida albicans* and *Saccharomyces cerevisiae*. Minimum Inhibitory Concentration (MIC) technique was used and medicaments included in the study are Amphotericin B and Fluconazole. The silver nanoparticles were produced by chemical reduction method. Antifungal sensitivity was determined and variations with responses of yeasts on membrane were found by Scanning Electron Microscopy. Thus the study concluded that silver nanoparticles showed appreciable antifungal effect in contrast to other antifungal drugs, hence suggests advanced evaluation for implementation in clinics.[37]

Fatemeh Noorbakhsh et al 2011 directed a study to find out the result of silver nanoparticles alone and along with fluconazole and geriseofulvin on dermatophyte pathogen *T.rubrum.*It was carried out by broth microdilution discussed in NCCLS data M38-A.They came to the conclusion that silver nanoparticles inhibited T.rubrum at 10µg/ml. Also silver nanoparticles showed less inhibitory efficiency compared to geriseofulvin (0.8 µg/ml), and was more efficient than flocunazol (40µg/ml). Also there was a rise in their antifungal activity in the combination test in presence of silver nanoparticles.[38]

Hema Kanathila et al in 2011 conducted a study to test the result of magnesium oxide (1%, 3%, 5%, and 7%) added with two tissue conditioners (Viscogel and GC Soft), in restricting the growth of *Candida albicans.* The study concluded that inhibitory result of magnesium oxide 1% added with tissue conditioners (VGC and GCC) was not remarkable in both of groups whereas the result of MgO 5% and 7%

added with tissue conditioners (VGC and GCC) was highly significant against Candida albicans. They also noted that with the increased concentration of magnesium oxide, production of candida albicans was reduced and the zone of inhibition was increased.[39]

D. R. Monteiro et al in 2011 led this study to examine the potential of silver nanoparticles in opposition to *Candida albicans* and *Candida glabrata* attached cells and biofilms. Minimal inhibitory concentration (MIC) tests were carried out and showed that silver nanoparticles were antifungal against both the species at 0.4–3.3 mg/ml concentrations. The results showed that silver nanoparticles were extremely effectual in decreasing biofilm biomass when exerted to attached cells in contrast to previously developed biofilms except C. glabrata wherein either case exhibited a decrease of 90%. The result was not so noticeable on Candida albicans but, a decrease in the total amount of biofilm cells. Thus they concluded that silver nanoparticles have the capability to prevent formation of biofilm and they exhibited higher antifungal activity in opposition to *C.albicans* and *C.glabrata*.[40]

Ki-Young Nam et al in 2011 investigated a study to recognise *in vitro* antimicrobial activity of silver nanoparticles integrated tissue conditioner on microbial species, *Streptococcus mutans, Candida albicans, and Staphylococcus aureus*. Disc shaped specimens were fabricated containing silver nanoparticles with concentration ranging from 0.1- 3% and were subjected to antimicrobial assay. Obtained result suggested that silver nanoparticle integrated tissue conditioner exhibited antimicrobial property and could be effectively used in denture plaque control.[41]

Ki-Young Nam, et al in 2012 conducted a study to determine the antifungal property and physical features of acrylic resins reinforced with silver nanoparticles. Silver nanoparticles of 0, 1.0, 5.0, 10.0, 20.0 and 30.0 wt% were added to acrylic resins to fabricate the specimens. Silver with 20.0 and 30.0 wt% showed a significant reduction of viable cells. And the characterized acrylic disc carrying silver nanoparticles was ruled out by TG/DTA and EDX analysis. Thus they came up with the conclusion that denture base acrylic along with silver nanoparticles have a potential antifungal effect but displayed a poor color stability and needs further studies for clinical applications.[32]

Laura Susana Acosta-Torres et al in 2012 conducted a study to produce an acrylic resin having an antifungal medium as silver nanoparticle and to find its biocompatible nature to make sure the fabrication of a nontoxic antifungal material for denture bases. Commercial acrylic resin was used to make PMMA control discs and PMMA-silver nanoparticle discs. For evaluating the antifungal activity luminescent microbial cell viability assay, NIH-3T3 mouse fibroblasts and human lymphocyte cell line was used to fulfill biocompatibility tests. To assess the dispersion of nanoparticles, samples were assessed mechanically and acrylic resin containing silver nanoparticle evaluated microscopically to assess the dissipation of nanoparticles. C.albicans attachment was reduced PMMA silver nanoparticle and it was neither genotoxic nor cytotoxic as per calculation of DNA duplication, mitochondrial enzymatic action and non-DNA genomic destruction in cultural cells.[42]

D.R. Monteiro, S. Silva, et al in 2012 carried out a study to examine the antifungal effect of colloidal silver nanoparticle suspension in opposition to C. albicans and C. glabrata biofilms. The present study used (5, 10 and 60 nm) average sizes of silver

nanoparticles, which was produced by chemical reaction. Minimal inhibitory concentration (MIC) tests were conducted utilizing micro-dilution technique. Anti-biofilm activity of silver nanoparticles was found by colony forming units and crystal violet staining. They have concluded that the silver nanoparticle colloidal suspension was effective towards all the tested strains in very low concentrations.[43]

S Ó Nia Silva et al in 2012 ran a study to contrast biofilm initiation by *C. glabrata* and *C. albicans* on denture base resin, and efficacy of nystatin and silver nanoparticles was tested on the biofilms for antimicrobial activity. Biofilm development and candidal adherence on acrylic surface was carried out in existence of artificial saliva for 2hr and 48 hrs, besides crystal violet staining was utilized as a measure of biofilm biomass, also to measure biofilm formation ability. Also colony forming unts helped in determining the candidal adherence. Candida species formed biofioms on acrylic surface and a higher rate of CFUs was seen with candida glabrata than those formed by C.albicans as per results. In addition to that silver nanoparticle was potent enough to reduce the c.glabrata biofilm in comparison to C. albicans. They concluded that nystatin and silver nanoparticle presented antifungal activity against both the species with preformed biofilms.[44]

Abbas Monzavi et al in 2014 directed a study to evaluate the in vitro and ex vivo antimicrobial efficacy of solution out of nano-magnesium oxide (MgO) in opposition to endodontic pathogens. To investigate the cytotoxicity of nanoparticle Lactate dehydrogenase cytotoxicity assay (LDH assay) was used. Direct contact method was used for comparing antimicrobial efficacy of varied concentrations of chlorhexidine gluconate, sodium hypochlorite and nano-MgO solution, in opposition to Candida albicans, Staphylococcus aureus and Enterococcus faecalis. Varied concentrations of

nano-MgO displayed no Cytotoxicity. The results showed that comparing to sodium hypochlorite, 5 mg/L of nano-MgO have long-lasting efficacy in riddance of E. Faecalis. Thus nano MgO considered as an effective root canal irrigant and further studies need to be done regarding the competency of nano MgO in eliminating smear film.[45]

Douglas Roberto Monteiro Delbem et al in 2014 Study aims to find out how silver nanoparticle's chemical stability affects its outcome against *C. albicans* and *C. glabrata biofilms*. They evaluated different varibles of silver nanoparticles stabiliy such as temperature, pH and duration of contact with biofilms and used colloidal suspensions at 54mg/ml to serve mature candida albicans adhered on acrylic. Determination of their potency was evaluated by overall biomass and colony-forming values. Therefore they concluded that temperature along with pH variability of silver nanoparticles unchanged their effect towards the viable cells of *Candida* biofilms. Thus silver nanoparticle gives a promising result against candida and can be applicable in care of denture stomatitis correlated with candida.[46]

Harini P et al in 2014 aimed to examine whether the incorporation of titanium dioxide nanoparticles in polymethyl methacrylate (PMMA) expands the flexural strength and to contrast the varied concentrations of titanium dioxide (1 wt%, 2 wt%, 5 wt %) nanoparticles and its association to flexural strength. Flexural strength was recorded by universal testing machine INSTRON. Addition of titanium dioxide to polymethyl methacrylate showed much better flexural strength than that of normal PMMA as per the study.[47]

S Suganya et al in 2014 directed a study to examine the anti-Candida property of heat cure acrylic resins integrated with silver nanoparticles in the proportion of 4:1, 3:1, 2:1 to the weight of denture base resins. Within the drawbacks of the study they concluded that characterized denture base with silver nanoparticles showed less C.albicans adherence apart from control group and can be used as an antimicrobial agent in denture bases for immune compromised and geriatric patients.[48]

Yoshiaki Kamikawa Tomofumi Hamada et al in 2014 determined to investigate the attachment of *Candida albicans* and *Candida glabrata* on a heat cure acrylic specimen with Ag NPs by low- vacuum scanning electron microscopy (SEM) and by calculating colony-forming units. Results suggested that both the candida species adhered to the control specimen but adhesion to the Ag NPs coated acrylic specimen was markedly inhibited. Thus gave a way to prevent denture associated oral candidiasis by using silver nanoparticle incorporated denture base resins.[31]

Zhe Li et al in 2014 conducted a study to determine the biofilm formation and adhesion of candida albicans onto acrylic resins containing silver nanoparticles. XTT assay and crystal violet assay used to measure the bioactivity and biomass of candida albicans biofilm. Confocal laser scanning microscopy helped in determining average thickness and live cell percentage within the biofilm on the specimens. They have concluded that with the increase in nano silver concentration there was a decline in biomass and bio activity of candida albicans. Biofilm architecture was found very less on the acrylic specimens with 5% nano silver and only a few fungal cells were seen.[49]

Hossein Najafzadeh et al in 2015 conducted an in-vitro study to determine antifungal activity of 4 nano-metal oxides namely zinc oxide, silicon oxide, magnesium oxide and copper oxide (MgO, SiO2, ZnO and CuO) were determined by

an in-vitro study against *Candida albicans* and compared with amphotericin B. Nanoparticles suspensions was made by using acetic acid. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the nano-particles were determined. Amphotericin B proved to have better antifungal effect than nano metals as per results, but nano-ZnO had higher effect than nano-CuO, wherein nano MgO and nano SiO2 did not show any antifungal effect towards *C. albicans* at that concentrations.[24]

Abdulrahman Syedahamed Haja Hameed et al in 2015 conducted a study to examine the antifungal activity of metal ions (Mg2+, Ca2+, Sr2+ and Ba2+) loaded ZnO nanoparticles (NPs). In the on-going study, doped ZnO NPs with metal ions are produced by the co-precipitation method and tried out on Candida albicans and yeasts. The outcomes concluded that Mg-loaded ZnO NPs gave superior outcome in contrast with different alkaline metal ion doped ZnO NPs. At 2000 μg/ml development of C.albicans declined by Mg doped ZnO NPS. Also ZnO:Mg specimen with different concentrations of histidine decreased the antifungal effect of nanoparticles in opposition to C. albicans and the binding of nanoparticles was viewed by FESEM analysis.[50]

Moamin I. Issa et al in 2015 aimed to study the outcome of adding silver nanoparticles into heat cured acrylic-based soft denture liner on the antifungal activity and solubility, water sorption, colour change and shear bond strength of the soft lining material. They also investigated the quantity of silver ion released. Different percentages (0.05%, 0.1% and 0.2%) of silver nanoparticles were added into soft denture liner. The antifungal property of the Ag NPs was determined by utilizing viable count of *C. albicans,* disk-diffusion test and atomic absorption spectroscopy to

detect the amount of silver released. The Solubility, water sorption, shear bond strength and color change were also checked. They concluded that soft denture liner incorporated with silver nanoparticles possessed antifungal property and the release of silver ion was not found. However the reinforcement of silver nanoparticle gave an added advantage in decreasing water sorption, and unaltered the shear bond strength and also increased the material opacity.[51]

Chiaki Tsutsumia et al in 2015 conducted a study to determine the effect of acrylic resin reinforced with pre-reacted glass ionomer (S-PRG) against Candida albicans adhesion. Acrylic discs were fabricated by the addition of S-PRG filler into a heat cure resin at 0, 5%, 10%, and 20% (w/w). The disc surfaces were used for evaluation of surface roughness. The study concluded that the addition of S-PRG filler moderately aggravates the surface roughness of acrylic resin and there was a reduction of C. albicans adherence.[52]

