"ANTIMICROBIAL EFFICACY AND OSTEOGENIC POTENTIAL OF TRIPHALA AND CISSUS QUADRANGULARIS COMBINATION, AS A HYDROGEL COATING ON IMPLANT SURFACE FOR PERI-IMPLANTITIS AND OSSEOINTEGRATION- AN IN-VITRO STUDY"

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LIST OF ABBREVIATIONS USED IN THE STUDY

ABBREVATIONS	FULL FORMS
TC	Triphala powder
CQ	Cissus Quadrangularis
TCQ	Triphala & Cissus Quadrangularis Hydrogel
Study Group	Disc coated with (0.5%) Novel Hydrogel
Control $_{(Ost)}$ Group	Disc without novel hydrogel coating
Control $_{(Chx)}$ Group	Disc coated with (2%) Chlorhexidine gel
HA	Hydroxyapatite
Ti6Al4V	Titanium-6 aluminum-4 vanadium
TiO2	Titanium dioxide
CO_2	Carbon dioxide
MOC	Murine osteoblastic cells
BIC	Bone Implant Contact
Ra	Average surface roughness value
MG-63	Human osteosarcoma cells
CC-2511	Human Fibroblast cells
RCT	Randomized Controlled trial
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

PMN	Polymorph neutrophil
MMP	Matrix Metallo proteinase
ECM	Extra cellular matrix
LPS	Lipopolysaccharides
EPS	Extra cellular polymeric substances
IL	Interleukins
TNF	Tumor necrosis factor
CRP	C-Reactive protein
DPPH	2,2—Diphenyl-1-Picrylhydrazyl
FRAP	Fluorescence recovery after photo bleaching
COX	Cyclo oxygenase
NF-Kb	Nuclear factor kappa B
RANKL	Receptor activator nuclear factor- kappa Ligand
CHX	Chlorhexidine
ALP	Alkaline Phosphatase
MAPK	Mitogen activated protein kinase
B.D.	Twice a Day
ASTM	American society of Testing and Materials
ATCC	American type culture collection
EDTA	Ethylenediaminetetraacetic acid
SEM	Scanning electron microscope

DMSO	Dimethyl sulfoxide
MIC	Minium inhibitory concentration
MBC	Minium bactericidal concentration
rpm	Rotations per minute
PBS	Phosphate buffer solution
hrs	Hours
µl	Microliter
mg	Milligram
ml	Milliliter
gm	Grams
°C	Degree Celsius
w/v	Weight by volume
%	Percentage
O.D.	Optical density
SD	Standard Deviation
SE	Standard of Error
ANOVA	Analysis of variance

ABSTRACT

STATEMENT OF PROBLEM

Implant dentistry provides essential functions such as normal shape, aesthetics, speech, and health, even in cases of atrophy, disease, or injury to the stomatognathic system. Ti6Al4V titanium alloys are preferred for their mechanical properties and biocompatibility over traditional materials like stainless steel 316L and cobalt-chromium alloys. However, titanium has limitations as an implant material. Such as lack of antimicrobial property, bio inertness which leads to increases the risk of implant failure or loosening.

Surface modification also seeks to improve the implant's antibacterial qualities, corrosion resistance, and bioactivity while maintaining its mechanical qualities and biocompatibility. Although commercially available antimicrobials are used to address early periimplantitis, their release at suboptimal concentrations may lead to bacterial resistance, while high doses can disrupt the oral microflora, cause cell toxicity, and hinder osseointegration.

This research study explores traditional natural medicines to achieve predictable results on specific parameters. Triphala Powder (TC), a blend of three fruits—Emblica officinalis, Terminalia bellerica, and Terminalia chebula—is rich in vitamin C, nicotinic acid, and Chebulinic acid, which have antimicrobial and antifungal properties. Incorporating TC into a hydrogel mixture can enhance the implant osteotomy site by decreasing microbial activity. Additionally, Cissus quadrangularis, another herbal remedy, has shown osteogenic potential, which may facilitate bone formation between the bone and implant, improving bone-implant contact and osseointegration. The study aims to assess how well TC and CQ integrate

in a hydrogel form to address osseointegration and antimicrobial efficacy, highlighting the potential benefits of these natural remedies in implant dentistry.

AIM

To Evaluate the efficacy of novel hydrogel containing Triphala and Cissus Quadrangularis extracts, for antimicrobial activity and osteogenic potential at the site of dental implant.

METHODOLOGY

The cytotoxicity, osteogenic potential, and antimicrobial efficacy of 216 titanium disc specimens were evaluated by dividing these samples into test and control groups for comparative analysis.

The hydroalcoholic extracts were prepared using the maceration method. Subsequently, the extracts were mixed and subjected to determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Based on these values, a gel was prepared using Carbopol as the base. The gel was then coated onto the disc using the dip coating method.

The coated samples were further subjected to various tests, including cell attachment, cell proliferation, and disc diffusion assay. The MG-63 cell line was utilized for the study. The collected data was analyzed using statistical methods.

RESULTS

Cytotoxicity was assessed using the MTT assay at 24, 48, and 72-hour intervals, and the proliferative index percentage was documented. Osteogenic potential was evaluated through cell attachment using MG-63 cell lines at three different time points (24, 48, 72hours). Cell counts were recorded after trypsin detachment. Lastly, the antimicrobial activity of the compounds was evaluated against *E. coli, P. aeruginosa, and S. aureus* using the disc diffusion assay. The diameter of the inhibition zones was measured and compared to the control groups.

The hydrogel exhibited a significant enhancement in MG-63 cell attachment and proliferation compared to the control $_{(Ost)}$ group. Statistically significant attachment and proliferation increase was observed over time, particularly at 72 hours. Minimal cytotoxic effects were observed, indicating the hydrogel's biocompatibility. It also demonstrated notable inhibition against *Staphylococcus aureus and Pseudomonas aeruginosa. E. coli* exhibited similar inhibition to the control group, suggesting comparable efficacy.

This TCQ Hydrogel formulation offers dual benefits in promoting osseointegration and preventing peri-implant infections. Its hydrogel structure supports cell adhesion and proliferation, while its antimicrobial properties target, key pathogens.

CONCLUSION

In-vitro research on titanium discs coated with a novel TCQ hydrogel showed substantial cell proliferation and attachment, suggesting potential for bone regeneration. The gel also exhibited significant antimicrobial activity, comparable to chlorhexidine, potentially reducing periimplantitis.

Further research and development in this area will pave the way for new discoveries and the potential of ancient drugs. This will enable us to identify and utilize similar or comparable drugs with more refined combinations and concentrations, which will lead to improved prospects in future developments.

KEYWORDS

Triphala, Cissus Quadrangularis, Antimicrobial efficacy, Osteogenic potential, Titanium Implants, Hydrogel

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INTRODUCTION

The widespread adoption of dental implants since their accidental discovery by Dr. Per-Ingvar Brånemark has transformed the field of implantology. Endo-osseous implants are the primary tool in implantology, enabling the restoration of lost oral structures. In essence, implantology has emerged as a well-established discipline with robust clinical protocols and techniques.[1] To achieve successful outcomes, clinicians have developed multiple criteria, including biocompatibility, macro and microscopic geometry, bone morphology, surgical site sterility, and the absence of undisturbed healing phases. Despite the advancements in implant dentistry over the decades, there remains a source of failure. [2]

Implant failures are influenced by various factors, including age, sex, patient immune response, bone quality, and rejection. Early failures may be attributed to periimplantitis, inadequate bone density, systemic diseases, poor primary stability, smoking, or surgical trauma. Conversely, late failures can result from excessive loading forces, incorrect prosthetic design, bruxism, traumatic occlusion, or neglect of implant-protected occlusion. Failures occurring in first few years after placement and are majorly related to periimplantitis & poor osseointegration. [2,3]

Biomaterial composition, implant geometry and surface properties, sandblasting, acid etching, anodizing, plasma spraying, laser ablation, and hydrogel coatings represent a few of the techniques used to improve implant longevity.[4] Osteointegration and the therapeutic/antibacterial function of the implant coating have been considered the most important factors for the continued functioning of dental implants.[5]

Evaluations of dental implants coatings with different materials and biomolecules have been carried out in recent decades in an effort to attain particular advantages.[6] For example, adding bioactive coatings to dental implants, like calcium phosphates and hydroxyapatite (HA), improves the surface bioactivity and the contact between the implant and bone.[5] Since the surface plays a crucial role in determining implant toxicity, loosening, and failure as well as it help enhance osseointegration, biocompatibility, and stability, while simultaneously addressing challenges such as pre-implantitis; variations in the surface of dental implant-based biomaterials are extremely important.[2]

Hydrophilic gels, or hydrogels for short, are frameworks of polymer chains that are sometimes found as colloidal gels with water providing as a dispersion medium. They also have an extent of plasticity that is quite similar to that found in living tissue due to their high-water content.[7] Depending on whether they are manufactured or natural, hydrogels can be divided into two categories. Numerous industrial and biological applications make use of hydrogels. One of the main problems with treating infectious diseases is microbial resistance, which is brought about by the over use of antibiotics. Using pure natural derivatives with hydrogel is a novel approach to managing microbial resistance. [8]

Triphala, an Ayurvedic formulation historically and currently utilized in India, comprises three fruits: *Terminalia chebula, Terminalia bellerica, and Phyllanthus emblica L;* It helps to treat microbial illnesses, and its effectiveness as an antibacterial agent has been the focus of several investigations. [9]

Phyllanthus emblica L. possesses carotene, nicotinic acid, D-glucose, D fructose, riboflavin, empicol, mucic, and phyllemblic acids in addition to being a great source of vitamin C. On the other hand, *Terminalia chebula* is used extensively in

traditional medicine because of the great variety of pharmacological activity linked to the plant's physiologically active components.[10]

Terminalia bellerica, a medicinal fruit, is a rich source of anthraquinone glycoside, chebulinic acid, tannic acid, terchebin, vitamin C, and various fatty acids. These compounds collectively exhibit anti-cancer properties by inhibiting the proliferation and apoptosis of cancer cells. Furthermore, *Terminalia bellerica* contains chebulagic acid, ellagic acid, and its ethyl ester, gallic acid, fructose, galactose, glucose, mannitol, and rhamnose. Notably, lower levels of lipid peroxide in treated wounds indicate that the extract has antioxidant effect. [10]

Extensive research has been conducted on alcoholic extracts of Triphala, a rich source of catechins, for its potential against a wide spectrum of microorganisms.[11] Various researches demonstrated the effectiveness of Triphala in combating various pathogens, including *Pseudomonas aeruginosa, Shigella sonnei, Shigella flexneri, Vibrio cholera, Escherichia coli, Enterococcus faecalis, and Staphylococcus aureus*. Notably, Triphala exhibits potent inhibitory activity against polymorphonuclear leukocytes-type collagenases (matrix metalloproteinase-9), which further supports its application in treating periodontal diseases. Including antimicrobial activity, triphala also possess other effects like antiseptic, anti-inflammatory, and antioxidant among others. [12]

There are over 800 species in the genus *Cissus*, which belongs to the Vitaceae family and is divided into 13 genera globally. Numerous tropical locales, including Africa, Arabia, South Asia, Sri Lanka, India, and others are home to these genera. There are 63 different species and eight genera in India. CQ is a member of the grape family, & is a dicotyledonous blooming annual herb with a thick, meaty, quadrangular twig.[13]

This herb is traditionally used to treat bone fractures, scurvy, tumours, haemorrhoids, and peptic ulcers.[14] In India, it is known by various names such as *Bonesetter, Hadjod,* or *Hadsanka*, depending on the region. The drug has demonstrated the anti-osteoporotic potential in various preclinical and clinical settings. [15]

In-vivo research with CQ extracts were done using an ovariectomy-induced osteoporotic rat model, as well as tests involving closed radius-ulna fractures and unilateral comminuted diaphyseal femoral osteotomies in rats and dogs. Here, the researchers utilized murine osteoblastic cell (MOC) lines to showcase its potential to promote bone formation.[16,17] The mechanisms by which CQ aids in fracture recovery are thought to involve its steroidal content, which likely functions as phytoestrogens to prevent or minimize bone loss. [17]

Bringing these widely used Ayurvedic drugs to the forefront of the dental and implantology fields is the main aim of this research. These medications possess the potential to address two significant challenges associated with implants: periimplantitis and osseointegration.

Triphala will act as a potent antimicrobial agent to prevent periimplantitis[9]; while cissus quadrangularis will assist in osseointegration[18] when combined in a hydrogel form. This Novel TCQ hydrogel will be coated onto the grade-V titanium surface to evaluate the efficacy of both drugs in an in-vitro study model, were cytotoxicity, osteogenic potential & antimicrobial efficacy will be evaluated.

NEED FOR STUDY

Implant dentistry offers normal function, shape, aesthetics, speech, and health, even with atrophy, disease, or injury to the stomatognathic system.[4] Ti6Al4V titanium alloys are favoured for their mechanical properties and biocompatibility over traditional materials like stainless steel 316L & cobalt-chromium alloys. Yet, titanium has disadvantages when it comes to implant materials. [19]

As an alloy, titanium is bioinert, which means it cannot form a chemical link with surrounding tissues. The chance of failure or implant loosening is increased since it only creates a physical bond with bone tissue, which is less robust than chemical osseous bonding. Additionally, titanium does not possess natural antimicrobial properties, making it susceptible to bacterial colonization during the initial implantation phase. Once colonized, bacteria can develop a biofilm that hinders antibiotic effectiveness, potentially leading to infection.[20]

With the materials and techniques available today, the titanium implant usually takes four to six months to fuse with the surrounding bone. However, in situations with poor bone quality or systemic diseases, longer waiting times are necessary. Bone response, which includes the rate, quantity, and quality of bone formation, is influenced by the implant surface. Consequently, most surface treatments focus on enhancing osteoprogenitor cell activity to boost new bone growth, facilitate early Osseo-integration, & ensure greater secondary implant stability.[21]

Surface modification aims to improve the implant's antibacterial qualities, corrosion resistance, and bioactivity while maintaining its exceptional mechanical qualities and biocompatibility. Although various commercially available antimicrobials are employed to address early periimplantitis, their release at

suboptimal concentrations may lead to bacterial resistance.[22] Conversely, high doses can disrupt the oral microflora, cause cell toxicity, and hinder osseointegration. An ethnopharmacological approach utilizing natural phytochemicals from medicinal plants is regarded as a safe, effective, and promising alternative to synthetic drugs.[23]

This research study aims to highlight some traditional natural medicines to achieve predictable results on the specified parameters. Triphala Powder (TC), a blend of three fruits—Emblica officinalis, Terminalia bellerica, and Terminalia chebula—is rich in vitamin C, nicotinic acid, and Chebulinic acid, which together have antimicrobial and antifungal properties. Incorporating this into a hydrogel mixture can enhance the implant osteotomy site by decreasing microbial activity.[9,24,25] Additionally, Cissus quadrangularis, another herbal remedy, has demonstrated osteogenic potential, which may facilitate bone formation between the bone and implant, resulting in improved BIC & osseointegration. [26]

The exploration of the potential of combining these drugs in the field of dentistry, particularly in implant dentistry, is currently insufficient. Their active ingredients have the capability to eliminate microbes and accelerate bone healing. In terms of application, these drugs can be easily incorporated into a hydrogel form and applied to the implant surface to achieve potential benefits. Consequently, it is essential to test these drugs in combination to address two critical parameters: osseointegration and antimicrobial efficacy. The purpose of this research thesis is to assess how well TC & CQ integrate in a hydrogel form for these parameters.

HYPOTHESIS

Null Hypothesis:

There is no difference in the antimicrobial efficacy & osteogenic potential of titanium coated with a hydrogel of Triphala and Cissus quadrangularis.

Research Hypothesis:

There is a difference in the antimicrobial efficacy & osteogenic potential of titanium coated with a hydrogel of Triphala and Cissus quadrangularis.

STUDY DESIGN

In-Vitro Study

AIMS AND OBJECTIVES

AIM

To Evaluate the efficacy of novel hydrogel containing Triphala and Cissus Quadrangularis extracts, for antimicrobial activity and osteogenic potential at the site of dental implant.

OBJECTIVES

1. To Evaluate the Antimicrobial efficacy of Triphala & Cissus Quadrangularis hydrogel.
2. To Evaluate the Osteogenic Potential of Triphala & Cissus Quadrangularis hydrogel.