Andrés Felipe Cartagena et al in 2016 directed a study by reinforcing polymeric miconazole nitrate (MN) micro particles on an investigational antifungal denture adhesive (DA) for the development of an oral drug delivery system. Denture adhesives were incorporated by Spray drying Eudragit L-100 (E) and Gantrez MS-955 (G) MN-micro particles. DAE1, DAG1, DAEG1, DAE2, DAG2, DAEG2 were the groups acquired from the mixture of polymers used in MN-micro particles (E, G and EG) and concentration of MN into DA (1% and 2%). Microbiological assay, adhesive force and toxicity were evaluated for all the groups. The results showed that DA containing 2% of MN loaded in micro particles presents better adhesive force , non-toxic and superior antifungal activity assuring an effective therapeutic for denture induced stomatitis.[53]

Ahila Singaravel Chidambaranathan et al in 2016 conducted a study to investigate the antifungal effect at different time periods with zirconium, aluminium and titanium nanoparticles against *C.albicans*. The samples were fabricated and splitted into four groups. The colonies formed on the samples were accounted in 24, 72 hours and one week intervals. The obtained result showed a remarkable difference in C.F.U in samples with titanium coating at different time intervals. Thus titanium plates coated with TiO2 nanoparticles showed notable anti candidal effect compared to ZrO2 and Al2O3 nanoparticles.[54]

Aysan Mirizadeh et al in 2017 investigated an in vitro study to determine antimicrobial and mechanical attributes of a denture base resin incorporated with quaternized N, N-dimethylaminoethyl Methacrylate (DMAEMA). Quaternized ammonium monomer (QAM) was made through the action of octyl bromide and DMAEMA. The denture base resin system was added with the synthesized QAM (8 to 12 wt %). The antimicrobial effect was evaluated by direct contact test against Candida albicans, Escherichia coli and Staphylococcus aureus, and for detecting the release of the QAM agar diffusion was done. The results concluded that there was a huge decline in the number of actively growing cells of E coli, S aureus and C albicans for 12% QAM denture base resins. QAM ensures increased antibacterial activity, but there was a decrease in flexural strength and modulus of the modified resin.[30]

Mahmoud Robati Anaraki et al in 2017 conducted a study to find out the antifungal effects of Zinc Oxide Nanoparticles in acrylic resin polymethyl methacrylate (PMMA) against C.albicans and to compare with acrylic resin containing silver nanoparticles. Different weight percent of nanoparticles (0.5, 2.5, 5, 10, and 20%)

were introduced to the denture base resin. They have concluded that both the nanoparticles effectively reduced the C.albicans population after 24 hours. However silver nanoparticles displayed strong anti candidal effect than that of zinc oxide nanoparticles.[55]

A Kurt et al in 2017 conducted an *in vitro* study to determine the antifungal effect and cytotoxicity of an acrylic resin reinforced with silver micro-particles. The silver microparticles were added to the polymethyl methacrylate (PMMA) denture base material in varied concentrations such as 0%, 0.25%, 0.5%, and 1% and their activity against C.albicans was tested by colony-forming units. A real-time cell analysis (RTCA) system and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay were the two Cytotoxicity assay techniques used here. The result revealed that as the percentage of silver nanoparticles increased antifungal effect against *C. albicans* also increased and no cytotoxic effect was seen.[33]

Mohammed M Gad et al in 2017 directed a study to find out the effect of zirconia nanoparticles incorporated into cold cure acrylic resins against *Candida albicans*. Zirconia nanoparticles were added to the resin in different percentages that is 0% wt, 2.5% wt, 5% wt, and 7.5%. Antifungal effect was assessed using a slide count method and a direct culture test. The results showed that addition of zirconia nanoparticles to the cold cure acrylic resin reduced *C. albicans* adherence and can be considered as a material of choice for repair of denture bases and prosthesis for ensuring prevention of denture stomatitis.[56]

Ali Abdul Hussein S. AL‑Janabi et al in 2018 aimed to determine the capability of antifungal mediums to reduce the formation of biofilm by *Candida albicans* after incorporating into heat‑cured acrylic denture base materials. Amphotericin B (AmB)

and Clotrimazole (CT) were added into polymethylmethacrylate (PMMA) specimens in varied concentrations. Porosity of PMMA specimens were measured in percentages and crystal violet and transmittance percentage assays helped in determining biofilm count. They came to a conclusion that addition of AmB and CT into denture base materials has a notable inhibitory activity on the biofilm formed by *C. albicans*, mainly at least concentrations. Also there was a decrease in porosity by inclusion of low concentrations of AmB and CT within denture base materials.[57]

Rama Krishna Alla et al in 2019 conducted a study to observe the antimicrobial effect of silver nanoparticles reinforced into heat cure acrylic resins. Different concentrations of silver nanoparticles that is 0.5, 1.0, 2.0 and 5.0 wt% were added into three heat cure denture base resins. Direct contact method was applied to assess the antimicrobial activity against C.albicans and colony forming units used for S.mutans. The results concluded that addition of silver nanoparticles into denture base resins reported higher resistance to C.albicans and a decrease in antimicrobial effect against S.mutans as the concentration of silver nanoparticle increased.[58]

Ghaith Darwish et al in 2019 conducted a study to assess the surface characteristics (chemical composition, roughness, morphology and wettability) of poly methyl methacrylate (PMMA) by addition of thin layer of titanium oxide (TiO2) developed by atomic layer deposition technique (ALD) and evaluated wear resistance of this novel coating with tooth brush abrasion. They also tested C.albicans attachment to denture base material after surface modification with TiO2. The results concluded that by the deposition of TiO2 layer denture base surface was capable enough to reduce the microbial adhesion and it improved the surface smoothness, resistance to wear and wettability.[59]

Mariusz Cierech Emilia Prochwicz et al in 2019 investigated a study to detect the amount of release of zinc oxide nanoparticles (ZnO NPs) from polymethyl methacrylate –ZnO nano composites (2.5%, 5%, and 7.5%), and also layer of ZnO NPs formed on the denture base resins and finally Cytotoxicity was checked. Optical emission spectrometry with inductively coupled plasma (ICP-OES) was used to calculate the amount of ZnO nanoparticles released. They came to the conclusion that MTT assay showed no cytotoxic effect up to 20mg/L concentrations of ZnO on human HeLa cell line and suggests a safe usage of new biomaterial in denture bases.[60]

MATERIAL AND METHODOLOGY:

SOURCE OF DATA:

This in-vitro study was conducted in-

- The Department of Prosthodontics, Crown and Bridge, KAHER'S KLE V.K Institute of Dental Sciences, Belagavi.
- KAHER'S Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research, Belagavi.

SAMPLE SIZE:

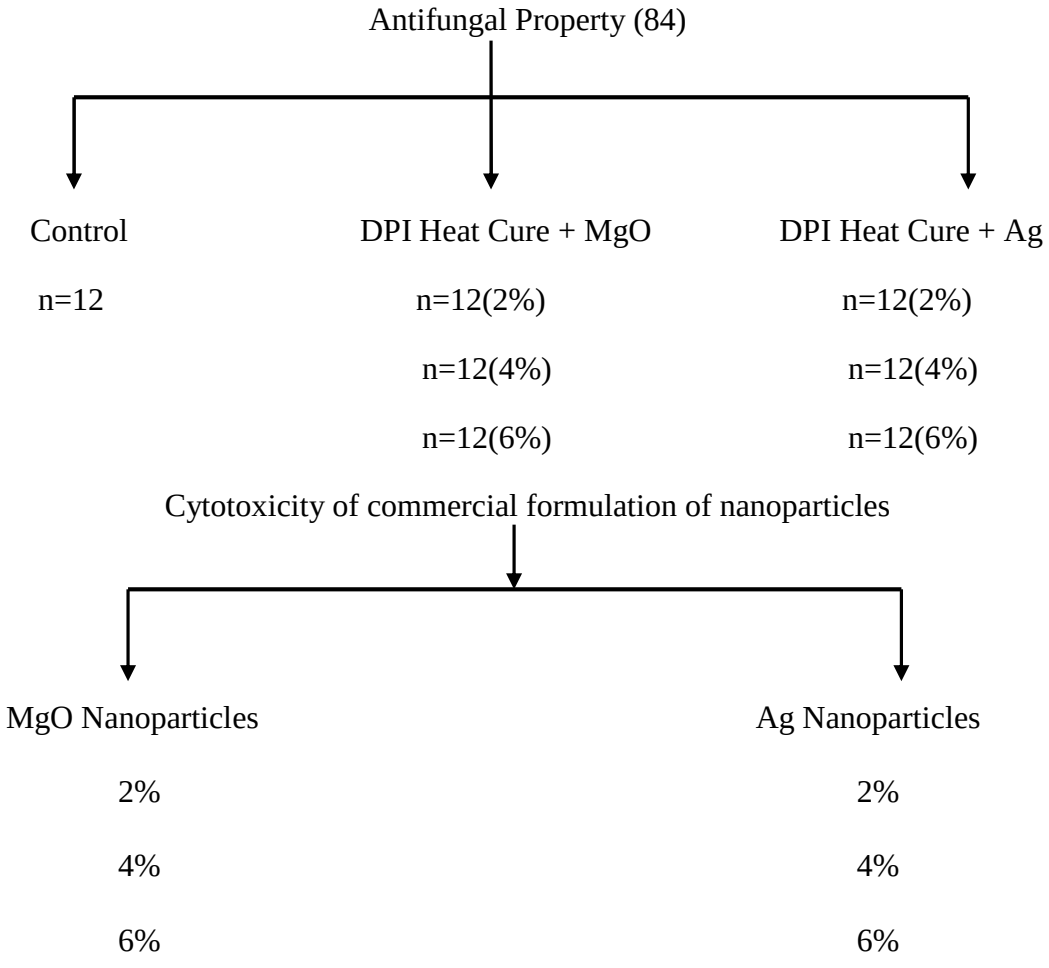


Table 1: ANTI-CANDIDAL PROPERTY OF ACRYLIC RESINS

Control group (12)	**Conc**	***Silver nanoparticles in DPI Heat cure* (36)**	**Magnesium oxide nanoparticles in DPI Heat cure (36)**
DPI Heat cure	**2%**	**12**	**12**
	4%	**12**	**12**
	6%	**12**	**12**

A total of 84 samples were taken to assess the antifungal property. To check for the antifungal property the samples were divided into 3 groups:

Group 1: Control group (DPI Heat cure).

Group 2: DPI Heat cure incorporated with Silver nanoparticles.

Group 3: DPI Heat cure incorporated with Magnesium oxide nanoparticles.

Each of these groups was divided into 3 subgroups of different concentrations 2%, 4%, 6% with 12 samples respectively.

INCLUSION CRITERIA:

1. Specimens of exact dimensions measuring 5×1mm acrylic discs.

EXCLUSION CRITERIA:

1. Specimens with microporosities and surface irregularities.

2. Incompletely polymerized discs.

MATERIALS USED IN THE STUDY

Table: 2

MATERIALS	DESCRIPTION	MANUFACTURER
Silver Nanoparticles	Purity : 99.9%, 30 nm	Ultrananotech Ltd, Bengaluru
Magnesium oxide Nanoparticles	20nm	Ultrananotech Ltd, Bengaluru
Heat polymerized acrylic resin	DPI –Heat cure	Dental products of India- Mumbai
Sabouraud dextrose agar	LOT 0000431253	Hi media, Mumbai
Candida albicans strain	90028	MTCC No. 2091
Petri plates	PW011	Hi-media
Addition silicon	Aquasil, soft putty/regular set Lot no: 1201001102	Dentsyply,U.S.A
Modelling wax	Hindustan modelling wax	Hindustan dental products, Hyderabad
Type II gypsum product	Kaladent	Kalabai, Mumbai
Separating medium	DPI Heat cure-Cold mould seal	Dental products of India-Mumbai
Sand paper	Oaky's abrasives	John oaky n Mohan Ltd.India
Cell lines	L929 Mouse fibroblasts	NCCS, Pune
DMEM Media	-	Hi media
Foetal Bovine Serum(FBS)	-	Hi-media
Distilled water	Ranken distilled water Batch No. : - 007M15	Avantor performance materials India limited
DMSO	-	Hi-media

ARMAMENTARIUM:

- Disk shaped metal molds of dimension 5mm× 1mm.
- Digital analytic balance: UniBloc (AUW220D) and Kern and Sohn GmbH- (240-3N).
- Bacteriological Incubator- Bio technics India (BTI-25).
- Laminar airflow cabinet (Yorco international Pvt. Ltd).
- ELISA Reader.
- CO_2 Incubator.

INSTRUMENTS

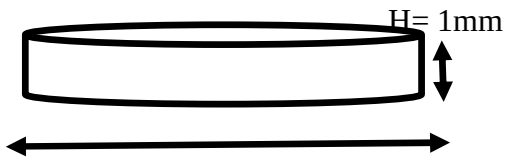
- Glass beakers.
- Flasks and Clamps (Jabbar & company).
- Bowl and Spatula (Prime Dental Products Pvt. Ltd).
- Heavy-duty micromotor (Handy 701 Marathon, Korea).
- Nickel- Chromium finishing and polishing kit (Shofu Inc, Japan).
- 96 Well Plates – Nest.
- Falcon Tubes.
- Micro pipettes.

METHODOLOGY:

1. FABRICATION OF THE SPECIMENS WITH THE NANOPARTICLES:

Master die fabrication: [48]

A metal mold of dimension 5mm diameter and 1 mm was fabricated and used for the making of specimens. (**Fig: 4)**



D= 5 mm

Sample fabrication:

- Modelling wax (Hindustan dental products, Hyderabad) was melted and flown into the metal mold to fabricate the wax samples. **(Fig: 5)**
- Dental stone type III and dental plaster was mixed in ratio of 1:1 and was used for investing of the wax samples (Kalastone, Kaladent & kalabai, Mumbai) in metallic flasks (Jabbar and company) **(Fig : 6)**. After setting of dental stone, de-waxing was carried out by immersing the flask in the boiling water at 100° C. After 4 min, it was removed from the water and segments were separated. The wax was carefully eliminated, and the stone mold was cleaned with hot water. Flask segments were permitted to cool down to room temperature.
- Two coats were applied of a separating medium (cold mould seal DPI) to both dental stone flask surfaces. The heat cure polymethyl methacrylate selected in the study was DPI heat cure material. The denture base resin was combined

according to manufacturer's instructions. For sample fabrication, 4 gms of powder was homogenized with 1.9 ml of liquid.

- Separately weighed Silver and Magnesium oxide nanoparticles was introduced to the monomer of the acrylic resin at concentrations of 2%, 5% and 6% based on the polymer mass and homogenized in an ultrasonic set for 5 minutes. The resin was packed using compression molding technique and pressed into the mold. Flasks were bench cured for 6 hours. The acrylization was done using short curing cycle at 74° C for 90 minutes, terminal curing at 94° C for 30 minutes in water. All flasks were allowed to bench cool for 30 minutes before opening. Similarly, samples devoid of nanoparticles were fabricated for control group.
- The excess resin was trimmed with a tungsten steel bur using a hand piece at low speed and finished with 320- grit sandpaper. **(Fig: 7, 8 & 9)**

2. TO CHECK FOR THE ANTI-CANDIDAL PROPERTY OF THESE NANOPARTICLES IN ACRYLIC RESINS: [39]

The anti-candidal effect of these nanoparticles was checked against *Candida albicans*.

Candida albicans (American Type Culture Collection catalog number 2091 strains) was obtained from Dr.Prabhakar Kore's Basic Science Research Centre, Belagavi. Sub culturing of *Candida albicans* was carried out in Sabouraud's dextrose agar and incubated at 37° C. The *Candida albicans* suspension after 24 hours of incubation was then mixed with BHI broth to a density of 0.5 McFarland to standardize the concentration **(Fig 10& 11).**

Antifungal susceptibility was tested with the disk diffusion method. Sabouraud's dextrose agar was filled into 90mm diameter petri plates inside the laminar airflow chamber. **(Fig: 12)** The culture plates will be then streaked with the inoculum. Using an inoculation loop *Candida albicans* was streaked on to these culture plates. **(Fig: 13)**

Once the inoculum dries, acrylic disc specimens integrated with Silver and Magnesium oxide nanoparticles were kept on the agar plates and incubated at 37˚±1˚C for 24 hours. **(Fig: 14)** Twelve agar plates were made for both groups and samples of each concentration were kept on the surface of culture plates. The diameter of inhibition zone (DIZ) was measured after 24 hours using a metallic scale and readings were noted. **(Fig: 15-18)**

TO CHECK FOR THE ANTIFUNGAL ACTIVITY:

PREPARATION OF ACRYLIC DISCS INCORPORATED WITH *SILVER NANOPARTICLES (Ag NPs)* AND *MAGNESIUM OXIDE NANOPARTICLES (MgO NPs)*:

Disk shaped wax pattern measuring 5mm in diameter and 1mm thickness was made.

⇩

2%, 4%, 6% of AgNPS and MgO NPs were separately weighed and mixed with monomer.

⇩

PMMA powder was weighed and mixed to the nanoparticle mixture.

⇩

Flasking procedure was carried out in the disk shaped mould achieved after de-waxing and bench cured for 6 hours.

⇩

Acrylization was done using short curing cycle at 74^0C for 90 minutes, terminal curing at 94^0C for 30 minutes in water.

⇩

Excess resin trimmed with a tungsten steel bur and finished with 320grit sand paper.

Candida albicans suspension was obtained and Antifungal Susceptibility test was done by disc diffusion method

⇩

Culture plate made using Sabouraud's dextrose agar on 90mm petri plates.

⇩

Cultural plates were streaked with inoculum and dried.

⇩

Control and the resin discs were placed into the agar plate at the concentration of 2%, 4%, 6%.

⇩

Agar plates were incubated at 37^0±1°C for 24hours.

⇩

Diameter (in mm) of inhibition zone was measured after 24hours.

⇩

All the culture plates were measured with a a metallic scale

TO EVALUATE THE CYTOTOXICITY OF DIFFERENT CONCENTRATION OF NANOPARTICLES: [26]

Mouse fibroblasts cells were obtained from NCCS, Pune. **(Fig: 19)** In vitro growth cessation effect of test compound i.e, Silver and Magnesium oxide nanoparticles was assessed by colorimetric or spectrophotometric determination of conversion of MTT into 'Formazan blue' by living cells.

Day 1:

The mouse fibroblasts were cultivated in Dulbecco's modified Eagle medium (DMEM-Gibco) boosted with 10% foetal bovine serum and Antibiotic–Antimycotic solution and waited until it reached 70%-80% Confluency. The cells were maintained in an incubator under optimal temperature and pressure control in a humid environment at 37^0C and in 95% oxygen and 5% CO_2 flow. The cell were counted using Tryphan Blue dye exclusion method. Tryphan blue was employed to decide the cell viability against the extracts procured. Around 1×10^5 cells/ml cell suspension was seeded into distinct wells in a 96 well micro titer plate and final volume was made up to 150µl by infusing DMEM media and incubated overnight. **(Fig: 20)**

Day 2:

- The test compounds (Silver and Magnesium oxide Nano particles) were prepared by dissolving in DMEM media. **(Fig: 21)**
- 100µl of the test compounds of silver and magnesium oxide nanoparticles was added to the wells and incubated for 24 hours, in presence of 5% CO_2, incubator maintained at 37^0 C. **(Fig: 22)**

Day 3:

- After 24 hours, 20µl of 5mg/ml MTT reagent was added to the wells. The plate was maintained for 4 hours for incubation in CO_2 chamber. **(Fig 23 -25)**
- Without interrupting the precipitated Formazan crystals supernatant was cautiously drawn out and 100µl of DMSO was added to melt the crystals developed.
- The optical density (OD) was recorded at wavelength of 492 nm using ELISA reader. **(Fig: 26)**

  Formula:

$$\textbf{Surviving cells (\%)} = \frac{\textbf{Mean OD of test compound}}{\textbf{Mean OD of control (untreated cells)}} \times 100$$

2. TO EVALUATE THE CYTOTOXICITY OF DIFFERENT CONCENTRATION OF NANOPARTICLES:

L929 Cell lines (Mouse fibroblasts)

⇩

Culturing of Cell Lines

⇩

Dulbeco's Modified Eagle Medium (DMEM- Gibco)

10% Foetal Bovine Serum

Antibiotic- Antimycotic Solution

⇩

70-80% Confluency

⇩

Suspensions of mouse fibroblasts cells in DMEM

⇩

Incubated for 24hours at 37^0 C in presence of 5 % CO_2

⇩

Cell concentration standardized to 1×10^5 cells

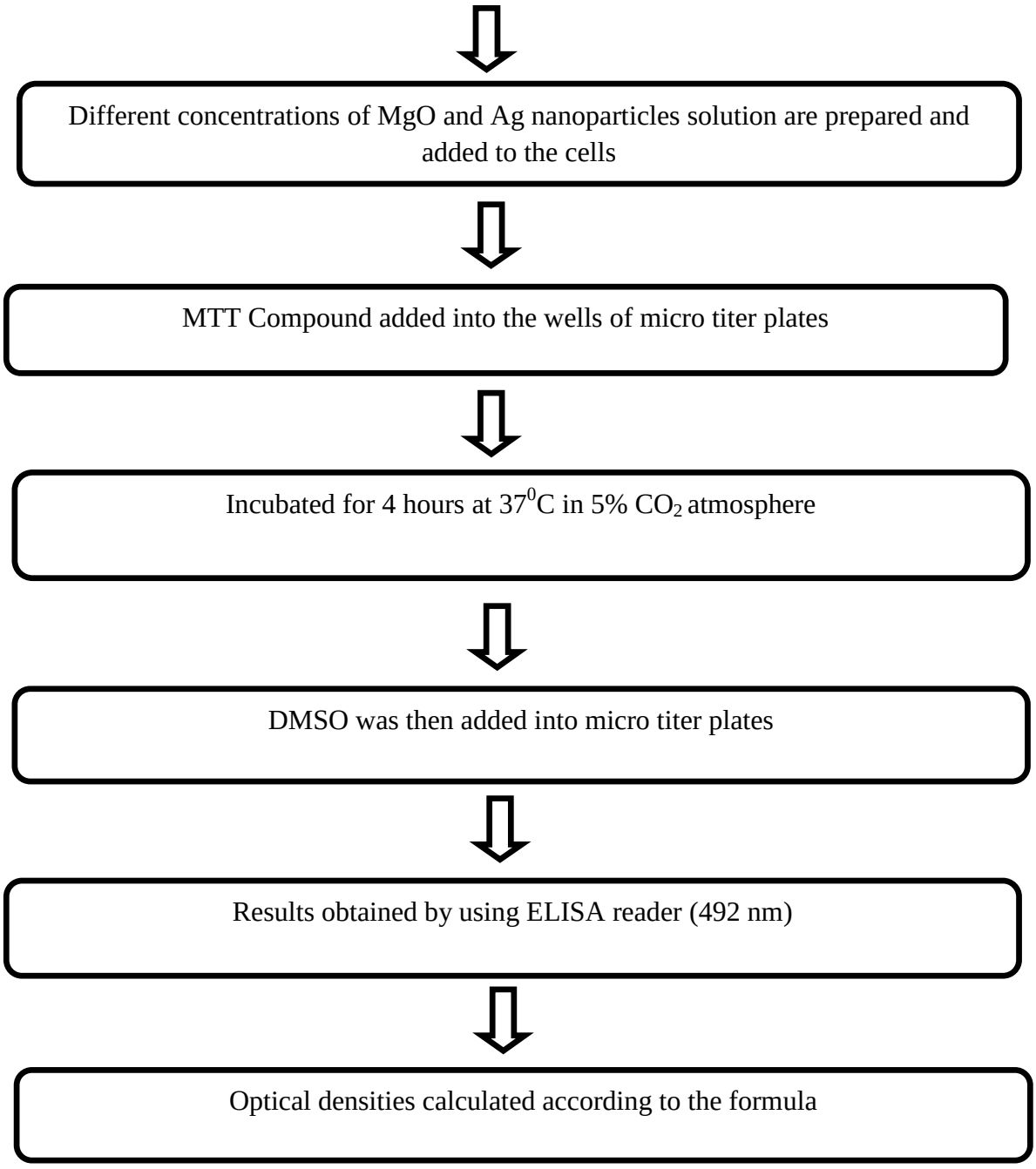

Different concentrations of MgO and Ag nanoparticles solution are prepared and added to the cells
MTT Compound added into the wells of micro titer plates
Incubated for 4 hours at 37^0C in 5% CO_2 atmosphere
DMSO was then added into micro titer plates
Results obtained by using ELISA reader (492 nm)
Optical densities calculated according to the formula