REVIEW OF LITERATURE

1. **John Nicholson in 2020** reviewed the properties of titanium and its alloy for their use in dentistry. This article highlighted some of the important aspects of titanium and its excellence in properties. These include biocompatibility, mechanical strength & exceptional clinical success rate which goes up to 99% at ten years.[1]

2. **Mombelli A et al. in 1987** conducted a study in patients with implant overdenture treatment done. They analyzed the patients by dividing them into two groups. One with health peri-implant tissue and another with inflamed tissue & failing implants. Their study concluded that, there are different bacteria associated with healthy and unhealthy tissue surrounding the implants & the failing implants have similar microbes which are found in chronic periodontitis.[27]

3. **Gerald McDonnell et al.** in 1999, investigated the antimicrobial activity of Chlorhexidine. It exhibits bactericidal and fungicidal properties, but it does not eliminate or hinder the growth of bacterial spores or mycobacteria. While it has a low level of effectiveness against viruses, it demonstrates efficacy in destroying cysts of Acanthamoeba species at high doses. [28]

4. **Lindhe J Meyle et al** did a study in 2008 to look into the reasons and treatment options for peri-implant mucositis and peri-implantitis. These disorders are caused by a number of circumstances, such as smoking cigarettes, dental cements, poor oral hygiene, and a history of periodontitis. In order to remove plaque from peri-implant mucositis, mechanical debridement is crucial. Non-surgical treatment which includes mechanical debridement, remains very

helpful for peri-implant lesions. However, it cannot effectively treat the conditions & adjuvant antimicrobial treatment is necessary.[29]

5. **Stefan Renvert et al in 2008** conducted a comprehensive review to assess non-surgical therapy options for peri-implantitis. Mechanical nonsurgical therapy are effective in management of peri-implantitis. Moreover, incorporating antibacterial mouth rinses alongside mechanical therapy enhances the outcomes of such lesions. However, nonsurgical treatment for peri-implantitis lesions has been found to be ineffective. Clinical and microbiological characteristics were only marginally influenced by the use of chlorhexidine.[30]

6. **Antonio Fernández-Barbero et al** conducted a study in 2009 to examine the hydrogel's polymer network's mechanical properties, internal framework, and refractive index. These substances are well known for their remarkable absorbency, which allows them to absorb solvents several times their own weight. They react quickly to changes in the immediate environment. Because of their capacity to change size in response to changes in their internal dimensions, microgels have been used as both diagnostic agents and carriers of medicinal drugs.[31]

7. **Wennerberg A et al in 2010** carried out research that emphasized the importance of implant roughness on the surface in attaining long-term implant success and osseointegration. In contrast to smoother or coarser surfaces, the study found that the optimal roughness value falls between 1-2 micrometers because this level of abrasion efficiently promotes osseointegration. Slightly rough surfaces (Sa between 1.0 and 2.0 micrometers) have consistently shown the best results in clinical trials.[32]

8. **Jemat A et al in 2015** did thorough analysis, that looked at several basic surface treatment options & their influence on titanium implants. Despite titanium's superior mechanical qualities, notable surface roughness was crucial in promoting efficient BIC, cell proliferation, & de-torquing. As a result, the most popular method for achieving the necessary surface roughness on titanium dental implants was acid etching of the surface. Successful dental implants can be created by fusing titanium's mechanical qualities with a good surface roughness.[33]

9. **Amparo Mendoza Arnau et al** did research that describes the surface characteristics of dental implants, in 2016. While a rough surface promotes cell adhesion, an overly rough surface inhibits biological response and osseointegration. There is a suggested optimal range for Ra roughness (0.77µm ± 0.05µm) and Rt roughness (5.25µm ± 0.55 µm). Eachimplant system that was looked at in the present investigation had a lower score. Additionally, the study found that the best cell adhesion was attained by a medium Ra, indicating that medium roughness is best for the optimum cell response.[34]

10. **Ramesh Chowdhary et al** in 2018 investigated cell viability of MG-63 cells exposed to hydrogel formulations containing acemannan and curcuminoids. The hydrogel was applied to titanium discs using the dipping method, and the MTT assay evaluated the cell viability. The study revealed that the hydrogel coated on titanium discs exhibited good cell viability at 24 hours. [35]

11. **Jyotsna Srinagesh et al. (2012)** performed an in vivo study for evaluation of antibacterial effectiveness of Triphala against oral streptococci. They compared the effects of mouthwash made of 6% Triphala & another made of 0.2% chlorhexidine. The study demonstrated that using 6% Triphala twice daily

significantly reduced the oral streptococci count by 17% after 48 hours and 44% after 7 days. Notably, the anti-streptococci action of Triphala closely resembled that of the well-established Gold Standard chlorhexidine.[24]

12. **Umey Salma et al. (2020)** highlighted in her review article that; Triphala, a blend of three plants, has been recognized for its beneficial roles in both medicine and dentistry. Its minimal side effects, lower cost compared to synthetic compounds, and longer shelf life contribute to its popularity as an alternative. Triphala's remarkable clinical benefits extend to its antimicrobial and antiplaque properties, which are highly valuable in dentistry.[9]
13. **Ritam S. Naiktari et al. (2014)** compared the potency of 0.2 percent CHX and Triphala mouthwash. To assess, a RCT was done in hospitalized patients with periodontitis. According to the study, triphala mouthwash works just as well as 0.2% chlorhexidine as an antiplaque agent. It efficiently controls periodontal disorders in all individuals by drastically lowering gingival inflammation and plaque formation. Triphala mouthwash is also reasonably priced, widely available, well-tolerated, and has no known negative effects.[36]
14. **Christine Tara Peterson et al. (2017)** in their review article on the therapeutic applications of Triphala in Ayurveda, emphasized the antimicrobial potential of Triphala Churna. Triphala research has shown the formula's effectiveness in various clinical settings, such as stimulating appetite, reducing hyperacidity, exhibiting antioxidant, anti-inflammatory, antibacterial, & various other along with preventing dental caries.[37]
15. **Yogesh S. Biradar et al. (2007)** assessed antimicrobial activity of Triphala Churna with hydro and ethanolic extracts. The extracts showed broad-spectrum antibiotic action against a variety of bacteria, including those obtained from pathology labs with established diagnoses and those present in human

secretions. Interestingly, broad spectrum inhibition was seen. In comparison to the ethanolic extracts, the aqueous extracts shown greater efficacy against every strain that was tested. Additionally, the extracts demonstrated a stronger inhibitory zone against bacteria including S. Aureus and E. Coli. The extracts' activity showed a dose-dependent pattern, indicating that higher concentrations were necessary for maximum effectiveness. Additionally, the extracts of triphala and triphala mashi showed susceptibility to all of the bacteria that showed resistance to several of the tested drugs.[38]

16. **Zaid Omran et al (2020)** performed a study that looked into the antibacterial activity of TAE extracts and other formulations for antimicrobial efficacy against a range of microorganisms. They came to the conclusion that the hydroalcoholic extract of triphala in the drug nano-formulation shown might be used as an adjuvant antibacterial agent to treat a variety of microbiological infections.[39]
17. **Sajith Abraham et al. (2005)** examined triphala's ability to decrease PMN-type Matrix-metallo-proteinase (MMP-9) expression in individuals suffering with periodontitis. They contrasted MMP-9's activity with that of doxycycline, which is known to have inhibitory action, and Kamillosan, another ayurvedic medication. According to the study, the medications use dramatically reduced MMP activity. Triphala showed an impressive 76.6% decrease in MMP-9 activity. These results imply that triphala has potent inhibitory action on PMN-type MMPs that contribute to the extracellular matrix's (ECM) breakdown in periodontitis.[40]
18. **D.H. Tambekar & S.B. Dahikar (2011) in their study** demonstrated, along with Triphala extracts other herbal extracts are beneficial when it comes to

antimicrobial activity. They suggested the use of these extracts as dietary supplements but also as an agent to control enteric bacterial infections.[12]

19. **V.N. Sumantaran et al. (2007**) concluded that Triphala guggulu inhibits hyaluronidase and collagenase activity, which may be a crucial mechanism behind its chondroprotective effects. It also exhibits exceptionally high potency as an inhibitor of both hyaluronidase and collagenase. The fact that T. chebula (0.10 mg/ml) totally inhibits both enzymes is noteworthy and has important medicinal implications for efforts to develop new drugs.[41]
20. **Aleksandra Tarasiuk et al. (2018)** in their review article highlighted that Triphala's anti-inflammatory and antioxidant qualities are attributed to its high content of flavonoids, polyphenols, and vitamin C. Triphala has various types of acids, including gallic, chebulagic, and chebulinic acids which have cytoprotective and antifungal effects.[11]
21. **Zhakaria Ahmed et al. (2019)** carried out a study to evaluate five fresh sample's antibacterial activity under varied circumstances. Using disc diffusion methods. They examined antimicrobial properties of triphala for gram - positive and gram - negative bacteria. According to this study, triphala may be used to make new oral antibiotics with a broad spectrum of activity.[25]
22. **K. Deepika et al. (2020)** concluded that a 0.6% triphala solution prevents plaque formation and demonstrates its positive impact on gingival health. Additionally, it exhibits inhibitory effects on the microbial count, particularly against Streptococcus mutans and Lactobacillus.[42]
23. **Duenpim Parisuthiman et al. (2009)** suggested that among its other possible medicinal uses, Cissus quadrangularis might help with ailments that call for the promotion of bone production. This plant's osteogenic potential may help prevent or postpone osteoporosis in addition to helping treat bone fractures. By

increasing MAPK-dependent alkaline phosphatase (ALP) activity, especially via the p38 MAPK pathway, CQ controls osteoblastic activity. This impact probably starts and intensifies the production of mineralized nodules in vitro, indicating that CQ has an osteogenic activity that promotes bone mending in vivo.[14]

24. **Hemal R. Brahmkshatriya et al. (2015)** carried out a study to see how C. quadrangularis affected the maxillofacial fracture healing process. According to their research, C. quadrangularis speeds up the healing of fractured jaw bones by dramatically lowering pain, edema, and fracture mobility.[43]
25. **Piyush S. Bafna et.al. (2020)** The review study provided a systematic overview of the botany, applications, phytochemistry, formulations, & patents to investigate the scientific and therapeutic use of CQ in the future. The findings showed that this herb, which goes by the name Hadjod, has analgesic, anti-inflammatory, anticonvulsant, antibacterial, anticancer, anti-osteoporotic, and bone-healing qualities when extracted using various techniques.[13]
26. **Somasundaram Ramachandran et.al. (2021)** evaluated the bone-healing activity of Cissus Quadrangularis (CQ) in Wistar albino rats. They proved that CQ ethanolic extracts restored bone strength and shortened the time it took for bones to heal. This was because of the high calcium content and other natural phytochemicals.[26]
27. **Nimisha Singh et al. (2013)** used ostepontin expression as a marker in a study to evaluate Cissus Quadrangularis (CQ) osteogenic potential. 60 patients with uncomplicated mandibular fractures participated in the trial; they were split into two groups. Group A were given 2 capsules of CQ, B.D., while Group B were given 2 capsules of placebo B.D. The conclusion was, CQ causes early remodelling of the fracture callus and speeds up fracture healing.[18]

28. **Srinidhi Bhat et al. (2022)** investigated the impact of CQ Hydrogel on improving the osseointegration of dental implants into bone in an in-vivo investigation. In this investigation, six male adult white rabbits were used. These six rabbits received 24 implants: 12 CQ hydrogel dip-coated (Test) and 12 uncoated (Control). The dip-coated implants showed significant increase in bone formation activity.[44]
29. **Praseetha R. Nair et al. (2021)** systematically examined CQ edible stem as a source of bioactive compounds capable of triggering osteogenesis and biomineralization in vitro. The work highlights the exceptional capacity of Cissus quadrangularis aqueous stem extracts to promote increased proliferation, biomineralization, and early osteogenesis.[45]
30. **Alaa Abdelqader Altaweel et al. (2021)** carried out a clinical study to check Cissus Quadrangularis's osteogenic potential in mandibular alveolar ridge distraction. According to their research, giving Cissus quadrangularis during the consolidation phase increases the distracted bone's capacity for osteogenesis. The current study's histology and radiographic findings also showed that Cissus quadrangularis enhances bone density in addition to speeding up the production of new bone. As a result, the bone grows stronger and better capable of withstanding the biomechanical demands of implant implantation in a shorter amount of time.[15]

MATERIALS AND METHODS

SOURCE OF THE DATA:

This In Vitro study was carried out in KAHER KLE VKIDS Department of Prosthodontics and Crown & Bridge, Department of Pharmaceutics KAHER KLE College of Pharmacy, Gogte Institute of Technology, KAHER Dr. Prabhakar Kore's Basic Science Research Centre and Goa University.

METHODS OF DATA COLLECTION

Inclusion Criteria:

- Identical Titanium disc shaped specimens measuring 10 mm in diameter and 2 mm in thickness were included in the study. (ASTM B348)

Exclusion Criteria:

- Specimens with internal and external porosities.
- Specimens with surface irregularities. (R_a> 4µm)

Table No. 1: List of Materials used for hydrogel formulation, assessment of osteogenic potential & microbial analysis.

Materials	Description	Manufacturer
Titanium alloy	TYPE-V (Ti-6Al-4V alloy)	Special Metals, Mumbai.
Triphala	Extract (Source- Fruit powder of *Emblica officinalis, Terminalia bellerica, and Terminalia chebula)*	Ayurvedic Rasashala, Pune
Cissus Quadrangularis	Extract (Source-Stem of the plant)	Ayurvedic Rasashala, Pune
Chlorhexidine Gel	Hexi gel 1%	ICPA Health products, India
Phosphate buffer solvent (6.8 pH)	Sodium chloride, sodium dihydrogen orthophosphate dihydrate, potassium chloride, distilled water	Hi-media, Mumbai, India
Carbopol gel base	Carbopol 940	OEM manufacturers
Distilled water	Batch No.: 007M15	Rankem Chemicals, Avantor, India
Nutrient Agar	Culture media	Hi-media, Mumbai
70% Ethanol	LOT No: 20151011	Changshu Hong sheng Fine Chemicals Co., Ltd.
Phosphate Buffer Saline	LOT No: 0000237353	Hi-media, Mumbai, India
Dulbecco's Eagle Medium	LOT No: 0000284912	Hi-media, Mumbai, India
Trypsin EDTA	LOT No: 0000297541	Hi-media, Mumbai, India
MTT Reagent	LOT No: 0000173725	Hi-media, Mumbai, India
Tryphan Blue	LOT No: 2024364	Hi-media, Mumbai, India
MG- 63 Cell Line	Human Like osteoblast cells	NCCS, Pune

Table No. 2: Armamentarium for determining surface characteristics, microbial analysis & evaluation of osteogenic potential for the test specimen.

Material	Description	Manufacturer
Profilometer	Contact profilometer Model: - Surtronic S–128	Taylor Hobson, Brazil.
Scanning Electron Microscope	FE- SEM	Carl- Zeiss
Laminar Air Flow	Model: - Vertical	Quest International, Bangalore.
Micropipette	Model No.: 299932	Riviera Glass Pvt, Ltd., Mumbai
Tissue Culture Plate	24 well plate	Tarsons, Korea
Electric Loop Sterilization	Model.: i-therm A1-401	Hi-Media, Mumbai
Anaerobic Jar	LOT No:14-1024	Hi-Media, Mumbai
Hemocytometer	-	Rohem, India
Microscope	TCM400	LABOMED, USA
CO_2 Incubator	Galaxy 170-R	Eppendorf, India
Micro Titer Plate Reader	Epoch	Bio-Tek, USA

Table No. 3 – List of Bacteria used for microbial analysis.

Bacteria	ATCC Number
Escherichia Coli	25922
Pseudomonas aeruginosa	15442
Staphylococcus aureus	25923

METHODOLOGY:

1. Preparation of Specimen

A total of 216 identical Titanium Grade-V 10 mm x 2 mm (ASTM B348) disc specimens were used. (Fig, 1)

These discs were sandblasted with 50 µm alumina for one minute at a steady pressure of 4 kg/cm2. To get rid of any remaining impurities, they were then ultrasonically cleaned for 180 seconds using acetone.

A. Cytotoxicity & Osteogenic potential:

144 discs were used for cytotoxicity & osteogenic potential which were subdivided as 72 discs in Study and $Control_{(Ost)}$ groups each.