FIGURE 1: Silver and Magnesium oxide nanoparticles

FIGURE 2: Materials used in the study

Figure 3: Digital analytical balance

Figure 4: Metal mould for fabrication of specimens

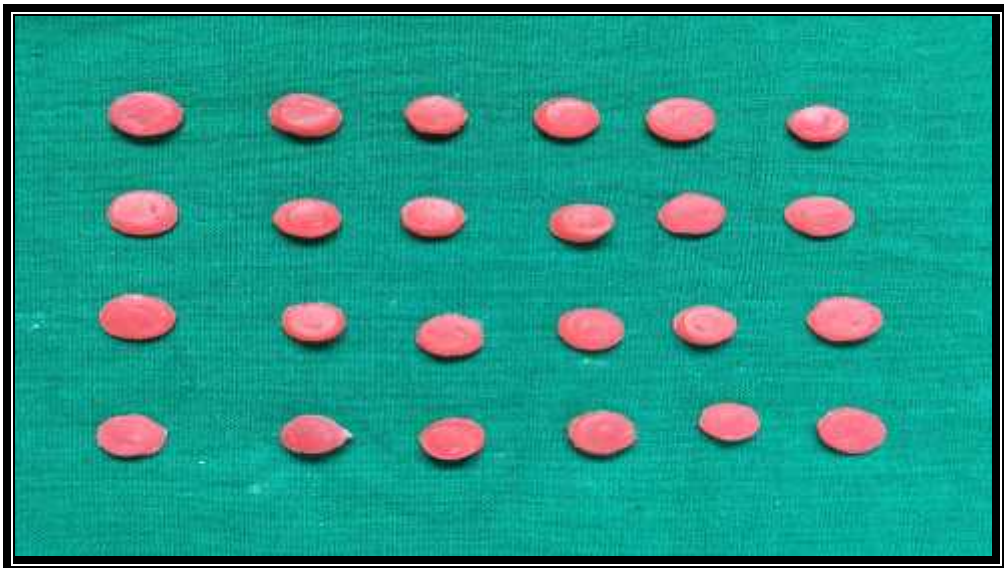


FIGURE 5: Wax patterns for the fabrication of specimens

FIGURE 6: Invested wax samples

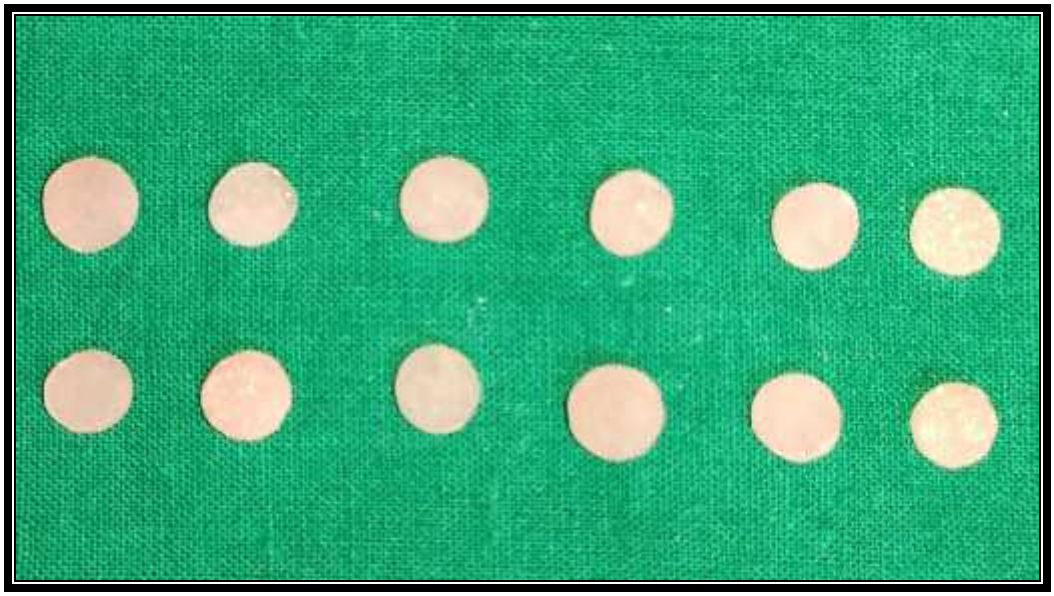


FIGURE: 7 Control disc specimens

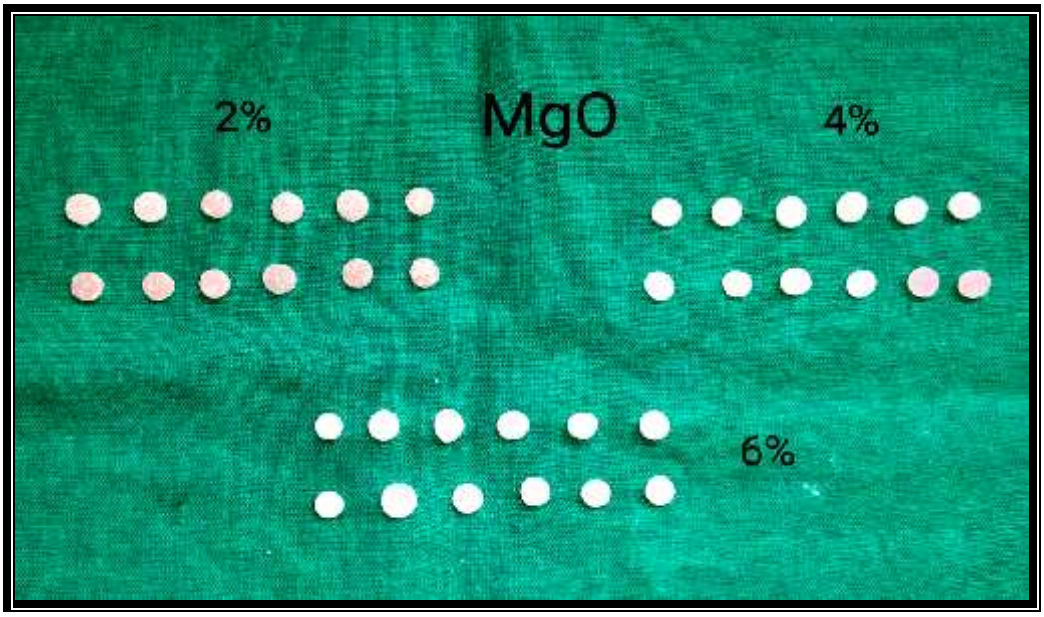


FIGURE 8: Acrylic resin disc incorporated with Magnesium oxide nanoparticles

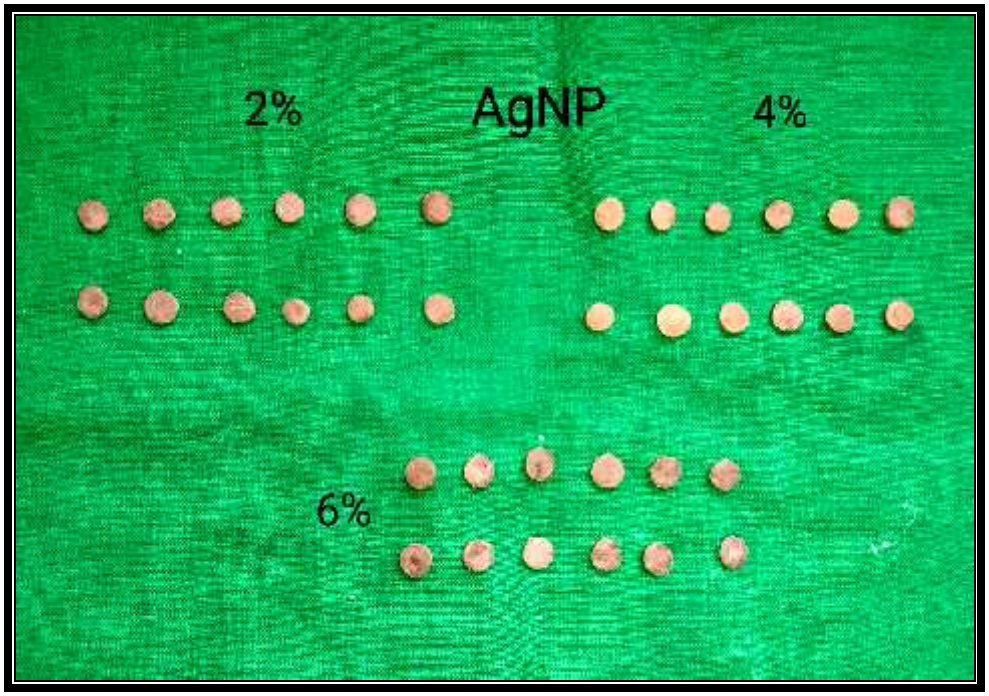


FIGURE 9: Acrylic resin disc incorporated with Silver nanoparticles

FIGURE 10: Sabouraud's dextrose agar

FIGURE 11: Candida albicans inoculum

FIGURE 12: Laminar airflow chamber

FIGURE 13: Streaking of Candida albicans inoculum on the Sabouraud's dextrose agar

FIGURE 14: Petri plates placed in an incubator at 37îC

Acrylic resin incorporated with Silver nanoparticles in 2%, 4% and 6%

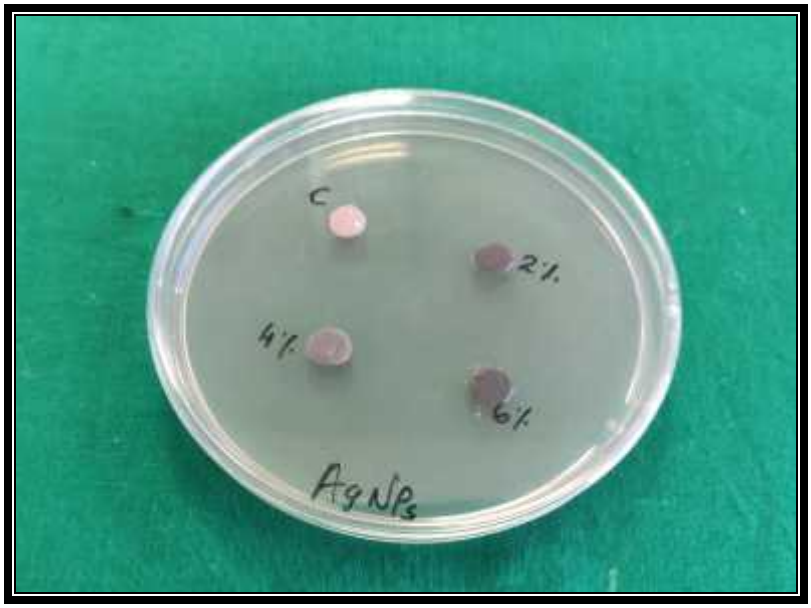


FIGURE 15: Acrylic disc placed on culture plates

FIGURE 16: Inhibition zone noted after 24 hours

Acrylic resin incorporated incorporated with Magnesium oxide nanoparticles in 2%, 4% and 6%

FIGURE 17: Acrylic disc placed on culture plates

FIGURE 18: Inhibition zone noted after 24 hours

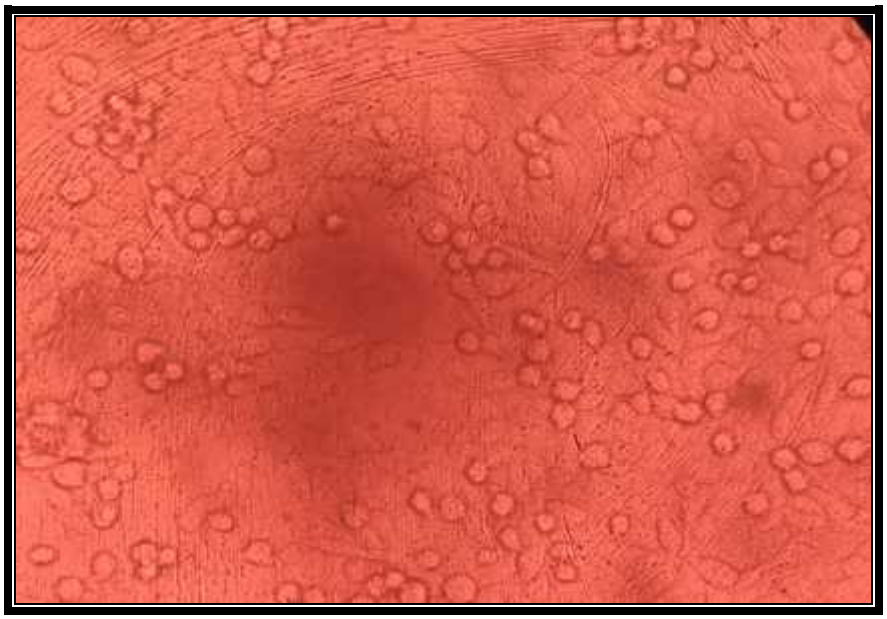


FIGURE 19: Photomicrograph showing Mouse fibroblast cell lines (L-929)

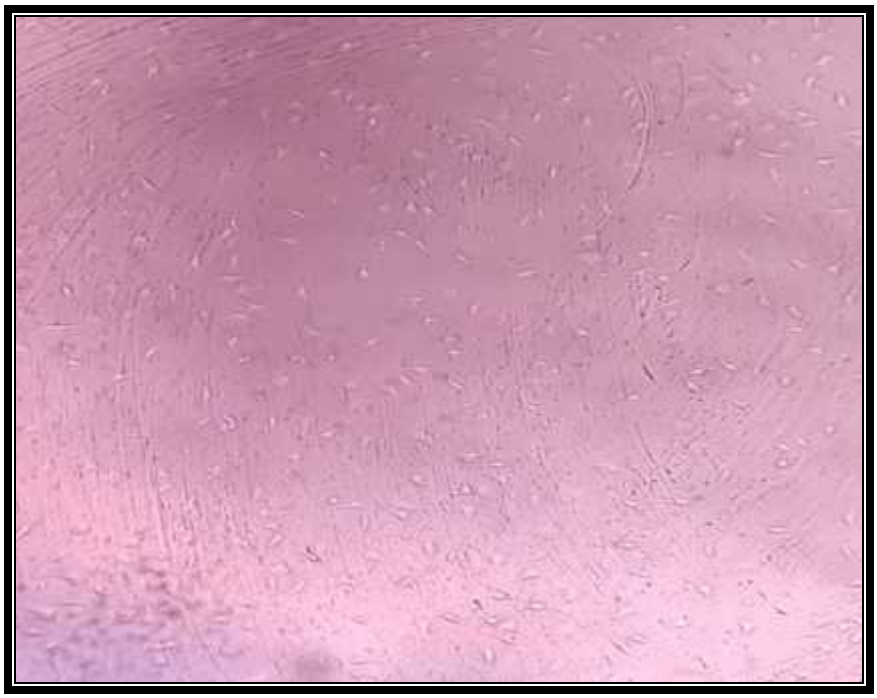


FIGURE 20: Mouse fibroblasts diluted with Dulbeco's modified eagle medium

FIGURE 21: Varied concentrations of nanoparticles

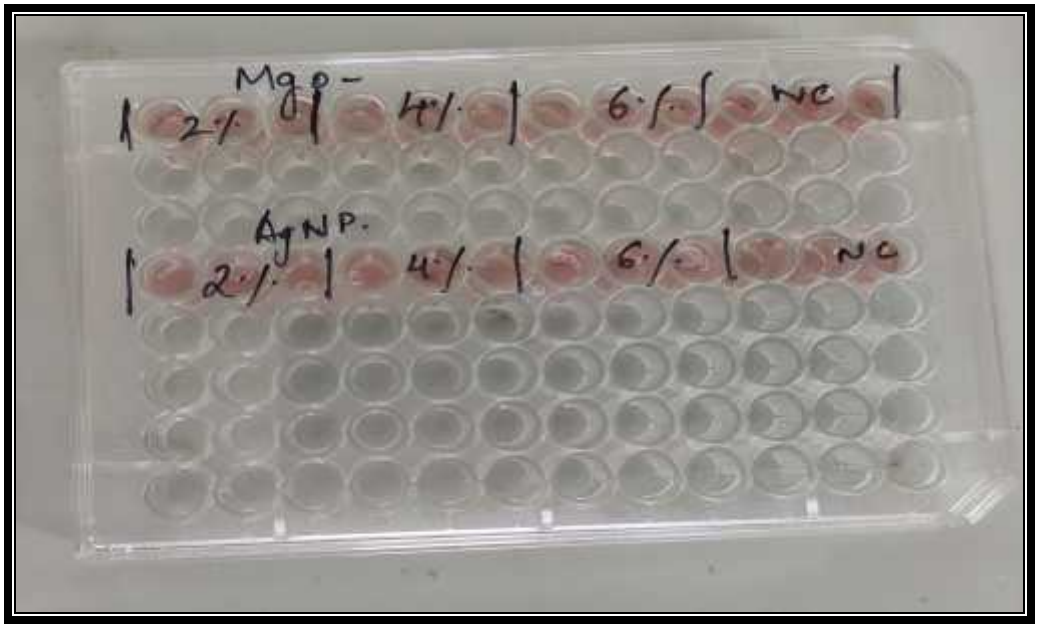


FIGURE 22: Cell lines in micro titer plates and treated with the test compound

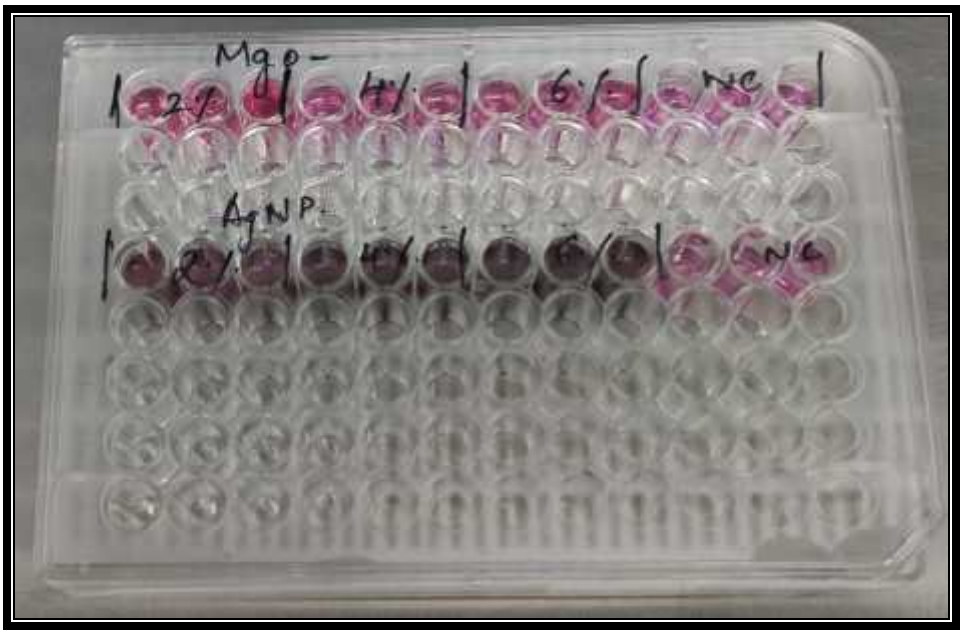


FIGURE 23: Micro titer plates after the addition of MTT compound

FIGURE 24: CO_2 Chamber for incubation of cell lines after the addition of MTT

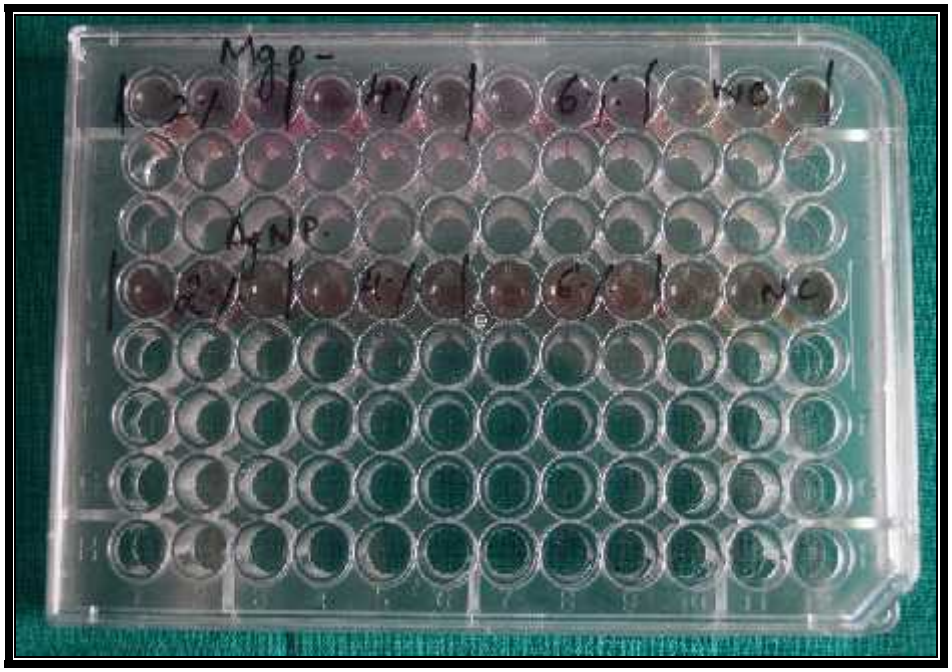


FIGURE 25: Post treatment with MTT compound and incubation

FIGURE 26: ELISA reader for optical density reading

RESULTS

The present study was conducted to evaluate and compare the anti-candidal property of acrylic resin reinforced with Silver and Magnesium oxide nanoparticles and its cytotoxic effects on mouse fibroblasts.

Statistical Analysis:

Data gained from the study was uploaded in Microsoft excel sheet and SPSS version 20 software, was used to perform the statistical analysis. Basic review of the data was illustrated by descriptive statistics.

Two-way ANOVA was used for comparison of two groups with mean **Zone of inhibition(DIZ)** in mm (anti-candidal activity) scores after 24 hours and pair wise comparison of two groups and sub groups with mean zone of inhibition (mm) scores after 24 hours by Tukey's multiple posthoc procedures.