- Study Group: Titanium Discs coated with TCQ hydrogel
- $Control_{(Ost)}$ Group: Titanium discs coated with blank hydrogel

They were subsequently separated (n=36) into time intervals of 24, 48, and 72 hours

B. Antimicrobial efficacy:

72 titanium discs were divided as study and $control_{(Chx)}$ groups (n=36)

- Study Group- Titanium Discs coated with TCQ hydrogel.
- $Control_{(Chx)}$ Group – Titanium discs coated with 1 % Chlorhexidine gel

They were subsequently separated (n=12) for test organisms

2. Surface Analysis

216 specimens were divided into 6 groups. In each group discs were numbered from 1 to 36. Quantitative and qualitative surface roughness evaluations were conducted on each specimen.

Contact Stylus Profilometer (Surtronic S–128, Taylor Hobson) was used for quantitative evaluation of surface roughness for the specimens. Each specimen was evaluated and average surface roughness was noted (Ra).

All the specimens were kept on a flat glass slab. Surface profile was evaluated with the tracking devices which had a diamond point stylus. This was moved over the disc transversely for 4mm with 0.8mm as cut off length. Average value for these distances from the center line was calculated. (Fig. 2)

Field emission Scanning Electron Microscope (Carl Zeiss) was used to evaluate the surface roughness of random specimen from each group. Their surface images were taken at 100X, 500X and 1000X magnification for clear visualization. (Fig. 3)

3. Preparation of Extracts

Powder form (5gm) of Triphala and Cissus Quadrangularis each were filtered through sieve no. 40 were subjected to extraction procedure. The procedure followed was maceration to produce hydroalcoholic extracts. [46]

Powders of both drugs were mixed with 10% distilled water and 80% Ethanol in individual Erlenmeyer flask (Fig. 4) and was loaded in shaking incubator (Excella E24, New Brunswick) at 36 ℃ and 80 rpm for 24hrs (Fig. 5). The supernatant of both drugs was collected (Fig.6) separately and was concentrated using evaporation

in water bath (Lab-go water bath) for 6hrs (Fig. 7). The dried extracts were collected and used for the further study (Fig. 8). Both Extracts $_{(mix)}$ were mixed together in 1:1 ratio for further research.

4. Determination of Minimum Inhibitory Concentrations for Extract $_{(mix)}$:

A standard protocol was use for testing the MIC. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* were revived by cultivating them on Brain Heart Infusion Agar and followed by aerobic incubation. The colonies were moved to a sterile BHI broth & grown at room temperature for 24 hours in aerobic conditions. The organisms were adjusted to 10^5 per ml by using 0.5 McFarland's turbidity standard. A stock solution was prepared by dissolving 1 gram of extract (mix) in 100 ml of solvent (1% DMSO and 99% distilled water). Six Minimum Inhibitory Concentration tubes were filled with 1 ml of BHI broth. In each tube, 1 ml of extract (mix) was added, starting with the first tube and doubling the concentration until the last tube. 50 µl of each previously prepared strain of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus was transferred to each test tube. The tubes were incubated at room temperature, aerobically for 24 hours. The turbidity was compared to the control, and the last visible tube with turbidity was noted as the MIC.

Based on the turbidity, the MIC was 12.5 mg/ml for Escherichia coli, 25 mg/ml for Pseudomonas aeruginosa, & 25 mg/ml for Staphylococcus aureus.

5. Determination of Minimum Bactericidal Concentrations for Extract $_{(mix)}$:

Two to three dilution tubes that showed no signs of growth (turbidity) and the control tube were subcultured onto blood agar and aerobically incubated for 48 hours at 37°C in order to measure the MBC. The colonies that resulted were tallied. The test

group's colony counts were contrasted with those of the control group. The following interpretation was made of the test results:

a) If there are similar numbers of colonies, it indicates bacteriostatic activity only.

b) A reduced number of colonies suggests partial or slow bactericidal activity.

c) If no growth is observed, it indicates a complete bactericidal effect.

Based on the results the MBC that was establishes was 50 mg/ml for Escherichia Coli, 100 mg/ml for Pseudomonas aeruginosa & 100 mg/ml for Staphylococcus aureus

6. Formulation of Hydrogel

A gel formulation of 0.5% w/v was developed. This gel contains 500mg of drug per 100ml. This concentration is equivalent to 200 times the MIC which was 50mg/ml and 50 times the MBC which was 100mg/ml. Therefore, this concentration was suitable for gel formulation.

Carbopol 930 was soaked in 50 ml distilled water for 24 hours. Glycerin was mixed with this solution at constant stirring, using propeller (100 rpm for 10 minutes). This solution was mixed with the polymeric solution using propeller at 100rpm for 5 minutes. Extracts $_{(Mix)}$ (0.5%) were slowly added to the same solution with constant stirring under propeller at 100 rpm for half an hour. To the above mixture 5gm Glycerin, 0.5% Sodium benzoate, and 0.01% Methyl paraben (Fig. 9) were added as preservatives. Prepared gel was stored in air tight containers at room temperature. (Fig. 10).

To coat the titanium disc with hydrogel, dip coating method was employed. (Fig. 11) The disc were immersed in freshly prepared hydrogel for 30 sec. Followed by which they were allowed to dry for 5 minutes.[72]

7. Determination of Cytotoxicity of Hydrogel:

Cytotoxicity was conducted using MTT assay in log phase for cell division. In current study this was recorded at three-point intervals (24, 48 & 72 hours). A 12 well plate was used initially for assessment.

After adding MTT dye, the samples were transferred to 96-well plates. Initially, markings were made on the 12-well plates based on the positive control. Trypan blue was used to count the viable cells. This was followed by seeding 50,000 cells / well. These wells were then completely filled with the media, with each well having a final volume of 1500microliters. After 24 hours, in each well 200 micro-liters of MTT dye was added, and the plates were covered in silver foil as MTT dye is prone to photosensitivity. The plates were incubated for four hours.

Before dissolving the formazan crystals by 1000 microliters of DMSO, the supernatant was discarded. This was followed by measurement of absorbance at around 570 nm using a spectrophotometer (Fig.11). The proliferation index was calculated by dividing the absorbance (Optical Density) of the test & control sample, multiplied by 100. This procedure was repeated for 48 hours and 72 hours. The percentage of proliferative index was recorded in an Excel spreadsheet for further statistical analysis.

Formula =

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

8. Osteogenic potential of Hydrogel

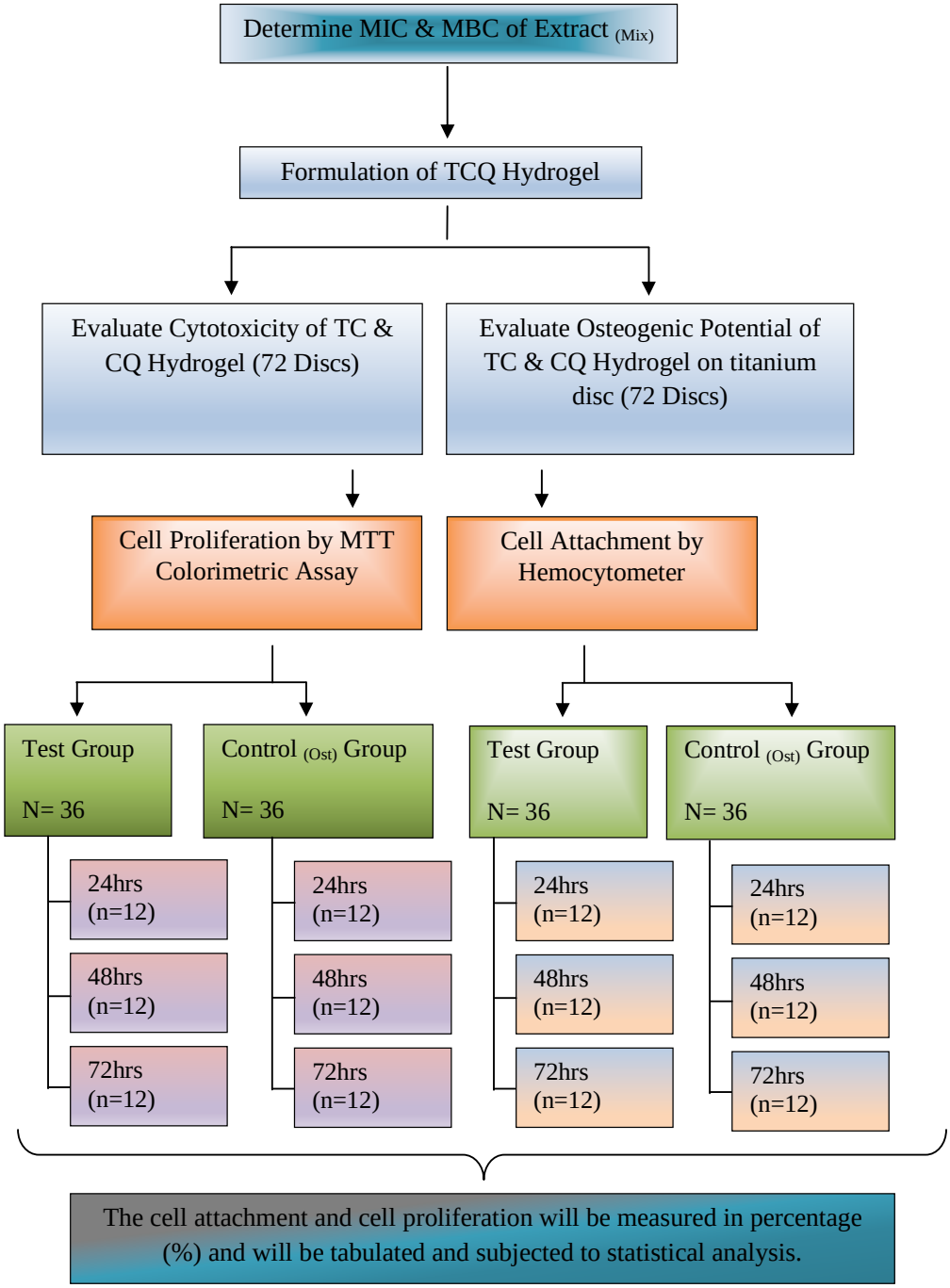
MG-63 Cell lines were procured for assessment of osteogenic potential. These are human osteosarcoma cell lines. Cell attachment was evaluated at three-time frames (24, 48, & 72 hours). For four hours, MG-63 cells were grown on Control $_{(Ost)}$ and Study group. After removing the growth medium, unattached cells were removed from the wells by washing them three times with PBS at 37°C. Trypsin was used to separate adherent cells. After gathering the cells and media in a Falcon tube, they were centrifuged for three minutes at 3000 rpm. After discarding the supernatant, a micropipette was used to break up the cell pellet. A Trypan blue assay was performed on the cell suspension, and a hemacytometer was used to count the cells. For every sample, the number of cells adhering was noted. (Figure 12).

The evaluation of cell attachment and cell proliferation was conducted as a percentage (%), organized into tables, and analysed statistically.

9. Microbial Analysis of Hydrogel

Zone of inhibition method was used to examine the specimens' antimicrobial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli*. After being moved to sterile Brain Heart Infusion broth, the organisms were cultured. The turbidity standard of 0.5 McFarland was used to adjust the growth concentration to 105 organisms per milliliter. On Muller Hinton agar, lawn cultures of Escherichia coli, Pseudomonas aeruginosa, and S. aureus were conducted. After being placed on Muller Hinton agar, the titanium discs coated with Triphala and Cissus Quadrangularis Hydrogel were incubated for 24 hours at 37°C in an aerobic environment. The antibacterial activity was evaluated by measuring the zones of bacterial growth inhibition around the titanium discs. The zone of inhibition (Fig. 13)

was measured in millimeters (mm) and compared with the control $_{(Chx)}$ group, which is disc coated with 2% chlorhexidine. The measured values will be tabulated and subjected to statistical analysis.



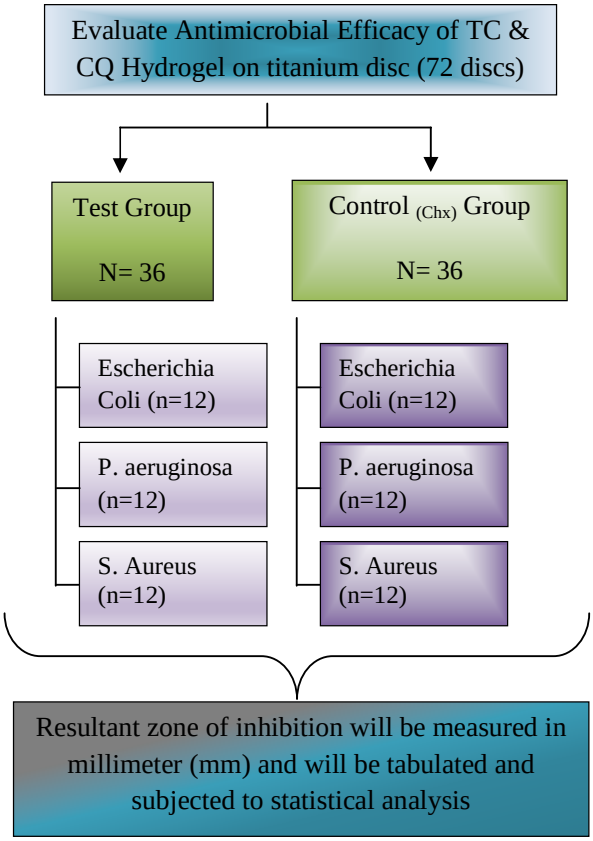

Evaluate Antimicrobial Efficacy of TC & CQ Hydrogel on titanium disc (72 discs)
Test Group
N= 36
Control (Chx) Group
N= 36
Escherichia Coli (n=12)
P. aeruginosa (n=12)
S. Aureus (n=12)
Escherichia Coli (n=12)
P. aeruginosa (n=12)
S. Aureus (n=12)
Resultant zone of inhibition will be measured in millimeter (mm) and will be tabulated and subjected to statistical analysis

Figure 1: Grade V - Surface treated Titanium disc

Figure 2: Disc assessment with Profilometer (Surtronic S–128, Taylor Hobson)