- **ANTI-CANDIDA TEST:**

Anti-candidal activity was evaluated for Silver and Magnesium oxide nanoparticles group for three different concentration 2%, 4% and 6% after 24 hours. Mean, standard deviation and standard error were calculated (Table 3). The mean values (DIZ) for Silver nanoparticles at 2%, 4% and 6% after 24 hours were 13.71(±2.07), 18.58 (±1.62) and 27.96 (±1.76) respectively. The mean values (DIZ) for Magnesium oxide nanoparticles at 2%, 4% and 6% after 24 hours were 11.63 (±1.35), 14.38 (±1.63) and 18.25 (±1.39) respectively. Thus Silver nanoparticles exhibited more anti-candidal activity as compared to the Magnesium oxide nanoparticlesgroup and control group had complete growth of Candida and therefore was not considered in the analysis.

Table 3: Summary of Anti-candidal activity in two nano particles (Silver and Magnesium oxide) and sub groups (2%, 4% and 6%).

Groups and concentrations	n	Mean	SD	SE
Silver Nano particles with 2%	12	13.71	2.07	0.60
Silver Nano particles with 4%	12	18.58	1.62	0.47
Silver Nano particles with 6%	12	27.96	1.76	0.51
Magnesium oxide Nano particles with 2%	12	11.63	1.35	0.39
Magnesium oxide Nano particles with 4%	12	14.38	1.63	0.47
Magnesium oxide Nano particles with 6%	12	18.25	1.39	0.40

Above table summarizes Anti-candidal activity in both the groups (Silver and Magnesium oxide nanoparticles) and sub groups (2%, 4%, 6% concentrations). Mean standard deviation and standard error was calculated.

Table 4: Comparison of two groups (Silver and Magnesium oxide nanoparticles) and sub groups of 3 concentrations (2%, 4% and 6%) with anti-candidal activity by two way ANOVA.

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Main effects					
Groups	1	512.00	512.00	186.9751	0.0001*
sub groups	2	1338.94	669.47	244.4804	0.0001*
2-way interaction effects					
Groups x sub groups	2	185.81	92.91	33.9280	0.0001*
Error	66	180.73	2.74		
Total	71	2217.48			

*$p<0.05$

Group 1= DPI Heat cure resin incorporated with Silver nanoparticles, Group 2= DPI Heat cure resin incorporated with Magnesium oxide nanoparticles.

Table 5: Pair wise comparison of two nano particles (silver and Magnesium oxide) and sub groups (2%, 4% and 6%) with anti-candidal activity by Tukeys multiple posthoc procedures.

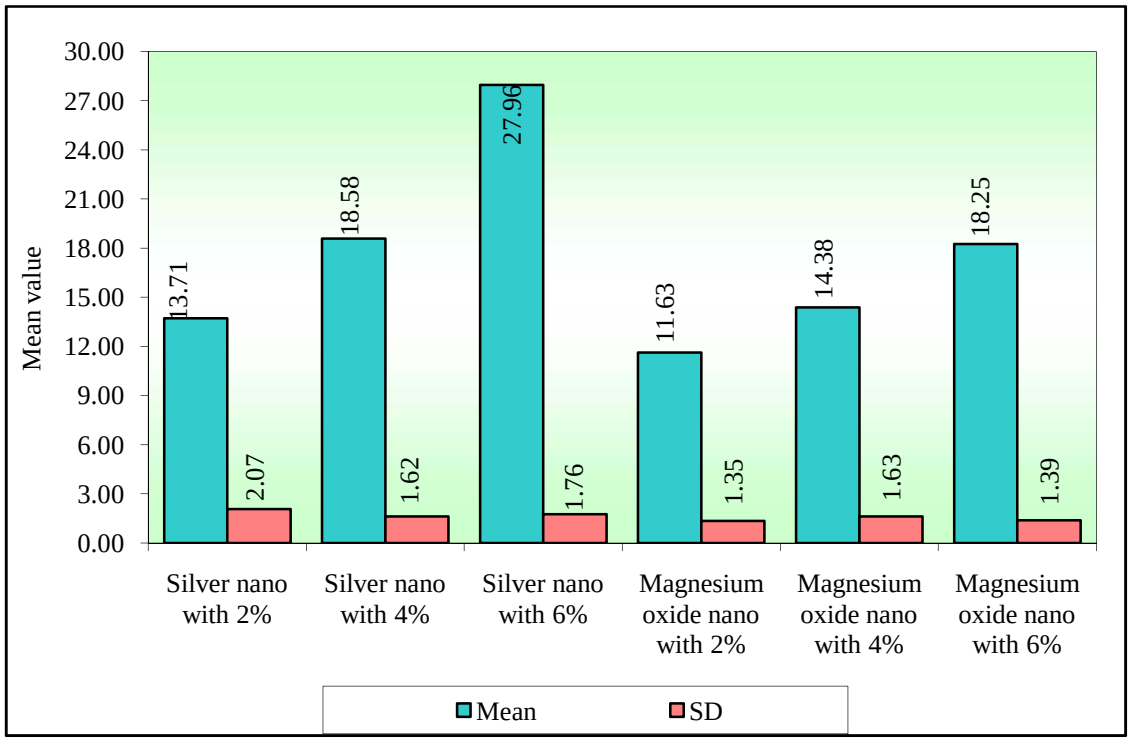
Groups and sub groups	Silver nano with 2%	Silver nano with 4%	Silver nano with 6%	Magnesium oxide nano with 2%	Magnesium oxide nano with 4%	Magnesium oxide nano with 6%
Mean	13.71	18.58	27.96	11.63	14.38	18.25
SD	2.07	1.62	1.76	1.35	1.63	1.39
Silver nano with 2%	-					
Silver nano with 4%	p=0.0001*	-				
Silver nano with 6%	p=0.0001*	p=0.0001*	-			
Magnesium oxide nano with 2%	p=0.0340*	p=0.0001*	p=0.0001*	-		
Magnesium oxide nano with 4%	p=0.9206	p=0.0001*	p=0.0001*	p=0.0018*	-	
Magnesium oxide nano with 6%	p=0.0001*	p=0.9963	p=0.0001*	p=0.0001*	p=0.0001*	-

*p<0.05

Pair wise comparison of two nanoparticles (Silver and Magnesium oxide) and subgroups (2%, 4% and 6%) with anti-candidal activity (DIZ) after 24 hours by Tukey's multiple posthoc procedures (Table 4). The table compares anti-

candidalactivity measured by the zone of inhibition between the groups at different concentration. Thus, there was a statistically significant difference ($p<0.05$) in zone of inhibition at different concentration within the groups. Highest zone of inhibition (anti-candidal activity) was seen in silver nanoparticles group with good inhibition zone.

Graph 1: Comparison of two nanoparticles (silver and magnesium oxide) and sub groups (2%,4% and 6%) with anti-candidal activity.



Above figure depicts graphical presentation of comparison of mean values and standard deviation of silver and magnesium oxide nanoparticles at different concentration (2%, 4% and 6%). Highest mean value for anti-candidal activity was observed with silver nanoparticles at 6% concentration (27.96) and lowest anti-candidal activity was observed with magnesium oxide nanoparticles at 2% concentration (1.35).

Graph 2: Comparison of two nano particles (silver and Magnesium oxide) and sub groups (2%, 4% and 6%) with anti-candidal activity.

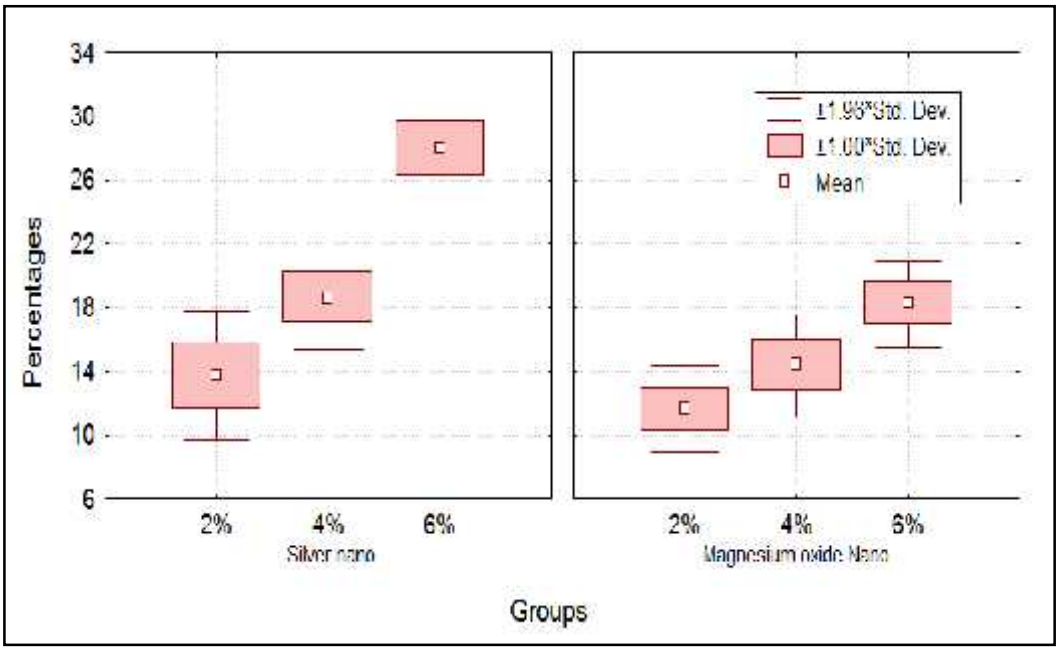
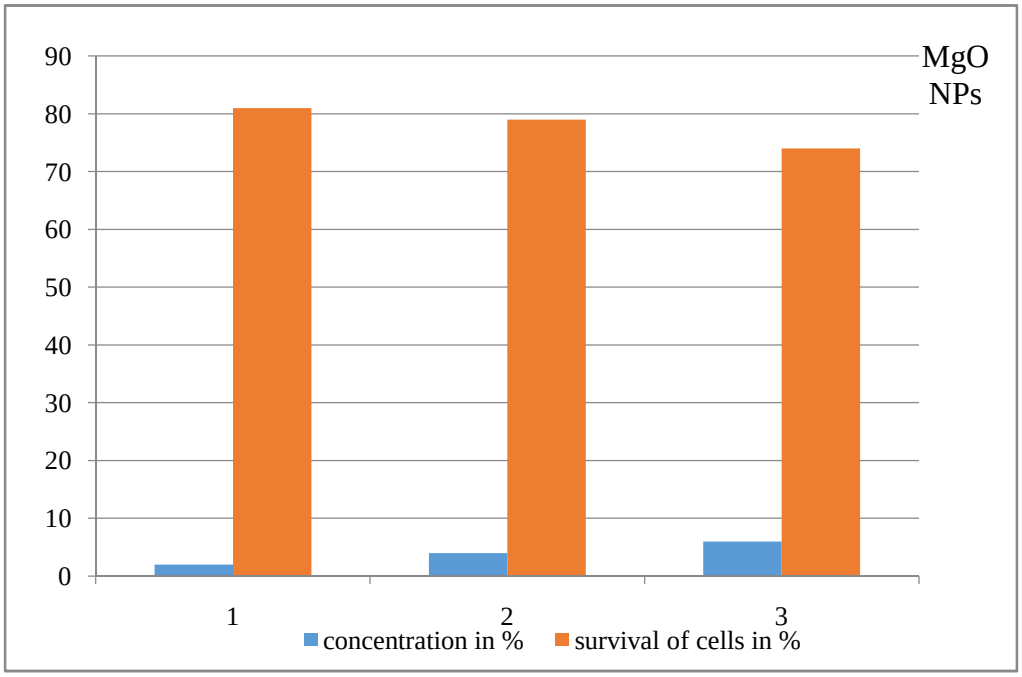