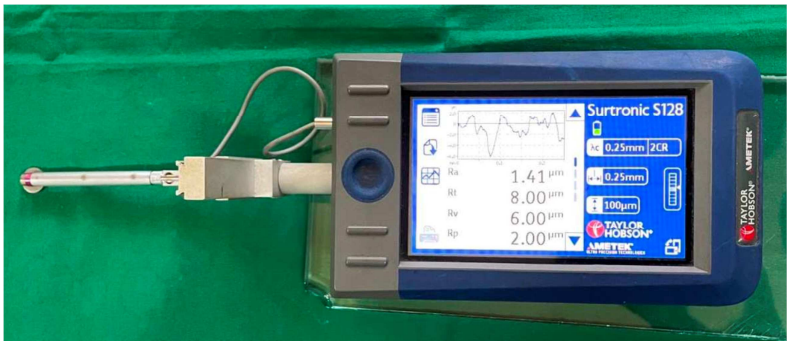


Figure 3: Scanning electron microscope images; A- 100X, B- 500X, C-1000X

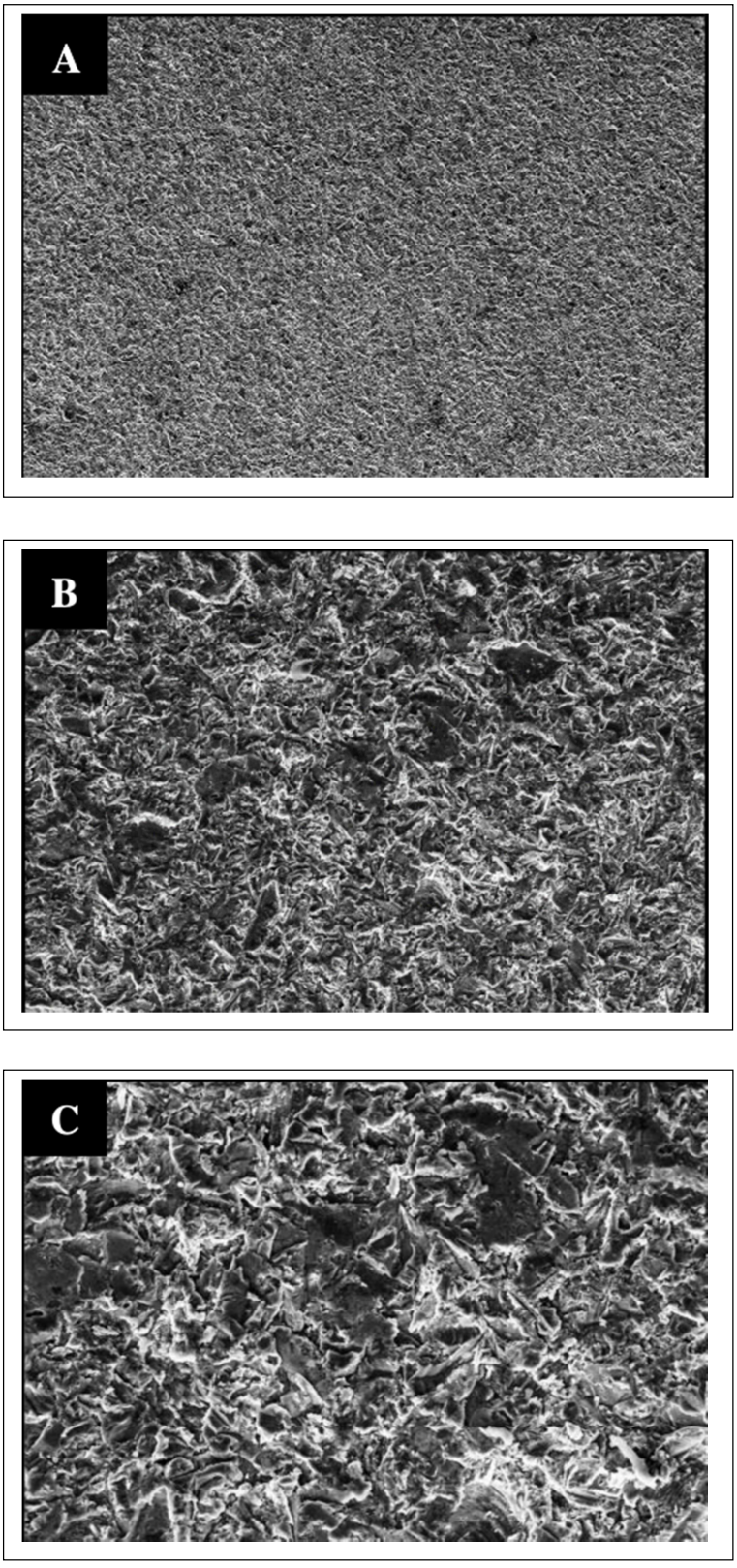


Figure 4: Powder of both drugs with water & Alcohol mixed in Erlenmeyer flask A- Triphala, B- Cissus quadrangularis

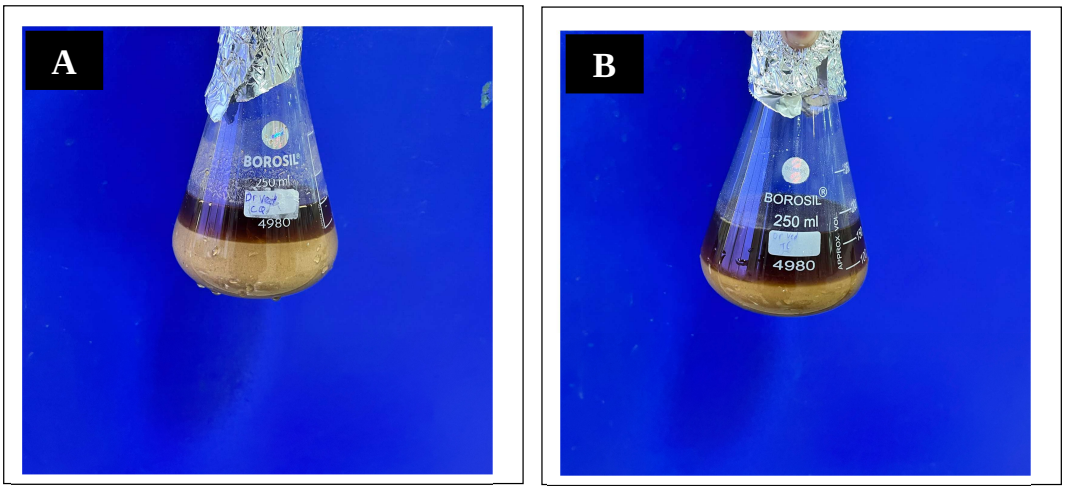


Figure 5: Shaking incubator (Excella E24, New Brunswick)

Figure 6: Supernatant collection; A- Triphala, B- Cissus Quadrangularis

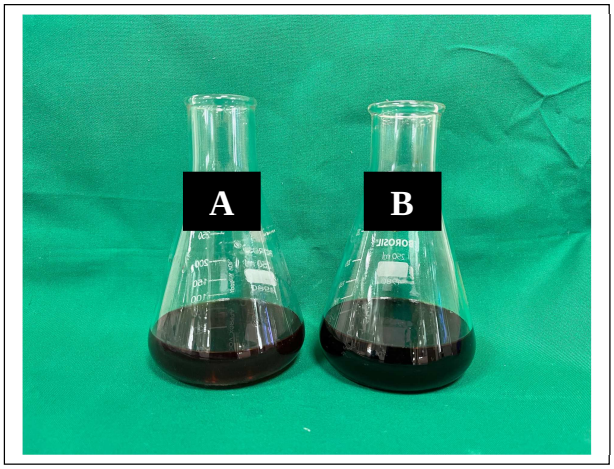


Figure 7: Water bath (Lab-go water bath)
A- Triphala Extract, B- Cissus Quadrangularis Extract

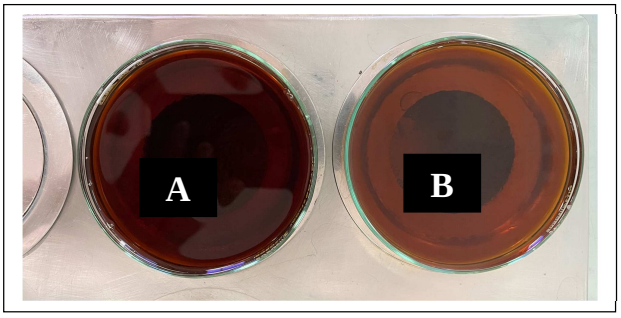


Figure 8: Powder Extracts A- Triphala, B- Cissus Quadrangularis

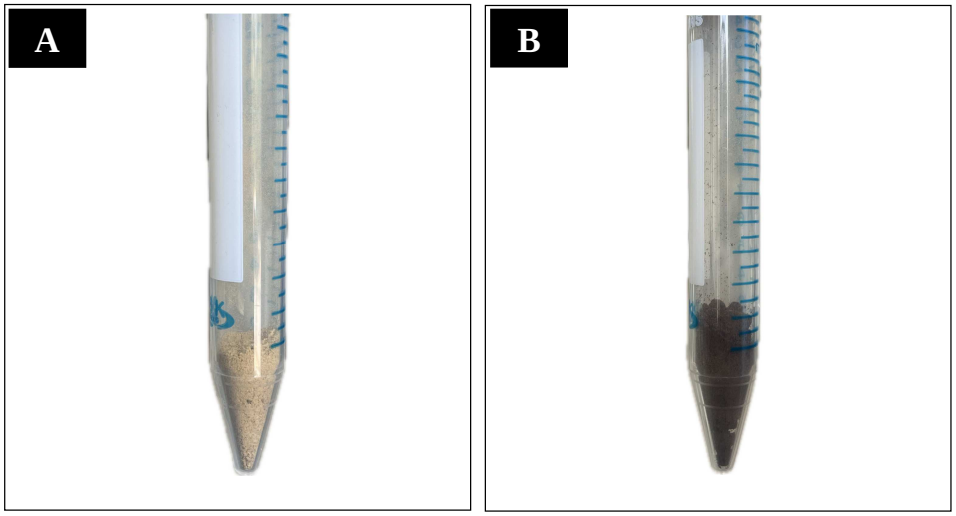


Figure 9: Materials for hydrogel preparation

Figure 10: A-TCQ Hydrogel, B-Control (Ost)

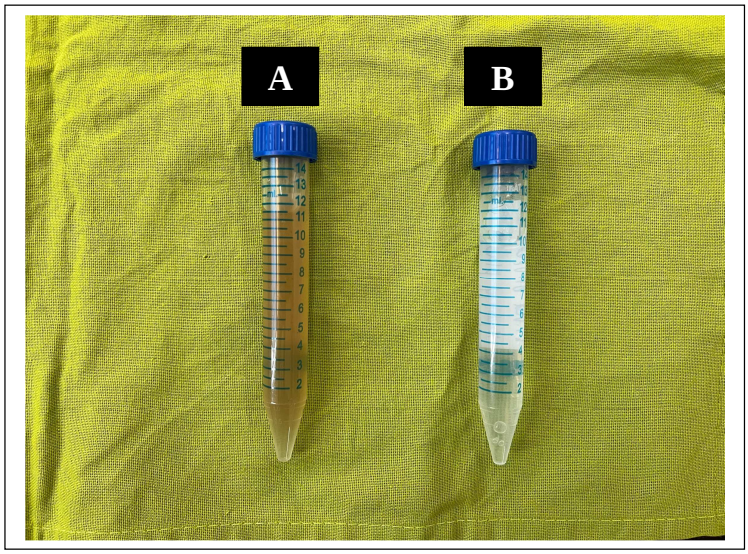
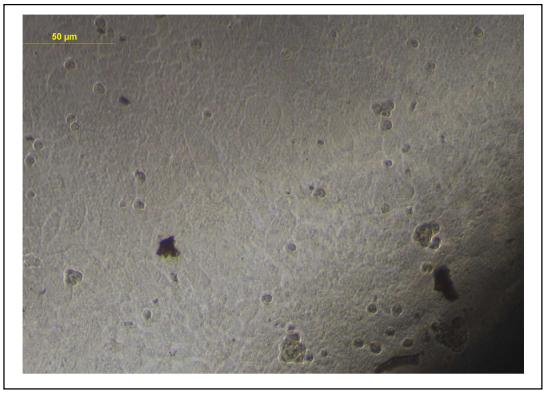
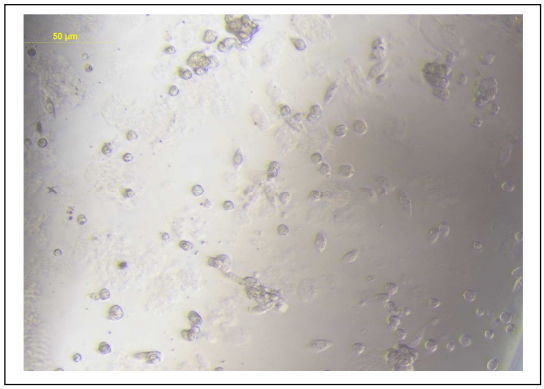


Figure 11: Dip Coating of titanium disc

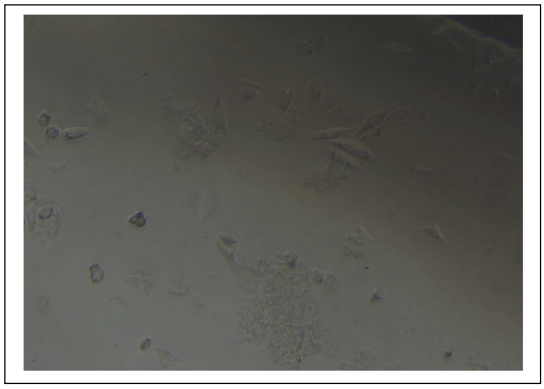
Figure 12: Cell Proliferation at 24, 48 & 72hrs



24hrs

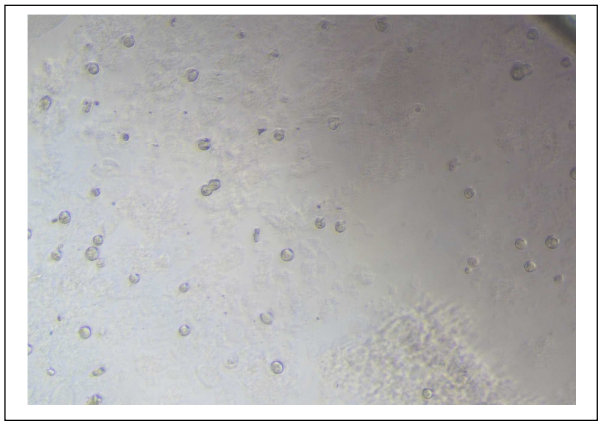


48hrs

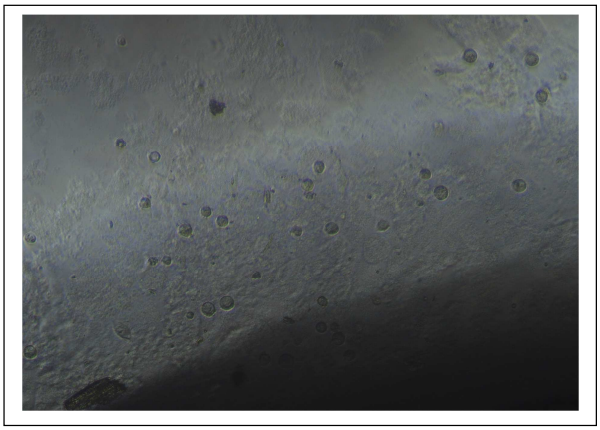


72hrs

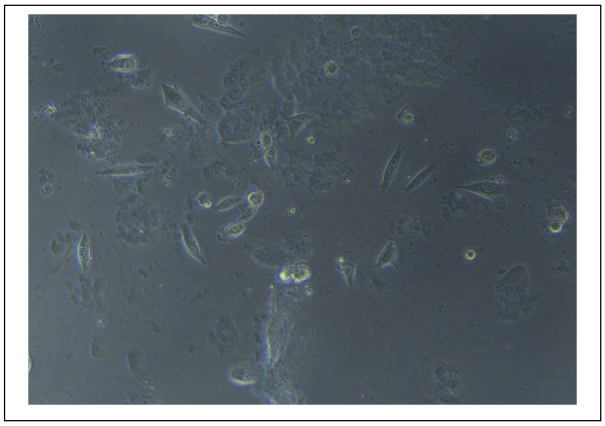
Figure 13: Cell Attachment at 24, 48 & 72hrs



24hrs

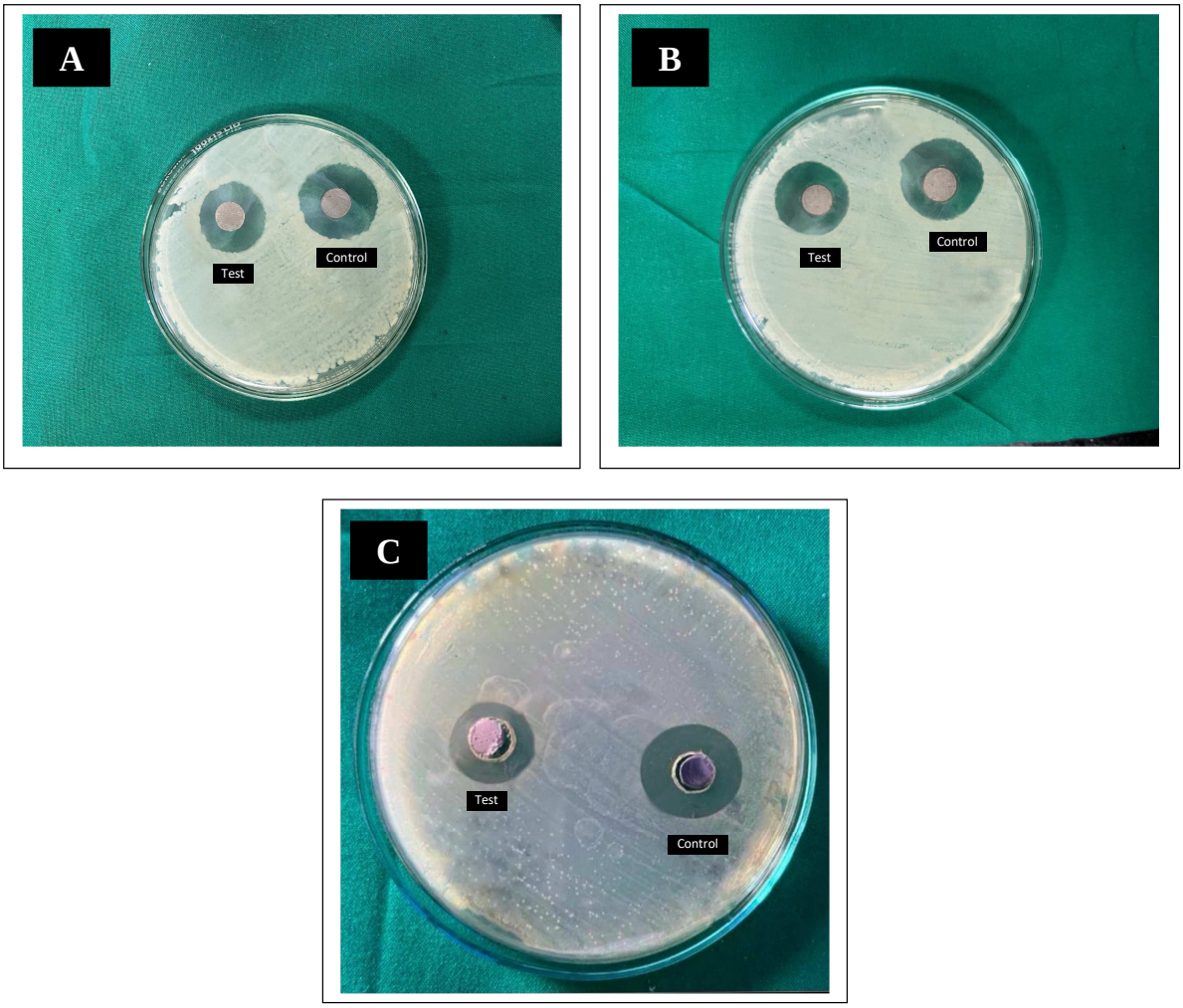


48hrs



72hrs

Figure 14: Zone of Inhibition A- Escherichia Coli, B-Pseudomonas aeruginosa, C- Staphylococcus aureus



RESULTS

The cytotoxicity, osteogenic potential and antibacterial efficacy of a novel gel formulation which include triphala and Cissus quadrangularis were examined in this current research. The osteogenic potential was assessed using a cell attachment procedure involving MG-63 cell lines, which exhibit comparable behaviour to human osteoblasts.

Cell cytotoxicity was evaluated employing a cell proliferation assay, specifically the MTT assay, to determine viable cell counts. Subsequently, the antimicrobial effectiveness was evaluated through a disc diffusion assay. All experiments were conducted at three distinct time points: 24, 48, and 72 hours, with a sample size of 216.

The resulting values of osteogenic potential, measured by the number of cells attached and the percentage of their proliferation, were analyzed for both the Study Group & Control $_{(ost)}$ Group and antimicrobial efficacy with the Study & Control $_{(Chx)}$ Groups. Based on the experimental results, conclusions were drawn using statistical analysis. For every group, descriptive statistical metrics like the mean and standard deviation were computed.

Table 4: Summary of Cell attachment in two groups and three time points

Factors	Levels	n	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	TCQ Hydrogel	36	1360120.0	92055.74	15342.62	1328972.8	1391267.2
	Control	36	1336668.3	84583.56	14097.26	1308049.3	1365287.2
Times	24hrs	24	1230179.6	10455.32	2134.18	1225764.7	1234594.5
	48hrs	24	1380001.4	7.86	1.60	1379998.1	1380004.7
	72hrs	24	1435001.4	25537.10	5212.74	1424218.0	1445784.8
Interaction	TCQ Hydrogel with 24hrs	12	1240358.3	1585.42	457.67	1239351.0	1241365.7
	TCQ Hydrogel with 48hrs	12	1380000.8	9.75	2.81	1379994.6	1380007.0
	TCQ Hydrogel with 72hrs	12	1460000.8	9.75	2.81	1459994.6	1460007.0
	Control with 24hrs	12	1220000.8	9.75	2.81	1219994.6	1220007.0
	Control with 48hrs	12	1380002.0	5.77	1.67	1379998.3	1380005.7
	Control with 72hrs	12	1410002.0	5.77	1.67	1409998.3	1410005.7

Table 5: Comparison of Cell attachment scores by two-way ANOVA

Sources of variation	Sum of squares	DF	Mean sum of squares	F-value	p-value
Main effects					
Group	9899698953.00	1	9899698953.00	23627.7904	0.0001*
Treatment times	539388521227.00	2	269694260614.00	643684.1660	0.0001*
2-way interaction effects					
Group*Treatment times	7586167900.00	2	3793083950.00	9053.0220	0.0001*
Error	27653035.70	66	418985.39		
Total	556902041115.70	71			

*$p<0.05$

A dependent t test and repeated measure ANOVA were used to compare the Study Group's osteogenic potential. Additionally, the antibacterial efficacy of the Study Group at various time intervals was compared using an independent t test. The study group's antibacterial efficacy was compared using a two-way ANOVA. P-values below 0.05 was considered as statistically significant.

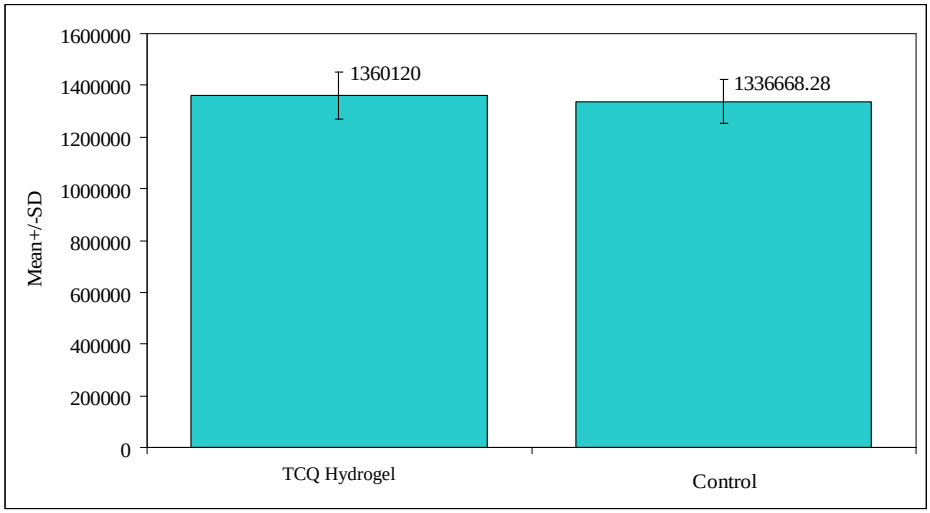
The Two-way ANOVA test performed for osteogenic potential showed significant differences in terms of MG-63 cell attachment in between both Study and $control_{(ost)}$ group for 24, 48 and 72hrs. The cell attachment is significant in study group, with p-value 0.0001. (Table 5).

Table 6: Comparison of two groups with Cell attachment scores by Tukey's multiple post hoc procedures

Groups	TCQ Hydrogel	Control
Mean	1360120.00	1336668.28
Std. Dev.	92055.74	84583.56
TCQ Hydrogel	-	
Control	P=0.0001*	-

*p<0.05

Graph 1: Comparison of two groups with Cell attachment scores



Comparison between the groups suggest that Study Group has a statistically significant effect compared to the $Control_{(Ost)}$ group. The higher mean value in the Study group (compared to the $Control_{(Ost)}$) suggests that this formulation have an osteogenic potential. Since p = 0.0001 (< 0.05), we can reject the null hypothesis (which assumes no difference between the groups) and conclude that this TCQ Hydrogel produces a significant impact. (Table 6 & Graph1)

Table 7: Pairs of comparison of three time points with Cell attachment scores by Tukey's multiple post hoc procedures

Times	24hrs	48hrs	72hrs
Mean	1230179.58	1380001.42	1435001.42
SD	10455.32	7.86	25537.10
24hrs	-		
48hrs	P=0.0001*	-	
72hrs	P=0.0001*	P=0.0001*	-

*p<0.05

Graph 2: Comparison of three time points with Cell attachment scores

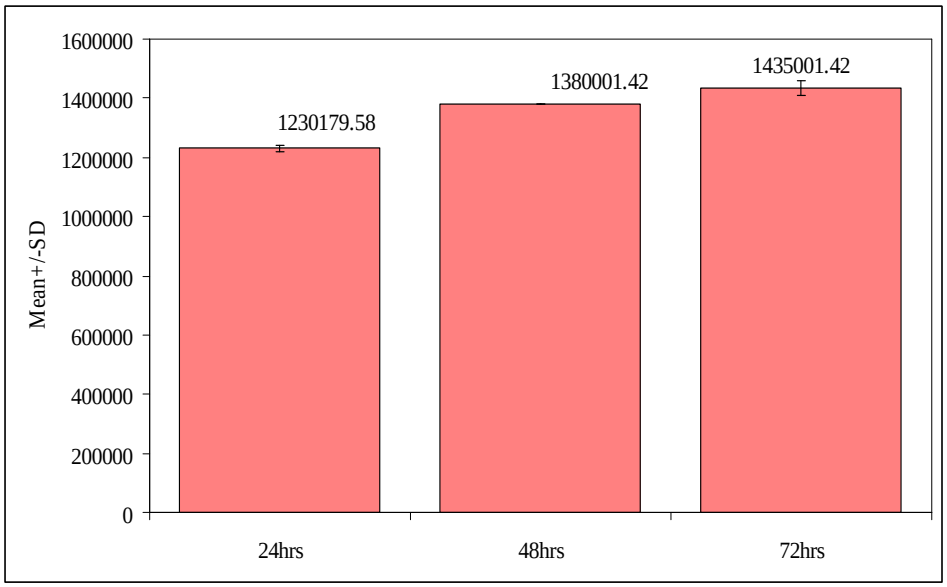
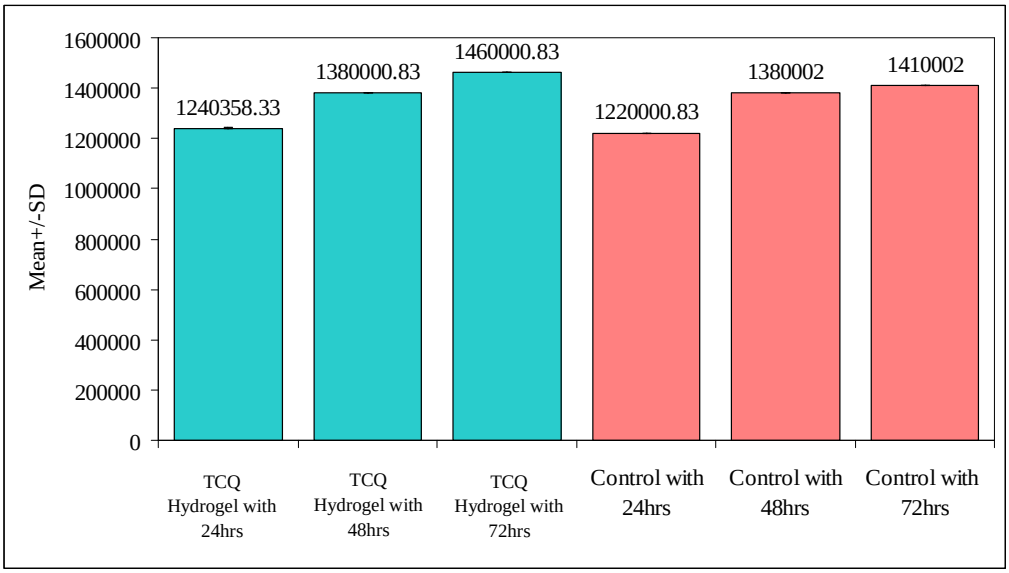


Table 8: Pairs of comparisons of interactions of two groups and three time points with Cell attachment scores by Tukey's multiple post hoc procedures

Interactions	TCQ Hydrogel with 24hrs	TCQ Hydrogel with 48hrs	TCQ Hydrogel with 72hrs	Control with 24hrs	Control with 48hrs	Control with 72hrs
Mean	1240358.33	1380000.83	1460000.83	1220000.83	1380002.00	1410002.00
SD	1585.42	9.75	9.75	9.75	5.77	5.77
TCQ Hydrogel with 24hrs	-					
TCQ Hydrogel with 48hrs	p=0.0001*	-				
TCQ Hydrogel with 72hrs	p=0.0001*		-			
Control with 24hrs	p=0.0001*	p=0.0001*	p=0.0001*	-		
Control with 48hrs	p=0.0001*	p=0.9999	p=0.0001*	p=0.0001*	-	
Control with 72hrs	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	p=0.0001*	-

*$p<0.05$

Graph 3: Comparisons of interactions of two groups and three time points with Cell attachment scores



The Table 8 & Graph 3 presents a comparison of cell attachment scores between two groups (Study Group and Control) over three time frames (24, 48, and 72 hours) using Tukey's multiple post hoc tests. The mean values indicate that Study group shows a progressive increase in cell attachment over time, with the highest mean observed at 72 hours (1,460,000.83). In contrast, the control group also exhibits an increase in attachment, but at a lower rate, with the highest mean at 72 hours (1,410,002.0). The standard deviations (SDs) are very small, suggesting low variability in the data. The p-values show that, except for the comparison between Study group (48 hours) and Control (48 hours) ($p=0.9999$, non-significant), all other comparisons are statistically significant ($p=0.0001$, $p<0.05$). This indicates that Study group significantly enhances cell attachment compared to the control at most time points, reinforcing its potential effectiveness in promoting cell adhesion.

Table 9: Summery of Cell proliferation in two groups and three time points

Factors	Levels	n	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	TCQ Hydrogel	36	86.7	7.41	1.24	84.2	89.2
	Control	36	94.7	5.24	0.87	92.9	96.4
Times	24hrs	24	84.5	6.33	1.29	81.8	87.2
	48hrs	24	91.0	6.47	1.32	88.3	93.7
	72hrs	24	96.5	4.26	0.87	94.7	98.3
Interaction	TCQ Hydrogel with 24hrs	12	79.0	1.13	0.33	78.3	79.7
	TCQ Hydrogel with 48hrs	12	86.0	4.07	1.17	83.4	88.6
	TCQ Hydrogel with 72hrs	12	95.0	4.07	1.17	92.4	97.6
	Control with 24hrs	12	90.0	4.07	1.17	87.4	92.6
	Control with 48hrs	12	96.0	4.07	1.17	93.4	98.6
	Control with 72hrs	12	98.0	4.07	1.17	95.4	100.6

Table 10: Comparison of two groups and three time points with Cell proliferation scores by two-way ANOVA

Sources of variation	Sum of squares	DF	Mean sum of squares	F-value	p-value
Main effects					
Group	1152.00	1	1152.00	82.2857	0.0001*
Treatment times	1732.00	2	866.00	61.8571	0.0001*
2-way interaction effects					
Group*Treatment times	228.00	2	114.00	8.1429	0.0007*
Error	924.00	66	14.00		
Total	4036.00	71			

*p<0.05

Table 10 presents the results of a two-way ANOVA analyzing the effects of group (Study vs. Control) and treatment time (24, 48, and 72 hours) on cell proliferation scores. The main effects for both group ($F = 82.2857$, $p = 0.0001$) and treatment time ($F = 61.8571$, $p = 0.0001$) are statistically significant ($p < 0.05$), indicating that both factors independently influence cell proliferation. Additionally, the interaction effect between group and treatment time ($F = 8.1429$, $p = 0.0007$) is also significant, suggesting that the effect of the treatment varies over time. The low error variance (924.00) and high total variance (4036.00) indicate a strong effect of the treatment.