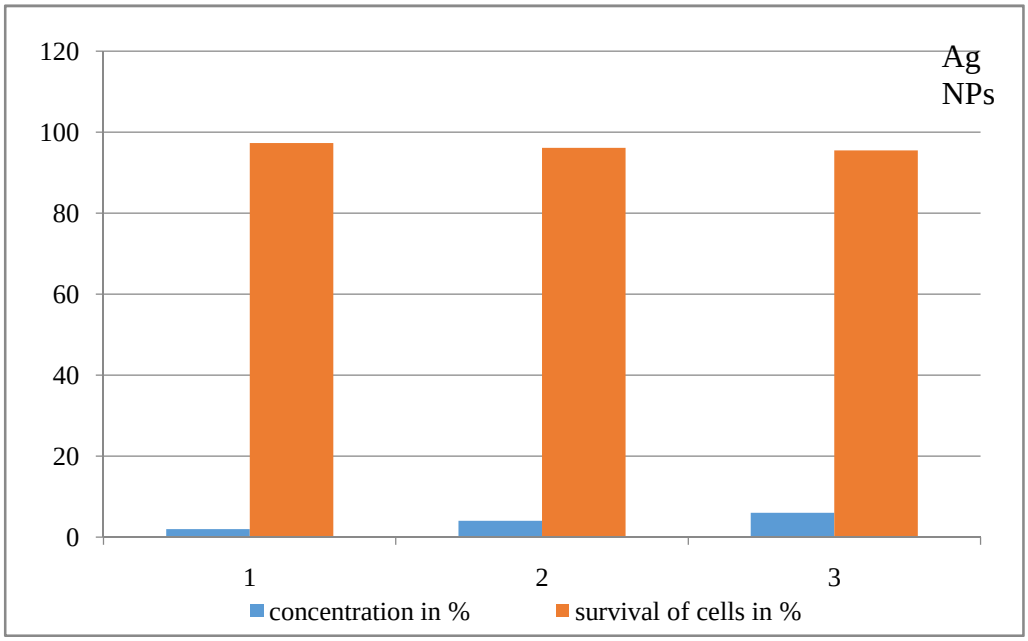
Table:6MTT Assay On L929 Cells

MgO COMPOUND	**CONCENTRATION**	**% SURVIVAL OF CELLS**
	2%	81
	4%	79
	6%	74
AgNP COMPOUND	**CONCENTRATION**	**% SURVIVAL OF CELLS**
	2%	**97.32**
	4%	96.10
	6%	95.53

The above table summarizes the percentages of survival of cells treated with different concentration of MgO and Silver nanoparticles suggesting overall that the Concentrations of MgO showed more Cytotoxicity on mouse fibroblasts with 74% of survival of cells at 6% MgO. Whereas the Concentrations of Ag NPs resulted less Cytotoxicity with 97.32% of cell survival at 2% concentration.

Graph 3: Graphical representation of Cytotoxicity of silver and magnesium oxide nanoparticles:





DISCUSSION

Denture induced erythematous candidiasis; an inflammatory oral mucosal lesion is a widely seen clinical condition in older adults and immune compromised patients. An increasing incidence of denture stomatitis is seen in recent years as candida albicans have an adhering tendency to mucosa as well as to the surfaces of denture infecting 60% of complete denture wearers.[61, 2]

Polymethyl methacrylate (PMMA) acrylic resin is a universally accepted denture base material because of its certain characteristics such as color stability, aesthetics and its simplicity in processing. Although PMMA attains all the specification of a flawless acrylic resin material, it lacks in resisting the microbial growth on its surface in the oral habitat. In addition to its rough intaglio surface local factors such as poorly fitting denture and poor oral hygiene contributes to denture stomatitis.[22] Considering the aforesaid factors there is a need to develop PMMA having inherent anti-candida effect to improve the quality of life of older individuals.

Nanoparticles (1 to 100 nm) have features unique to their properties due to reduced size of the atomic-grade products. They have distinctive physicochemical properties, Optical and biological features that are manipulated for the suitable medical applications we wish.[9] In fact, metallic nanoparticles infused in PMMA perhaps upgrades mechanical, antimicrobial, antifungal and physical properties of acrylic resin.[55] The potential of metallic nanoparticles has fascinated in the field of medicine due to its assuring antibacterial activity in small concentration as well as its non-cytotoxic nature with mammalian cells. Also, microorganisms are unlikely to develop resistance as seen in antibiotics against nanoparticles.[45]

In this study, metallic nanoparticles like Silver and Magnesium oxide nanoparticles have been incorporated into PMMA acrylic resin to evaluate the anti-candidal property and also assessed the Cytotoxicity of three different concentrations (2%, 4%and 6%) of silver and magnesium oxide nanoparticles used for anti-candidal test in L929 mouse fibroblasts. Incorporation of these nanoparticles into acrylic resin resulted to have an antifungal effect at different concentrations thus rejecting the null hypothesis.

For determining the anti-candida effect, *Candida albicans* was used as the reference strain as it is the common causative factor for denture stomatitis. Incorporation of 2%, 4%, 6% concentration of silver and magnesium oxide nanoparticles into denture base resin (PMMA) resulted in antifungal effect against *Candida albicans*.

The results of the study showed that silver and magnesium oxide nanoparticles of different concentration integrated into PMMA both found to have anti-candidal activity but silver nanoparticles exhibited to be more active in the inhibition of the Candida when compared with magnesium oxide nanoparticles. The mean value (DIZ in mm) for silver nanoparticles group at the concentrations of 2%, 4% and 6% after 24 hours were 13.71, 18.58 and 27.96 respectively. The mean value (DIZ in mm) for magnesium oxide nanoparticles group at the concentrations of 2%, 4% and 6% after 24 hours were 11.63, 14.38, and 18.25 respectively. Control group samples didn't show any anti-candida activity which was in accordance to Wady AF et al stating that acrylic resin has no or little anti-candida activity.[62] (Table 3)

Silver nanoparticles utilized in dental materials are reinforced through different modes, based on the material type. Generally in adhesive systems and resins,

the usual method is incorporating a monomer 2-(tert-butylamino) ethyl methacrylate, so as to ensure Ag salts are dissolved in the resin solution. In some studies Ag NPs are obtained through reduction of $AgNO_3$, sodium citrate, gallic acid and $NaBO_4$ whereas in this study AgNPs are procured commercially from the producer (ultra-nanotech Ltd).[22]

Casemiro et al considered agar well diffusion for the antifungal assay with 2.5%, 5.0%, 7.5% and 10% of silver zeolite and demonstrated that increase in percentage of silver zeolite accelerates the antimicrobial effect. Even though it gave excellent results, PMMA gained opacity by the addition of compound resulting in an undesirable appearance supporting the present study.[35] The acrylic resin integrated with silver nanoparticles sustained a colour change which is considered as an inevitable functional attribute of dental base materials due to the plasmon effect of nanoparticles. This paradox was very significant for concentrations of silver nanoparticles above 80 ppm. [63]

Casemiro et al also noted a turn down in flexural and impact strength by the integration of zeolites to denture base resins paving a way to breakage of prosthesis when it endures cyclic deformation.[35] Along with that, Mirizadeh et al confirmed that by the addition of N,N-dimethylaminoethyl methacrylate (DMAEMA) monomer; an antimicrobial agent to an acrylic resin tends to decrease the flexural strength as well as flexural modulus of the modified acrylic acrylic resin. Here Quartenized ammonium monomer incorporated in the denture base resin give way to increased water sorption which then reacted as a plasticizer and thus dismissing the mechanical property of the resin which can contribute midline fracture due to frequent flexural forces.[30] Contradicting to that Harini et al upgraded PMMA with titanium oxide

nanoparticles and exhibited higher grade flexural strength than that of conventional acrylic resin.[47]

Thereby suggesting that mechanical property is of greater importance in complete denture fabrication and need to be investigated in future studies along with antimicrobial or antifungal assays.

The current study carried out disc diffusion method as stated by Suganya et al and suggested that antimicrobial activity by silver integrated acrylic resin could not be confirmed whether the silver ions have leached out or there was an immediate contact with the silver and cells.[48] Whereas Rama Krishna et al suggested the direct contact technique for the antimicrobial assay of acrylic resin reinforced silver in varied concentrations against c. albicans and s. mutans and found out higher activity to C albicans and proposed that its antimicrobial effect rely on dispersal of nanoparticles in the acrylic resin and its particle size.[58]

As there is a strong antifungal activity noted with 2%, 4% and 6% of silver nanoparticles, it can be depicted that reduced size of nanoparticles owns a broad dissemination in PMMA matrix and creates wide area for oxidation.[63]

Mechanism of action of silver is that they sensitize oxygen and transforms to active oxygen by catalytic activity and causes structural damage to fungal membrane creating an 'oligodynamic action' as explained by Nam et al.[41] Also Li et al suggested that AgNPs can disrupt the cell membrane of fungus along with inhibition of normal cell replication and higher concentration of Ag depletes biofilm formation.[49]

According to Monteiro et al, silver nanoparticles are highly reactive and have a greater surface area which helps in binding to biological molecules with sulfur

contents as well as with phosphorous constituting elements such as DNA. This can bring about depletion of certain enzymes, deformed cell membranes and erosion of intracellular contents.[40]

Magnesium nanoparticle has been selected for this study because of its biocompatibility, good thermal stability and its feasible production. The principal aim of magnesium oxide nanoparticles is the cell wall of the bacteria. In aqueous conditions MgO possess bactericidal activity causing superoxide anion formation which in turn results in derangement of cell membrane and resulted in agglomeration of nanoparticle within the bacterial membrane.[45, 64]

Hajahameed et al carried out disc diffusion method to determine the antifungal effect of ZnO and alkali metal ions Mg, Ca, Sr and Ba doped ZnO NPs against C. albicans. Mg doped ZnO nanoparticles was found positive after incubation and was operated by electrostatic interaction which gave way to an increased antifungal effect than the other metal ions interacted with ZnO.[50] They also suggested the release of reactive oxygen species (ROS) which could be the causative agent for the elimination of C. albicans.

On utilization of metallic nanoparticles to restorative mediums cytotoxicity is one of the prime concerns. Zhang et al assessed the Cytotoxicity in human gingival fibroblast after the addition of 0.05% of silver nanoparticles in primer and adhesives where they have discovered it as a non toxic antimicrobial agent.[65] Agreeing to that, Acosta-torres et al developed a denture base incorporated with 1 μg/mL silver nanoparticles in which they have evaluated the antifungal activity as well as checked the biocompatibility of novel compound with human lymphocytes and mouse fibroblasts and found to be non-cytotoxic or genotoxic.[42] Similarly Cierech et al used zinc oxide nanoparticles (ZnO NPs) and tested its release from polymethyl

methacrylate –ZnO nano composites (2.5%, 5%, and 7.5% w/w). MTT assay exhibited no cytotoxic effect up to 20mg/L concentrations of ZnO on human HeLa cell line.[60]

In the present study mouse fibroblast (L929 Cells) was used as it is identical with that of human fibroblast. The Cytotoxicity of silver and magnesium oxide nanoparticles was assessed and we found that magnesium oxide nanoparticles with 6% concentration was cytotoxic with 74% of cells survived and rest all the concentration of silver nanoparticles were non toxic with survival rate of cells was more than 95%. Hence our MTT assay results showed that the concentrations of Ag nanoparticles are nontoxic to the normal mouse fibroblasts when compared with MgO nanoparticles. (Table 6)

Supporting to the results Mahmoud et al explained that MgO NPs induced cell death occurs generally by oxidative stress. For the nanoparticle activated apoptosis mitochondrial pathway is the commonest as mitochondria is the leading organelle for oxidative stress. They have used various assays to determine toxic levels of MgO nanoparticle on different cell lines such as intestine (Caco-2), lung (A549), liver (HepG2) and kidney (NRK-52E) resulted as oxidative damage, cell death and DNA impairment.[66]

Hence to sum up the results of the present study, anti-candida property was exhibited by test samples when compared to control samples. Since silver and magnesium oxide nanoparticles can act as a cure from denture stomatitis, the addition of it into acrylic resins should be considered as it can effectively act against fungi. However, the biocompatibility of the material clinically should be checked and implemented for future use.