Table 11: Comparison of two groups with Cell proliferation scores by Tukey's multiple post hoc procedures

Groups	TCQ Hydrogel	Control
Mean	86.67	94.67
Std. Dev.	7.41	5.24
TCQ Hydrogel	-	
Control	P=0.0001*	-

*p<0.05

Graph 4: Comparison of two groups with Cell proliferation scores

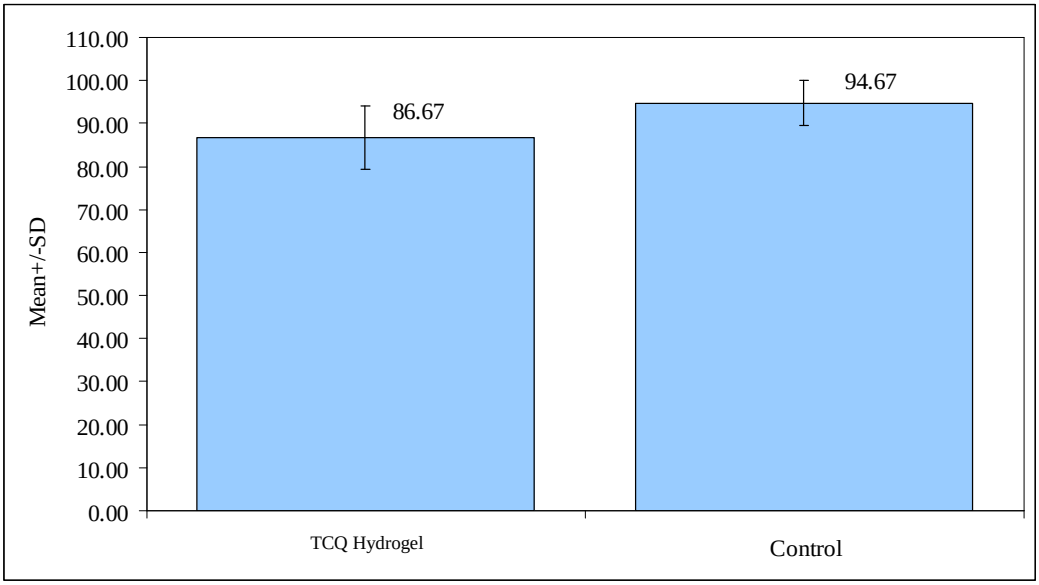


Table 12: Pairs of comparison of three time points with Cell proliferation scores by Tukey's multiple post hoc procedures

Times	24hrs	48hrs	72hrs
Mean	84.50	91.00	96.50
SD	6.33	6.47	4.26
24hrs	-		
48hrs	P=0.0001*	-	
72hrs	P=0.0001*	P=0.0001*	-

*p<0.05

Graph 5: Comparison of three time points with Cell proliferation scores

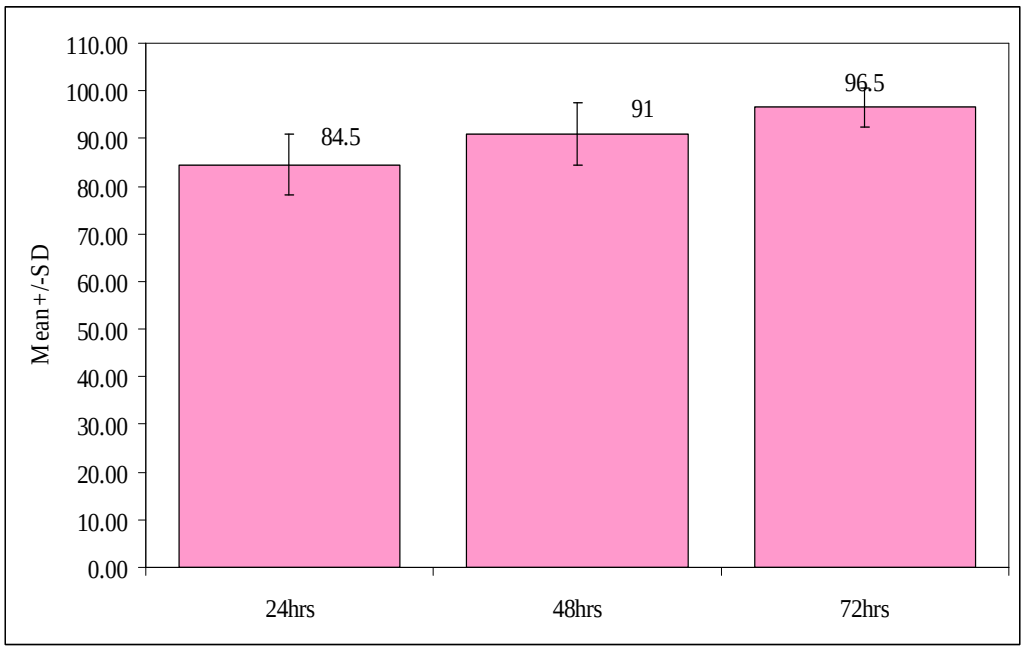


Table 13: Pairs of comparisons of interactions of two groups and three time points with Cell proliferation scores by Tukey's multiple post hoc procedures

Interactions	TCQ Hydrogel with 24hrs	TCQ Hydrogel with 48hrs	TCQ Hydrogel with 72hrs	Control with 24hrs	Control with 48hrs	Control with 72hrs
Mean	79.00	86.00	95.00	90.00	96.00	98.00
SD	1.13	4.07	4.07	4.07	4.07	4.07
TCQ Hydrogel with 24hrs	-					
TCQ Hydrogel with 48hrs	p=0.0004*	-				
TCQ Hydrogel with 72hrs	p=0.0001*	p=0.0001*	-			
Control with 24hrs	p=0.0001*	p=0.1072	p=0.0202*	-		
Control with 48hrs	p=0.0001*	p=0.0001*	p=0.9862	p=0.0028*	-	
Control with 72hrs	p=0.0001*	p=0.0001*	p=0.3739	p=0.0002*	p=0.7789	-

*p<0.05

Table 13 presents the pairwise comparisons of cell proliferation scores between Study & control $_{(ost)}$ over three time frames (24, 48, and 72 hours) using Tukey's multiple post hoc-test. The mean values indicate that cell proliferation increases over time in both groups, with the control group consistently showing higher values. Statistical significance (p-value < 0.05) is observed in multiple comparisons suggesting that treatment influences cell proliferation differently across time. Notably, Study group at 72 hours is significantly different from its earlier time points (p = 0.0001).

Graph 6: Comparisons of interactions of two groups and three time points with Cell proliferation scores

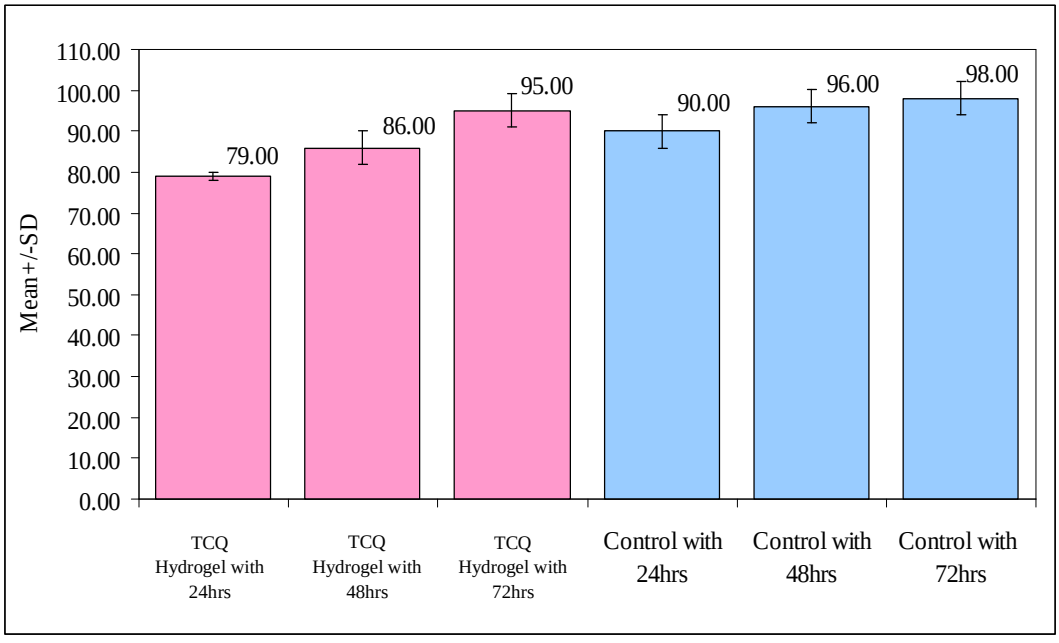


Table 14: Summary of zones of inhibition in two groups and three organisms

Factors	Levels	n	Mean	SD	SE	95% CI for mean	
						Lower	Upper
Groups	TCQ Hydrogel	36	19.4	2.69	0.45	18.5	20.3
	Control	36	21.3	2.61	0.43	20.5	22.2
Organisms	E. coli	24	20.5	2.70	0.55	19.4	21.7
	S. aureus	24	18.4	1.74	0.36	17.6	19.1
	P. aeruginosa	24	22.2	2.53	0.52	21.1	23.2
Interaction	TCQ Hydrogel with *E. coli*	12	20.0	2.76	0.80	18.2	21.8
	TCQ Hydrogel with *S. aureus*	12	17.3	1.42	0.41	16.3	18.2
	TCQ Hydrogel with *P. aeruginosa*	12	20.9	2.31	0.67	19.4	22.4
	Control with *E. coli*	12	21.1	2.64	0.76	19.4	22.8
	Control with *S. aureus*	12	19.5	1.24	0.36	18.7	20.3
	Control with *P. aeruginosa*	12	23.4	2.15	0.62	22.0	24.8

Table 15: Comparison of two groups and three organisms with zones of inhibition scores by two-way ANOVA

Sources of variation	Sum of squares	DF	Mean sum of squares	F-value	p-value
Main effects					
Group	68.0556	1	68.0556	14.4892	0.0003*
Organisms	173.6944	2	86.8472	18.4901	0.0001*
2-way interaction effects					
Group*Organisms	6.8611	2	3.4306	0.7304	0.4856
Error	310.0000	66	4.6970		
Total	558.6111	71			

*$p<0.05$

Table 15 presents the results of a two-way ANOVA analyzing the effects of two groups (study vs. control) and three organisms on zones of inhibition scores. The main effects for both group ($F = 14.4892$, $p = 0.0003$) and organisms ($F = 18.4901$, $p = 0.0001$) are statistically significant ($p < 0.05$), indicating that both factors independently influence the zones of inhibition. However, the interaction effect between group and organisms ($F = 0.7304$, $p = 0.4856$) is not significant, suggesting that the effect of the treatment does not vary significantly between the different organisms. The relatively high error variance (310.0000) suggests some variability in the data. Overall, the results indicate that both the treatment significantly antimicrobial impact.

Table 16: Comparison of two groups with zones of inhibition scores by Tukey's multiple post hoc procedures

Groups	TCQ Hydrogel	Control
Mean	19.39	21.33
Std. Dev.	2.69	2.61
TCQ Hydrogel	-	
Control	P=0.0004*	-

*p<0.05

Graph 7: Comparison of two groups with zones of inhibition scores

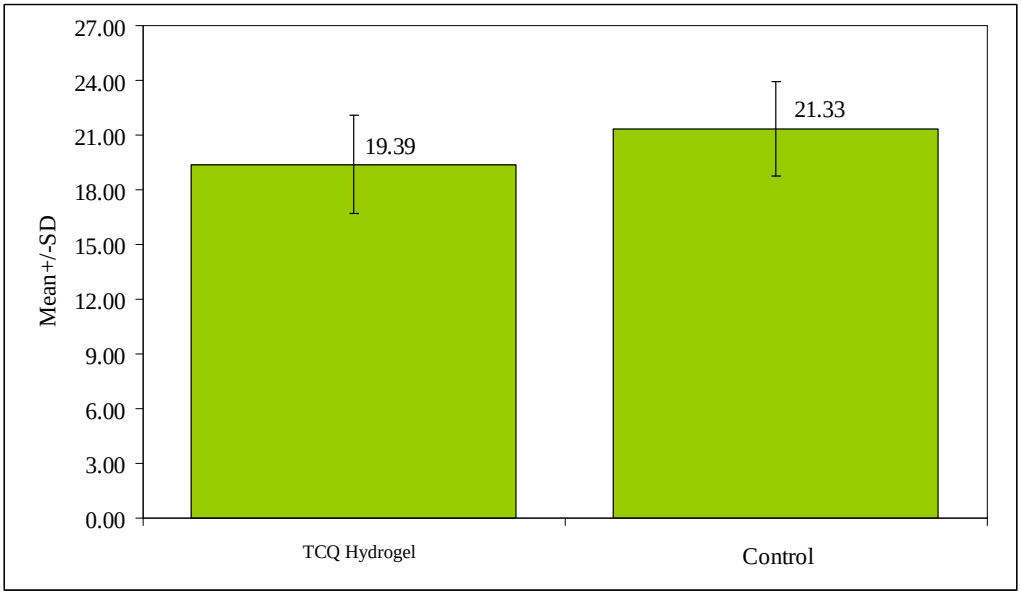


Table 17: Pairs of comparison of three organisms with zones of inhibition scores by Tukey's multiple post hoc procedures

Times	E coli	S aureus	P. aeruginosa
Mean	20.54	18.38	22.17
SD	2.70	1.74	2.53
E coli			
S aureus	p=0.0028*		
P. aeruginosa	p=0.0308*	p=0.0001*	

*p<0.05

Graph 8: Comparison of three organisms with zones of inhibition scores

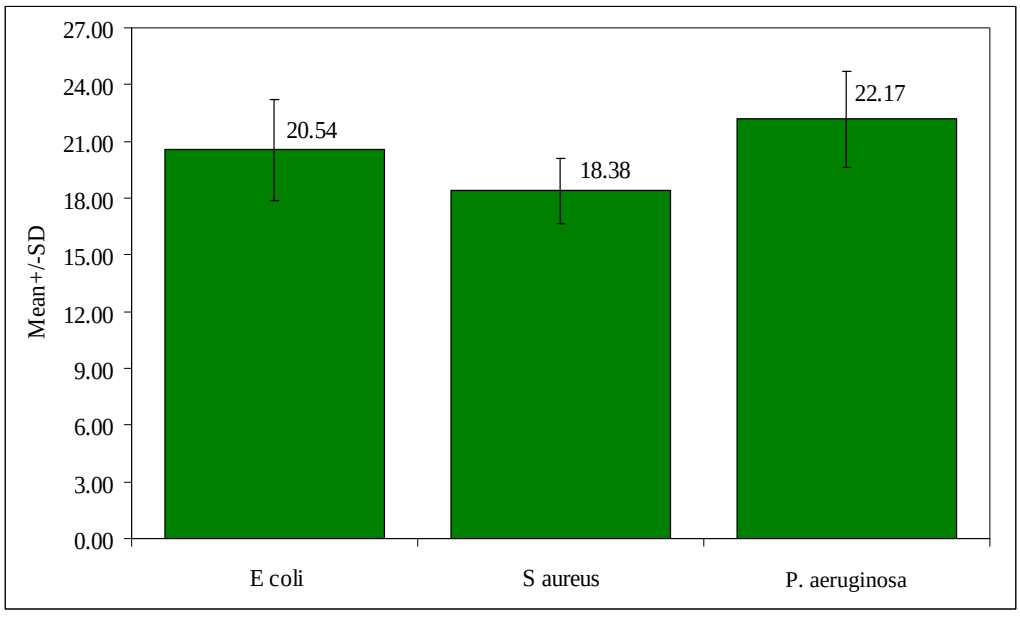


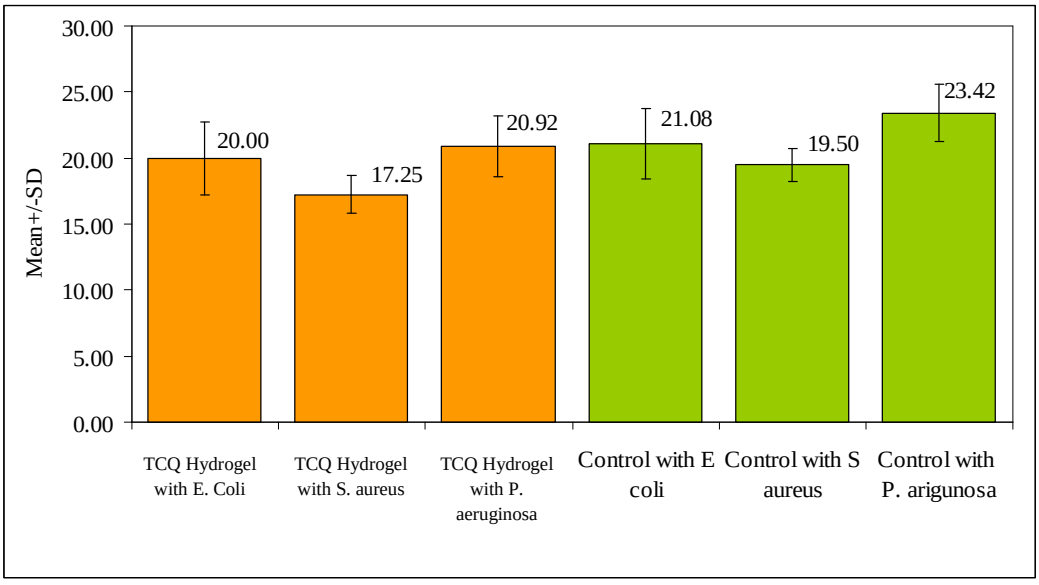
Table 18: Pairs of comparisons of interactions of two groups and three organisms with zones of inhibition scores by Tukey's multiple post hoc procedures

Interactions	TCQ Hydrogel with E coli	TCQ Hydrogel with S aureus	TCQ Hydrogel with P. aeruginosa	Control with E coli	Control with S aureus	Control with P. aeruginosa
Mean	20.00	17.25	20.92	21.08	19.50	23.42
SD	2.76	1.42	2.31	2.64	1.24	2.15
TCQ Hydrogel with E coli	-					
TCQ Hydrogel with S aureus	p=0.0318*	-				
TCQ Hydrogel with P. aeruginosa	p=0.9041	p=0.0014*	-			
Control with E coli	p=0.8234	p=0.0008*	p=0.9998	-		
Control with S aureus	p=0.9930	p=0.1268	p=0.6008	p=0.4795	-	
Control with P. aeruginosa	p=0.0035*	p=0.0001*	p=0.0659	p=0.1028	p=0.0006*	-

*p<0.05

In Table 18, the mean values indicate that the control group generally exhibits larger inhibition zones, particularly against P. aeruginosa (23.42 mm), compared to the Study group. Statistically significant differences ($p < 0.05$) are observed in multiple comparisons, particularly between Study Group and Control for S. aureus ($p = 0.0008$) and P. aeruginosa ($p = 0.0001$),. However, the differences between Triphala Churna Hydro Gel and Control for E. coli ($p = 0.8234$, non-significant) suggest that both treatments have a similar inhibitory effect against this organism. Overall, the results indicate that while Study group has antibacterial properties, its efficacy varies by bacterial species and is showing potent antimicrobial properties.

Graph 9: Comparisons of interactions of two groups and three organisms with zones of inhibition scores



DISCUSSION

Osseointegration, a concept introduced by Per-Ingvar Brånemark has made it possible to create a strong base for modern implantology. Use of implants in dentistry has made a paradigm shift in rehabilitating patients and has proven to be a reliable solution for missing teeth. The sterile structural connection Bone implant connection ensures a long-term stability of implants. In past decades the design modifications and advancements in them have significantly improved the success rates.[22,47]

Osseointegration is a dynamic bone remodelling process which is influenced by mechanical and biologic factors. This process starts immediately after the placement of implant in a cascade, involving osteoblastic bone formation and osteoclastic resorption. This ensures a structural and functional connection, ultimately stabilizing the implant. The degree of remodelling is influenced by implant design, loading conditions, & factors specific to patients such as bone quality & systemic health.[48]

However, osseointegration and bone implant contact (BIC) are primarily improved by surface characteristics, including surface, topography, roughness, & energy.[49]

Bone-implant contact (BIC) is a crucial factor that determines the success of osseointegration. A higher BIC percentage indicates better implant stability and integration with the surrounding bone.[50] Histomorphometry studies have revealed that implant surface modifications, such as roughened surfaces and bioactive coatings, enhance BIC by promoting osteoblast adhesion. Furthermore, mechanical loading plays a significant role in BIC, with early functional loading facilitating the adaptation

of bone to the implant. Therefore, achieving optimal BIC is necessary for long-term implant success and minimizing the risk of failure.[51]

Albrektsson and Wennerberg classified implant surfaces roughness (Sa) as Smooth surfaces (Sa < 0.5 μm), Minimal roughness (Sa = 0.5–1 μm), Moderate roughness (Sa = 1–2 μm), High roughness (Sa > 2 μm) and the authors concluded that moderately roughened surfaces appear to offer some clinical benefits than surfaces. [47]

Surface roughness plays a crucial role when it comes to cell attachment and osseointegration. Micro & Nano-rough surfaces promote osteoblast adhesion, proliferation, and differentiation, leading to faster bone formation. Studies have shown surfaces with moderate roughness (Ra value of 1-2 μm) enhance BIC & improve implant stability. Techniques such as sandblasting, acid etching, and plasma spraying create controlled roughness, improving cellular response. Enhanced roughness facilitates protein adsorption, which further regulates cellular interactions at the implant interface.[52]

Marta Romero-Serrano et al conducted a systematic review on dental implants on 3 months prospective studies after the placement. They concluded that surface roughness plays a crucial role and is directly correlated with the amount of osseointegration.[52]

The current study evaluated the surface roughness values using a profilometer (Surtronic S–128, Taylor Hobson). This device provides the roughness profile from the center line within the measuring length. The values obtained had a mean range between 1.2 and 1.7 μm. These values coincide with those found in literature with similar measurements. To quantitatively assess these results, scanning electron

microscope evaluation was performed to obtain images at 100X, 500X, and 1000X magnification.

Lukaszewska et al in his research proved that the dispersion of cells on smooth surface is more than rough surface, but the cells show better cytoplasmic extensions and interconnections among them on rough surfaces suggesting that they exhibit better adhesion properties. Along with this there was significant increase in vitality of the cells as well.[53]

Santos et al proposed that implants with a rough surface promote better osseointegration. However, careful manipulation is crucial, as this surface treatment can have adverse effects or alter the titanium oxide surface. Therefore, it's essential to standardize the surface roughness of titanium to ensure consistent use for evaluating hydrogel characteristics.[54]

To achieve high clinical success rates, various coatings have been proposed. An ideal coating surface along with therapeutic effects should possess the following capabilities: enhanced cell attachment & differentiation, bone apposition & fixation & minimal dissolution in body fluids.[55]

The success of oral rehabilitation using dental implants hinges on several factors. The implantation process demands favourable interactions between the titanium surface and the surrounding bone, as well as resistance against bacterial colonization. Implant-related infections significantly contribute to implant failure.

Peri-implantitis is a pathology in which inflammation is followed by continuous bone loss around dental implants. This is primarily caused by bacterial infection. This infection leads to the formation of peri-implant pockets and subsequent destruction of supporting bone. [56]

Derks & Tomasi (2015) conducted a systematic review which revealed that peri-implantitis affects approximately 10-20% of cases within 5-10 years of placement. Risk factors like poor oral hygiene, smoking, diabetes, including implant surface contamination, contribute to the development of peri-implantitis. Early detection and intervention are crucial in preventing severe complications and implant failure.[57]

To prevent peri-implantitis, several methods are employed, focusing on patient education, maintenance protocols, and implant surface modifications. Regular professional cleanings, antimicrobial rinses, and improved oral hygiene practices significantly reduce bacterial load around implants. Additionally, systemic and local antibiotic therapies have been explored as adjunctive treatments. Proper implant placement, ensuring adequate keratinized tissue, and minimizing excess cement during prosthesis delivery are also essential preventive strategies.[58]

Different implant coatings have been developed to reduce the incidence of peri-implantitis by inhibiting bacterial colonization and promoting healthy osseointegration. Silver coatings exhibit strong antibacterial properties, preventing biofilm formation on implant surfaces.[55] Similarly, titanium dioxide (TiO2) coatings possess antimicrobial and Osseo integrative benefits.[59] Hydroxyapatite coatings enhance BIC while reducing bacterial adhesion, making them a popular choice in implantology. The integration of bioactive and antimicrobial coatings represents a promising approach to reducing peri-implantitis incidence.

Innovative coatings such as antimicrobial peptides, drug-eluting surfaces, and graphene-based materials are under investigation for their potential to enhance implant longevity.[60] Hans Erling Skallevold et al in their literature review stated that

bioactive glass coatings promote osteogenesis while simultaneously inhibiting bacterial growth. Additionally, surface-functionalized implants with controlled-release antimicrobial agents hold promise for reducing peri-implantitis-related complications. [61]

Chlorhexidine, the most widely used and primary agent for chemical plaque control, has earned its reputation as the gold standard.[62] However, its long-term use is limited due to the increasing resistance it develops and the potential side effects that can disrupt the oral microflora and cause cellular toxicity.[63] Consequently, the ongoing research and development efforts have shifted the focus towards biogenic agents as potential alternatives.

James X. Liu et al evaluated the cytotoxic nature of chlorhexidine at clinical level concentrations on human fibroblast cells (CC-2511). This study proved that even clinically used concentrations can stop the cell migration and its survivability.[63]

Peri-implant infections are primarily caused by microbial colonization of implant surfaces.[64] The most commonly isolated organisms include *Escherichia coli* (approximately 20%), *Staphylococcus aureus* (up to 40%), and *Pseudomonas aeruginosa* (around 25%) in various in vivo and in vitro models of peri-implantitis. These opportunistic pathogens possess strong adhesion capabilities on biomaterial surfaces, particularly titanium, where micro-topographic features facilitate bacterial retention and biofilm development.[56]

Escherichia coli is capable of adhering to implant surfaces via fimbriae and pili. It produces extracellular polymeric substances (EPS) which facilitates formation of mature biofilms. The lipopolysaccharide (LPS) of E. coli triggers the host immune response, contributing to peri-implant mucositis and subsequent bone loss.

Staphylococcus aureus, a Gram-positive coccus, utilizes adhesins such as fibronectin-binding proteins to colonize surfaces. It secretes toxins and enzymes that degrade peri-implant tissue, leading to a cascade of inflammatory cytokines (IL-1β & TNF-α). This initiates osteoclastic activity and peri-implant bone resorption.[65]

Pseudomonas aeruginosa is a particularly virulent pathogen characterized by robust quorum-sensing mechanisms and multidrug resistance. It adheres to implant surfaces via flagella and pili, forming thick biofilms with an alginate matrix, rendering it resistant to antimicrobial treatments. These bacteria secrete proteases and exotoxins that impair neutrophil function and retard wound healing, thereby intensifying the inflammatory milieu surrounding the implant and accelerating the progression to peri-implantitis.[66]

Triphala, an ancient Ayurvedic formulation comprising Terminalia chebula, Terminalia bellerica, & Emblica officinalis, has demonstrated broad-spectrum antimicrobial activity. It exhibits substantial inhibition against both Gram-positive & negative bacteria implicated in oral infections, including Staphylococcus aureus[67] and Escherichia coli. Its active constituents, like gallic acid, chebulagic acid, and ellagic acid, interfere with bacterial cell wall integrity and inhibit biofilm formation, rendering it an appealing phytotherapeutic option for peri-implant infections.[11,12,39]

Studies have demonstrated that Triphala effectively modulates inflammatory markers, including IL-6, TNF-α, and CRP. By reducing the cytokine-induced destruction of peri-implant tissues, Triphala effectively mitigates inflammation.[68]

Studies conducted by Vinay Rayudu et al in rat models, further support these findings, revealing reduced inflammatory infiltrates and the preservation of alveolar bone when Triphala is topically applied. These observations align with the

suppression of osteoclast activity and the enhanced expression of osteoblast-related genes, suggesting Triphala's dual anti-inflammatory and pro-regenerative properties.[69]

Sowmiya Kalaiselvan et al in their research, have shed light on the molecular mechanisms that underpin Triphala's anti-inflammatory properties. Specifically, Triphala effectively downregulates the activation of the NF-kB pathway, a key mediator of chronic inflammation associated with peri-implantitis. Moreover, Triphala-loaded scaffolds or coatings on titanium implants have demonstrated substantial antibacterial activity in laboratory experiments, exhibiting sustained release of bioactive compounds over time. This finding underscores the potential of Triphala as a coating agent in dental implant applications.[70]

Deepa Babu et al measured Triphala's antioxidant capacity, using DPPH and FRAP assays. They suggested the potentiality of antioxidative properties. This will help to reduce oxidative stress around implants, which can be exacerbated during bacterial infections. When combined with hydroxyapatite or polymer-based coatings. Triphala demonstrates potential, in creating bioactive implant surfaces that resist microbial adhesion while promoting osteogenesis.[68]

Cissus quadrangularis, a traditional medicinal plant renowned for its potent osteogenic and anti-inflammatory properties, has gained significant attention due to its significant role in bone healing. This plant extract has demonstrated the proliferation and differentiation of osteoblasts while simultaneously suppressing osteoclastic activity, this is because it is packed with flavonoids, ketosteroids, and ascorbic acid. Numerous animal studies have consistently demonstrated that Cissus

accelerates the healing of bone fractures and promotes the formation of callus, particularly when administered locally or systemically.[13]

Histological investigations done by Rahul Mahajan et al, in rabbit and rat models have shown that bones treated with Cissus quadrangularis extract exhibit early mineral deposition, a dense trabecular structure, and increased expression of bone morphogenetic proteins (BMPs) compared to control groups. Additionally, inflammatory markers such as IL-1β and COX-2 are significantly reduced in experimental animals, indicating its anti-inflammatory effect in bone microenvironments.[71]

In vitro assays on human osteoblast-like cells (MG-63) have revealed that Cissus extract enhances osteoblastic function by upregulating alkaline phosphatase activity, osteocalcin, and collagen type I expression. These cellular responses are attributed to the synergistic effects of its bioactive molecules, such as quercetin, β-sitosterol, and calcium oxalate. Furthermore, Cissus's role in bone tissue engineering has been investigated using Cissus-infused scaffolds, which exhibit superior cell adhesion and mineralization.[72]

Cissus quadrangularis-coated titanium surfaces are gaining traction in dental applications, as evidenced by studies demonstrating their ability to enhance bone-implant contact (BIC) in vivo. These surfaces facilitate osseointegration while simultaneously potentially mitigating inflammatory responses, positioning them as ideal candidates for extending implant longevity, particularly in patients with compromised bone health or diminished healing abilities.[44]

In this study, dip coating was used to functionalize titanium discs with a bioactive plant extract coating. Dip coating is a widely used technique because it's

simple, uniform, and reproducible for thin film deposition.[73] The titanium discs were first ultrasonically cleaned and then immersed in the prepared extract solution for 30 seconds, ensuring complete surface contact and proper adhesion of the coating layer. Afterward, the discs were air dried for 5 minutes under sterile conditions to allow solvent evaporation and layer stabilization.

The dipping process was uniformly repeated to ensure consistent coating across all disc surfaces. This method not only allows the incorporation of thermolabile compounds but also enables multiple coatings for controlled drug release and surface modification. [73]

Katja Andrina Kravanja & Matjaž Finšgar in their review also stated that Dip-coating is advantageous for implant applications due to its compatibility with bioactive molecules and its ability to conform to complex surface geometries.[55]

The current investigation delved into the osteogenic and antimicrobial properties of a novel TCQ Hydrogel, uncovering its potential to enhance peri-implant healing and infection control. For osteogenic potential, cell attachment and proliferation assays were conducted using MG-63 cells (as they behave similar to human osteoblast cells)[74],Over a time frame of 24, 48 and 72 hours, significant differences were observed between the study group and the control group. Notably, the MG-63 cell attachment in the study group was markedly enhanced, as evidenced by highly significant p-values ($p = 0.0001$). These findings suggest that the TCQ Hydrogel facilitates initial cellular adhesion, a pivotal step in the early stages of osseointegration and tissue remodelling.

A progressive elevation in cell attachment was observed over three days in the study group, with the highest mean value recorded at 72 hours. The consistently low

standard deviations in both groups indicate high reproducibility and minimal variability. Most intergroup comparisons across time points revealed statistically significant differences, which further supports the idea that the hydrogel formulation creates an environment conducive to cell adhesion and, consequently, promotes osteogenic activity.

Cell proliferation analysis further confirmed the osteogenic efficacy of the hydrogel. Both treatment duration and treatment group significantly impacted proliferation, as evidenced by the main effects in the two-way ANOVA. Additionally, a significant interaction between treatment type and time was discovered, suggesting that the proliferative response varied across time points in a group-dependent manner. Notably, the TCQ Hydrogel group exhibited a substantial increase in proliferation over time, while the control group showed relatively higher values. The increase at 72 hours, which emphasizes the gel's time-dependent osteogenic effect, was one of the statistically significant changes across time points within the research group which the multiple comparisons confirmed.