SCOPE OF THE STUDY

The study evaluated and compared the anti-candidal property of acrylic resin reinforced with silver and magnesium oxide nanoparticles and their effect on cytotoxic levels in different concentrations (2%, 4% and 6%).

Further research is suggested to determine the specific mechanism of action of silver and magnesium oxide nanoparticles and its systemic effect due to release of silver as well as magnesium ions for a safe clinical application.

Further investigations evaluating mechanical property and physical stability may add relevant new information on this matter.

This study should be broadened by the use of various other microbial strains in the oral cavity and various other concentrations of silver and magnesium oxide nanoparticles.

Other mechanical properties like tensile strength, compressive strength, impact strength and color stability need to be evaluated.

As it was an in vitro study, further in vivo parameters should be considered with varied clinical conditions and long term follow up can be carried out.

LIMITATIONS OF THE STUDY

- Since this is an in vitro study application of the results in clinical conditions might yield different result.
- In the present study for anti-candida test only one strain of Candida species – *Candida albicans* was considered.
- It could not be concluded whether anti-candida effect which was revealed by test samples was because of silver and magnesium ion release into the surrounding media or direct contact between silver and magnesium oxide nanoparticles with *Candida* cells.
- Only one type of heat cure polymethylmethacrylate resin was included in the study, inclusion of different PMMA resins and various other concentrations of silver and magnesium oxide nanoparticles might give different results.
- Physical stability of silver and magnesium oxide nanoparticles were not studied, and must be carried out before clinical use since there was a change in color of specimens which affects the esthetics of complete dentures and will not be pleasing for the patients.

CLINICAL IMPLICATIONS

PMMA reinforced with silver and magnesium oxide nanoparticles can be considered as a promising denture base material with inherent anti-candidal property which could be practiced as a possible treatment regimen to improve oral health status of geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity.

Because of increase in health care cost as well as resistance exhibited by the microorganisms to the local and systemic antibiotics, it could also be used as a remedy for Candida associated denture stomatitis not responding to conventional therapy thereby helps to improve the quality of life of older individuals.

CONCLUSION

Within the limitations of the present in-vitro study, the following conclusions can be drawn.

- Silver and magnesium oxide nanoparticles can serve as a potential anti-candidal agent for treatment against denture stomatitis.
- The three concentrations of silver nanoparticles showed good anti-candidal activity as well as survival of cells were more with silver.
- However, Magnesium oxide nanoparticles showed to be more cytotoxic than silver nanoparticles.
- For clinical use further studies are needed to determine the color stability of acrylic resin and to assess the systemic toxicity.

SUMMARY

The present in-vitro study was carried out to evaluate and compare the anti-candidal property of acrylic resin reinforced with silver and magnesium oxide nanoparticles and their effect on cytotoxic levels.

The silver and magnesium oxide nanoparticles obtained commercially and separately weighed to three different concentrations (2%, 4% and 6%). A total of 84 samples were fabricated to evaluate the anti-candidal effect through disk diffusion method and Cytotoxicity of different concentrations of nanoparticles was checked by MTT assay.

Samples were fabricated of 12 control samples (without any nanoparticles incorporated), 36 samples of silver nanoparticles and 36 samples of magnesium oxide nanoparticles. Three concentrations of samples fabricated and candida albicans was streaked and then incubated for 24 hours.

The resultant data was tabulated and subjected to statistical analysis using SPSS software version 20. Two-way ANOVA was used for comparison of two groups with both mean score at different concentrations and pair wise comparison of two groups with both mean scores at different concentrations by Tukey's multiple posthoc procedures.

According to the results obtained, there was statistically significant increase in antifungal activity after incorporation of silver nanoparticles and magnesium oxide nanoparticles, evaluated at 24 hours. However magnesium oxide nanoparticles showed higher Cytotoxicity and cannot be considered for clinical applications. Thus,

according to the study and obtained results we can recommend the use of silver nanoparticles when incorporated at 2%, 4% and 6% into the denture base resins can prove to be beneficial to improve oral health status of the geriatric patients with cognitive disturbances, medically compromised conditions and reduced manual dexterity.

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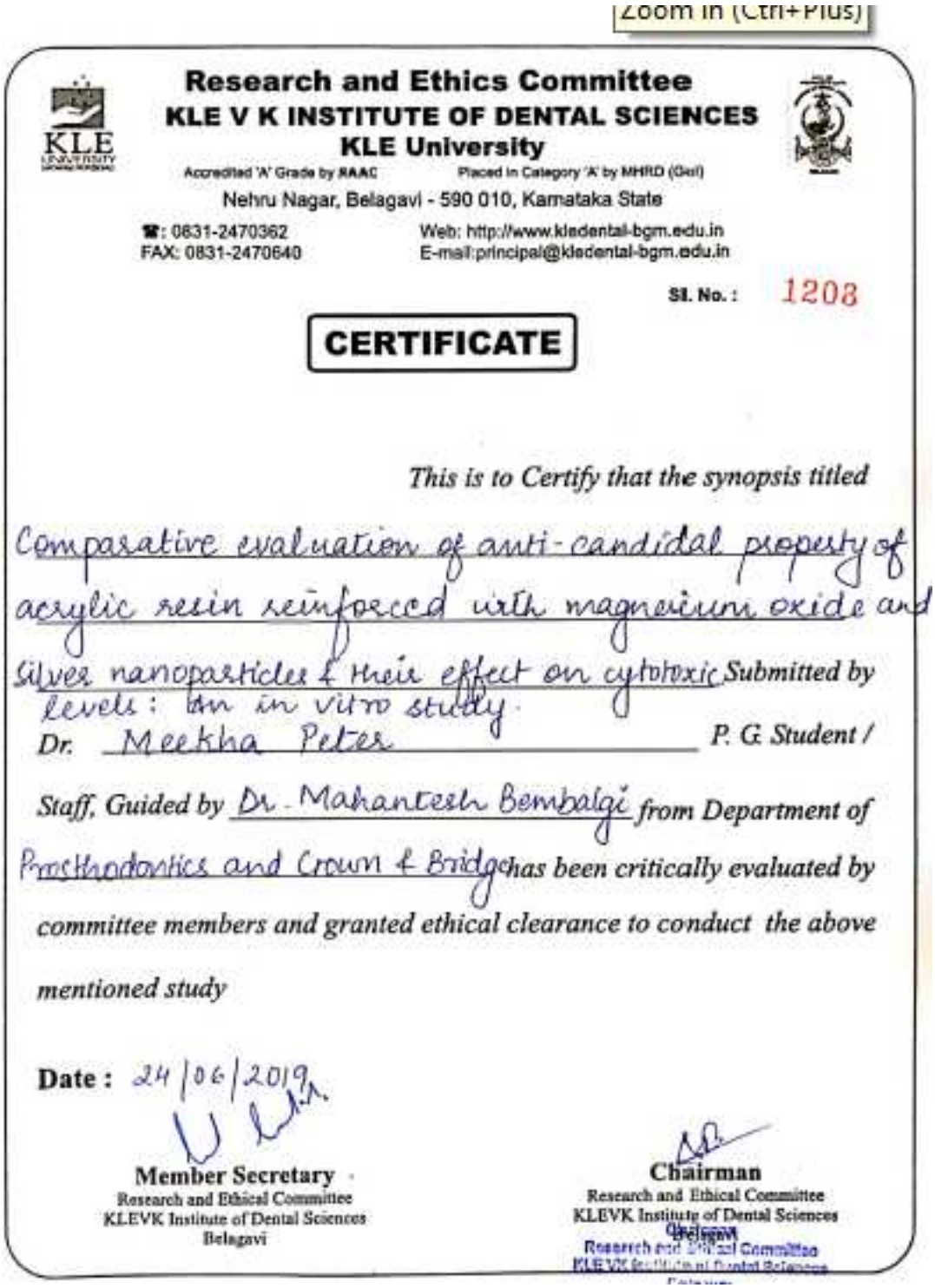
ANNEXURE I

ETHICAL CLEARANCE



Zoom in (Ctrl+Plus)

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (Govt)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Sl. No.: 1208

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of anti-candidal property of acrylic resin reinforced with magnesium oxide and silver nanoparticles & their effect on cytotoxic levels: An in vitro study.

Submitted by Dr. Meekha Peter *P. G. Student / Staff, Guided by* Dr. Mahantesh Bembalgi *from Department of* Prosthodontics and Crown & Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 24/06/2019

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belgaum

ANNEXURE II

Anti-candidal activity with silver nanoparticles

Silver nano particles	**Ag NPs**		
	In mm		
2% (12 samples)	**2%- 12 samples**	**4% - 12 samples**	**6 % - 12 samples**
	1 day	1 day	1 day
plate-1	12	20	28.5
plate 2	15	19	30
plate 3	16.2	18	26
plate 4	12.3	20	25.5
plate 5	15	19	28
plate 6	11.5	18	29.5
plate 7	12.5	17	24.5
plate 8	10	21	29
plate 9	15	16	28
plate 10	13	19	30
plate 11	16	16	28
plate 12	16	20	28.5

ANNEXURE III

Anti-candidal activity with Magnesium oxide nanoparticles

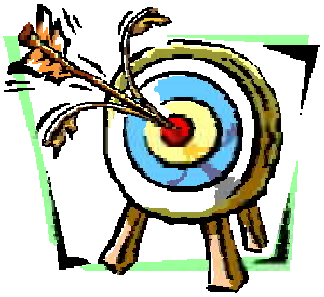
Magnesium oxide nanoparticles			
	In mm	**12 samples**	
2%- 12 samples	**2% - 12 samples**	**4%- 12 samples**	**6%- 12 samples**
	1 day	**1 day**	1 day
plate 1	10	14	18
plate 2	12	15	**19**
plate 3	10	13	**20**
plate 4	13	12.5	16
plate 5	12.5	16	17
plate 6	13	14	16.5
plate 7	14	15.5	20.5
plate 8	10.5	16.5	19
plate 9	12	17	19.5
plate 10	10	12	18
plate 11	11.5	13	17.5
plate 12	11	14	18

ANNEXURE III

Cytotoxicity of different concentration of nanoparticles:

	MTT ASSAY	**L929 cells**		
	conc	OD	Mean	% viability
	nc	0.455	0.368	100
		0.339		
		0.31		
	2	0.203	0.213	81
		0.241		
MgO		0.195		
	4	0.235	0.233333	79
		0.211		
		0.254		
	6	0.285	0.26	74
		0.312		
		0.183		
	2	0.298	0.248	97.32
AgNP		0.135		
		0.311		
	4	0.157	0.242667	96.1
		0.271		
		0.3		
	6	0.241	0.276333	95.53
		0.304		
		0.284		

Introduction



Need For the Study

Hypothesis

Aim and Objectives

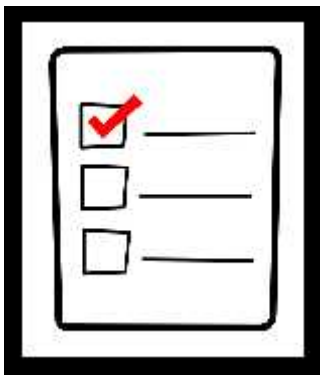
Review of Literature

Methodology

Results

Discussion

Scope of the Study



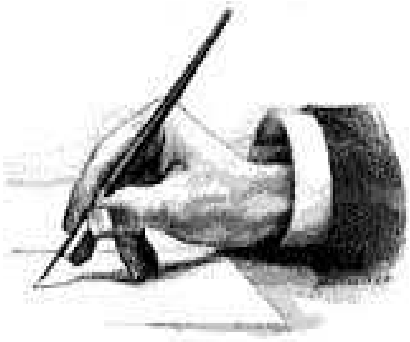
Limitations

Clinical Implication

Conclusion

Summary

Bibliography



Annexures