In terms of antimicrobial activity, the analysis revealed that both the treatment group and the type of bacterial organism significantly influenced the zone of inhibition. While the control treatment generally produced larger zones, particularly against Pseudomonas aeruginosa, the TCQ hydrogel also exhibited effective antibacterial properties. The study group showed significant inhibition against Staphylococcus aureus and Pseudomonas aeruginosa, suggesting that the hydrogel formulation has selective but potent antimicrobial efficacy. These results correlate with study done by Yogesh Biradar et al where they concluded the inhibition of potential periimplantitis microflora with Triphala Extracts.[38]

Collectively, these findings support the hypothesis that TCQ Hydrogel possesses both osteogenic and antimicrobial properties. The enhanced MG-63 cell adhesion and proliferation, coupled with its significant antibacterial activity against key peri-implant pathogens, indicate that this novel formulation could provide dual benefits in promoting osseointegration and preventing peri-implant infections. These properties hold particular relevance in the context of implant dentistry, where microbial colonization and inadequate bone regeneration continue to pose significant challenges.

SCOPE OF THE STUDY

- This research was aimed to assess the efficacy of two Ayurvedic drugs, Triphala and Cissus quadrangularis, when combined to evaluate their potency in two clinically relevant parameters in implant dentistry: osteogenic potential and antimicrobial efficacy against periimplantitis.
- The results demonstrate the potent osteogenic and antimicrobial potential of the hydrogel, which can be further validated in an In-Vivo setting. Additionally, the hydrogel dip coating method can be tested with various implants, and the responses observed can be analysed.
- These parameters can be tested with other implant biomaterials such as PEEK (Polyether Ether Ketone) & Zirconia.
- Drugs present in this gel were in their free-form state. Further research can be conducted to develop these drugs in microspheres for a sustained release.
- Parameters such as Alkaline Phosphatase activity (ALP), RANKL, Alizarin red, & Von Kossa staining can be used to assess both bone formation and antimicrobial activity.

LIMITATIONS

- This in vitro study lacks clinical validation and has a relatively small sample size.
- Since peri-implantitis is caused by a diverse range of microorganisms, the study focused only on the predominant microbial organisms in peri-implantitis and did not investigate the effect of TCQ Hydrogel on varying microbial strains.
- The study utilized MG-63 cells, which are osteoblasts-like cells that lack the ability to differentiate coherently.
- While the dipping method is a commonly used hydrogel coating technique, there may be variations in the uniformity of the coating's surface on the substrate. An ideal hydrogel coating method should exhibit strong adhesion to the substrate and conform to any arbitrary shape.

CLINICAL IMPLICATIONS

Periimplantitis and poor osseointegration lead to early failure of dental implants. To address this, various methods have been employed over the years. Similarly, this In Vitro study was conducted to assess the osteogenic & antimicrobial efficacy of the Novel TCQ hydrogel. This study proved that the novel TCQ hydrogel is non-cytotoxic as well as effective in promoting new MG-63 cell adhesion which in turn is an indicator for new bone formation. The gel also has antimicrobial properties to eliminate the significant colonizers in periimplantitis.

The TCQ Hydrogel is composed of naturally occurring products, making it more biocompatible and reducing the likelihood of microbial resistance. These natural drugs are safe and offer a promising alternative to synthetically derived drugs.

The primary objective of these hydrogels is to enhance the success rate in cases of bone deficiency and systemic conditions such as osteoporosis. By expediting bone healing, they enable immediate or early loading protocols & stimulate bone growth, allowing for implant placement in sites deficient in residual ridge.

With these advantages, TCQ hydrogel presents a beneficial, promising, and alternative treatment option to prevent periimplantitis and facilitate early osseointegration.

CONCLUSION

This in-vitro research was performed on titanium discs for evaluating, osteogenic potential & antimicrobial efficacy of Novel TCQ Hydrogel. This study concluded that the proliferation of cells at 24,48 and 72 hours was substantial, suggesting that the gel exhibited no cytotoxicity and promoted cell proliferation, which is crucial for bone regeneration. Increased surface roughness values, exhibited more cell attachment and proliferation, suggesting to substantial increases in bone formation & BIC.

The dip coated surface of a titanium disc with the novel gel, when seeded with MG-63 cells, exhibited a significant level of attachment at 24, 48, and 72 hours, suggesting the osteogenic properties of the hydrogel.

Additionally, the antimicrobial activity of the novel gel was assessed and compared with chlorhexidine gel (2%). The study group (TCQ Hydrogel) zones were comparable to those of the control $_{(Chx)}$ group, indicating that the gel possessed a significant antimicrobial activity. This property could potentially reduce the incidence of periimplantitis.

Further research and development in this area will pave the way for new discoveries and the potential of ancient drugs. This will enable us to identify and utilize similar or comparable drugs with more refined combinations and concentrations, which will lead to improved prospects in future developments.

SUMMARY

The study investigates the cytotoxicity, osteogenic potential and antimicrobial efficacy of a hydrogel composed of combination of Triphala and Cissus Quadrangularis extracts. This research seeks to improve osseointegration & peri-implant infection control.

A total of 216 identical Titanium Grade V discs were procured, each measuring 10 millimeters by 2 millimeters were used in the study. The specimens underwent sandblasting and ultrasonic cleaning. They were then divided into Study and Control $_{(Ost)}$ & Control $_{(Chx)}$ groups to assess the hydrogel's osteogenic potential and antimicrobial efficacy.

A 0.5% w/v TCQ gel was formulated. The gel's concentration was 500mg per 100ml, which is 200 times the MIC and 50 times the MBC.

The specimens underwent surface roughness evaluation using a contact stylus profilometer. Qualitative surface analysis was assessed using SEM. The roughness values ranged between 1.2 and 1.7 µm, which aligns with literature indicating that moderately rough surfaces enhance osseointegration.

Cytotoxicity was assessed using the MTT assay at 24, 48, and 72-hour intervals, and the proliferative index percentage was documented.

Osteogenic potential was evaluated through cell attachment using MG-63 cell lines at three different time points (24, 48, 72hours). Cell counts were recorded after trypsin detachment.

Lastly, the antimicrobial activity of the compounds was evaluated against *E. coli, P. aeruginosa, and S. aureus* using the disc diffusion assay. The diameter of the inhibition zones was measured and compared to the control groups.

The hydrogel exhibited a significant enhancement in MG-63 cell attachment and proliferation compared to the $control_{(Ost)}$ group. Statistically significant attachment and proliferation increase was observed over time, particularly at 72 hours. Minimal cytotoxic effects were observed, indicating the hydrogel's biocompatibility. It also demonstrated notable inhibition against Staphylococcus aureus and Pseudomonas aeruginosa. E. coli exhibited similar inhibition to the control group, suggesting comparable efficacy.

This TCQ Hydrogel formulation offers dual benefits in promoting osseointegration and preventing peri-implant infections. Its hydrogel structure supports cell adhesion and proliferation, while its antimicrobial properties target, key pathogens.

A study conducted on TCQ hydrogel formulation for dental implants highlights its potential to enhance implant longevity, reduce infection risk, and improve osseointegration.

By integrating bioactive and antimicrobial coatings, such as Triphala & Cissus quadrangularis, researchers can address the dual challenges of microbial colonization and bone regeneration, leading to improved implant success rates and patient outcomes.

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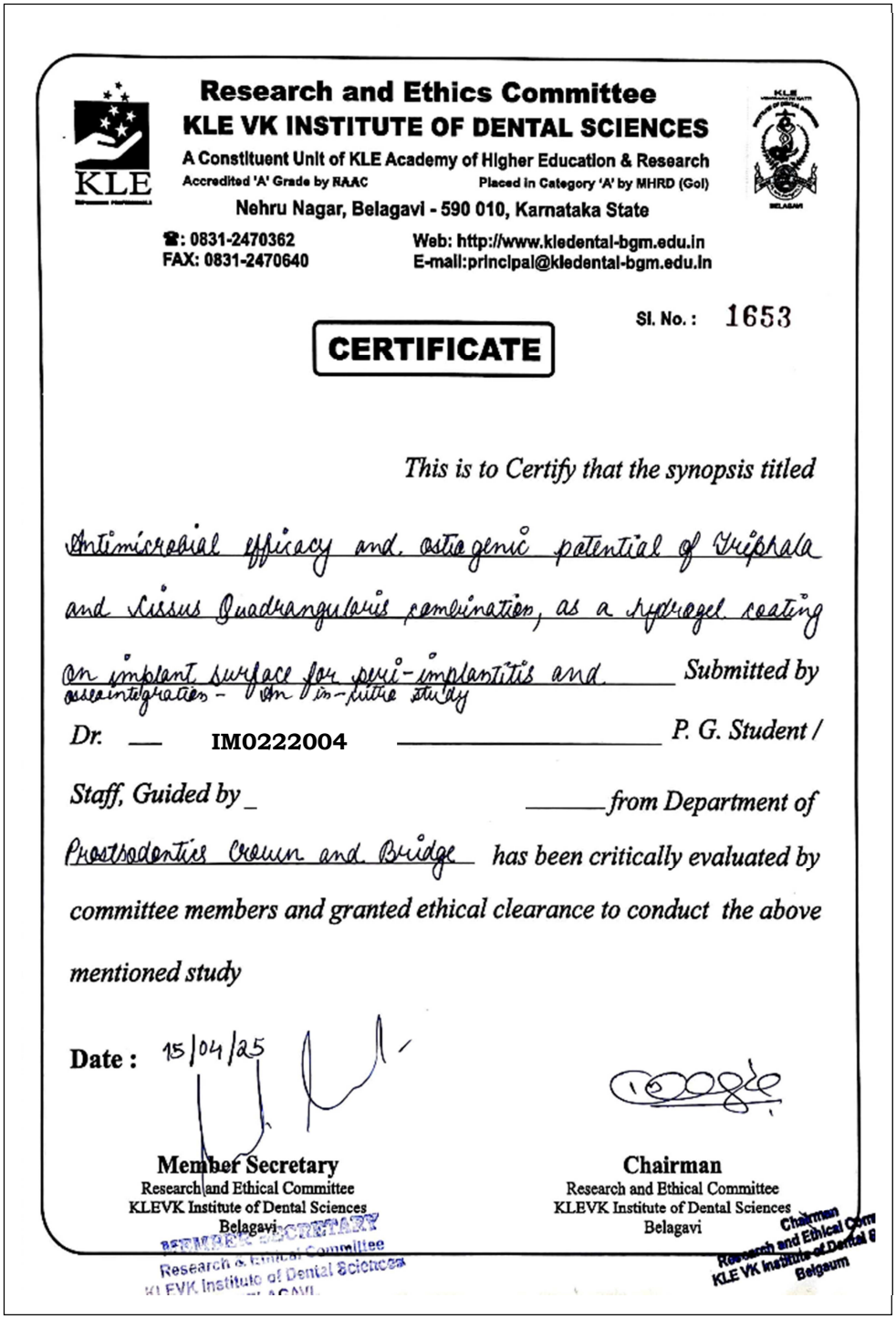
ANNEXURES

ANNEXURE I – ETHICAL CLEARENCE CERTIFICATE



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Sl. No.: 1653

CERTIFICATE

This is to Certify that the synopsis titled

Antimicrobial efficacy and osteogenic potential of Triphala and Cissus Quadrangularis combination, as a hydrogel coating on implant surface for peri-implantitis and osseointegration - An in-vitro study *Submitted by*

Dr. IM0222004 *P. G. Student / Staff, Guided by* _____ *from Department of* Prosthodontics Crown and Bridge *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 15/04/25

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURES II – SAMPLE SIZE ESTIMATION

SAMPLE SIZE CALCULATION

Title: Antimicrobial efficacy and Osteogenic potential of Triphala with Cissus Quadrangularis hydrogel mixture for periimplantitis and osseointegration"- an in-vitro study

Formula

$$n=\frac{(Z_{1-\frac{\alpha}{2}}+Z_{1-\beta})^2(p_1q_1+p_2q_2)}{(p_1-p_2)^2}$$

p_1 = Prevalence in the 1st group

p_2 = Prevalence in the 2nd group

q_1 = 1-p_1

q_2 = 1-p_2

Z_{1-a} = Significance level

Z_{1-B} = Power

Where,

p_1 = 0.570

p_2 = 0.107

q_1 = 0.430

q_2 = 0.890

Z_{1-a} = Alpha error at 5%

Z_{1-B} = Beta error at 80% power

Sample Size = 12 (each group)

Total Sample Size = 24 (2 groups for antimicrobial efficacy)

= 48 (4 groups for osteogenic potential)

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ANNEXURES III – TITANIUM DISC QUALITATIVE

EVALUATION

UQSR. ISO 9001:2015

ISO 9001:2015 Certified
- Optical Emission Spectrometry
- PMI
- Hardness Testing
- Ultrasonic Flaw Detection
- Ultrasonic Thickness Gauging
- Dye Penetrant Testing

MTL **METAL TEST LAB**
(Recognised By Government Deptts & Undertakings)

Office : Gr. Flr. Bhavnagari Bldg., 72, Nanubhai Desai Rd., Khetwadi Main Road, Mumbai - 400 004.
Phone : 6743 7546 • Mobile : 9224778882 / 9223371637 • E-mail : metaltestlab2016@gmail.com

TEST REPORT

T/C No : 1820 DATE 04/03/2022

PARTY NAME : SPECIAL METALS
125, C.P. TANK ROAD,
MUMBAI - 400 004.

REFERENCE : -
MATERIAL DESCRIPTION: TITANIUM DISC
GRADE : TI GR 5

%	C %	Si %	Mn %	P %	S %	Cr %	Mo %	Ni %	Al %
COMP	0.0600								6.10
REQD	-- 0.0800	--	--	--	--				5.5000 6.7500

%	Co %	Cu %	Nb %	Ti %	V %	W %	Pb %	Fe %	N %
COMP				87.88	4.40		--	0.069	--
REQD	-- --	-- --	-- --	-- --	3.5000 4.5000	-- --	-- --	-- 0.4000	-- --

REMARK: THE ABOVE MATERIAL CONFIRMS TO TITANIUM GR. 5 W.R.T. ELEMENTS SPECIFIED.

For METAL TEST LAB

METAL TEST LAB MUMBAI

AUTHORISED SIGNATORY

1. The above Test Reports relate only to the sample submitted.
2. The above samples are not drawn by the laboratory.
3. The company or its partners shall in no way responsible for any financial liability due to any act of omission or error made.
4. No part of this Test Report shall be reproduced without the written permission of this laboratory.

QUALITY IS OUR MOTTO

ANNEXURES IV – CELL ATTACHMENT READINGS

Cell Attachment			
	24hrs	48hrs	72hrs
Study Group (TCQ Hydrogel)	1238500	1379990	1459990
	1241200	1380010	1460010
	1239800	1380005	1460005
	1240300	1379995	1459995
	1242100	1380002	1460002
	1237900	1379998	1459998
	1243500	1380007	1460007
	1239600	1380003	1460003
	1240700	1380012	1460012
	1238800	1379988	1459988
	1241400	1380015	1460015
	1240500	1379985	1459985
Control (Ost) Group	1219990	1379995	1409995
	1220010	1380005	1410005
	1220005	1380008	1410008
	1219995	1379992	1409992
	1220002	1380003	1410003
	1219998	1380001	1410001
	1220007	1380007	1410007
	1220003	1380004	1410004
	1220012	1380006	1410006
	1219988	1379997	1409997
	1220015	1380010	1410010
	1219985	1379996	1409996

ANNEXURES V – CELL PROLIFERATION READINGS

Cell Proliferation (MTT)			
	24hrs	48hrs	72hrs
Study Group (TCQ Hydrogel)	78	84	93
	80	88	97
	79	83	92
	77	89	98
	81	85	94
	79	87	96
	78	82	91
	80	90	99
	79	81	90
	78	91	100
	80	80	89
	79	92	101
Control (Ost) Group	88	94	96
	92	98	100
	87	93	95
	93	99	101
	89	95	97
	91	97	99
	86	92	94
	94	100	102
	85	91	93
	95	101	103
	84	90	92
	96	102	104

ANNEXURES VI – ZONE OF INHIBITION READINGS

Zone of inhibition (mm)			
	Study Group (TCQ Hydrogel		Control (Chx) Group
E coli	18		20
	20		22
	23		23
	17		18
	16		18
	18		17
	22		24
	20		22
	22		20
	23		24
	24		25
	17		20
S aureus	18		19
	20		22
	16		19
	18		20
	15		18
	15		20
	17		18
	18		20
	17		20
	18		21
	17		19
	18		18
P. aeruginosa	20		22
	22		26
	21		24
	21		21
	17		26
	22		24
	25		26
	20		22
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