Fabrication of a Novel Implantoplasty Varnish Using *Cissus Quadrangularis* and *Vitex Negundo* Formulation Mediated Titanium Dioxide Nanoparticles

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**Abstract:**To fabricate and access the antimicrobial, anti-inflammatory, antioxidant, cytotoxic, and embryonic toxicology properties of a novel implantoplasty varnish using *Cissus quadrangularis* and *Vitex negundo* formulation mediated with Titanium dioxide nanoparticles.The plant extracts of Cissus *quadrangularis* and *Vitex negundo* incorporated in Titanium dioxide nanoparticles to synthesize a novel implantoplasty varnish. The varnish was subjected to a variety of tests to assess its antimicrobial, anti-inflammatory, anti-oxidant, cytotoxic, and embryonic toxicology. Further characterization of the nanoparticles was done with UV visible spectroscopy.Nanoparticles inherited important antibacterial properties against Staphylococcus aureus, whereas the zone of inhibition for *Enterococcus faecalis* and *Streptococcus mutans* was less. They also possess anti-inflammatory, antioxidant activity and also prove to be biocompatible. It showed no malformation in embryonic toxicology.This novel synthetic implantoplasty varnish may aid in reducing the inflammatory response in the soft tissues surrounding the implant as well as the harmful effects of pathogenic organisms. This can also be helpful in the healing of the soft tissues after implantoplasty is done.Peri-implantitis is the common reason for bone loss around dental implants for which implantoplasty is the last resort as a treatment choice. The purpose of this study is to lessen plaque adhesion and make it easier to clean the implant surfaces and avoid further bacterial invasion after implantoplasty.

**Keywords:** Implantoplasty varnish, Herbal extract, *Vitex negundo* , *Cissus quadrangularis*, Titanium dioxide nanoparticles, Zebrafish

# INTRODUCTION

Dental implants have established themselves as a crucial treatment option in dentistry for the replacement of lost teeth in a variety of clinical settings. Simonis P, Dufour T, and Tenenbaum H reported in 2014, the success rates of 82.9% after 16 years of follow-up (Simonis P et al., 2010). Dental implants are vulnerable to a variety of risk factors that might result in the emergence of infectious peri-implant illnesses and are not immune to inflammatory processes (Alani A., 2014). The occurrence of peri-implant inflammations, one of the most common problems affecting the surrounding soft and hard tissues that might result in the implant's loss, has come to light in recent decades due to mounting evidence. The term "peri-implantitis" refers to an inflammatory condition that causes the bone supporting an implant to deteriorate. In contrast to mucositis, which is reversible and does not include bone loss, this condition involves inflammation of the mucosa around an implant (Heitz‐Mayfield LJ et al., 2018). Following implant therapy, peri-implant conditions are not unusual. In this study, 98.69% of implant sites displayed no symptoms of an infection; 0.66% of implants displayed peri-implantitis; and 0.3% displayed peri-implant mucositis (Lee CT et al., 2018). Within these parameters, the researchers found that 1.31% of implant patients at a dental university hospital in Chennai, India, had incidents of peri-implantitis and peri-implant mucositis. Inserting dental implants appears to be a "safe" treatment option when done with caution and attention to indications and intra-individual limiting factors. As a result, new rehabilitation concepts in dentistry should include methods for peri-implant disease prevention and therapy (Lang NP., 2000).The maintenance of peri-implantitis has been suggested for several different treatment techniques. Chemical and/or mechanical debridement is utilized to guarantee the cleaning of the compromised implant surfaces (Sun TC., 2023). Peri-implantitis has been treated with implantoplasty, a surgery used to clean infected implant surfaces.The primary method should involve surface modification, including decontamination, debridement, and removal of implant threads, to remove biofilm from the implant surface. The term "implantoplasty" refers to a technique that involves rotating tools to smooth off uneven implant surfaces that are visible to the oral cavity. The purpose of this operation is to lessen plaque adhesion and make it easier to clean the implant surfaces (Kominami H., 2022). Titanium dioxide (TiO2) nanoparticles (NPs), *Vitex negundo*, and *Cissus quadrangularis* plant extracts make up the varnish's innovative composition. When titanium is implanted into the alveolar bone, a thin coating of dioxide quickly develops on the metal, enhancing its biocompatibility and passivation (Vanaja M et al., 2013). Because this titanium dioxide modifies bacterial adhesion and colonization during biofilm maturation, the electrostatic forces and ionic bonding are different between biofilms bound to teeth and those bound to implants (Ojha DK., 2020). The antibacterial ability of TiO2 nanoparticles has been investigated in a variety of bacteria, including *Escherichia coli*, *Streptococcus mutans, Candida albicans, Staphylococcus aureus,* and *Pseudomonas aeruginosa* (Rajeshkumar S et al., 2017). TiO2 exhibits an antibacterial effect without harming mechanical properties. Adding a tiny amount of TiO2 NPs to dental resin significantly increased the resin's degree of conversion and mechanical qualities. Due to its photocatalytic properties as well as the fact that it is chemically stable, non-toxic, affordable, and generally recognized as safe (GRAS), titanium dioxide (TiO2) has also been regarded as a desirable antibacterial molecule (Stohs SJ, 2021). The inclusion of titanium dioxide nanoparticles is justified by their potential benefits in healing improvement and acceleration through stimulation of angiogenesis, fibroblast proliferation, and the development of granulation tissue in the early stages of healing (Sawangjit R.; 2017).As the main source of novel compounds and medications, plants continue to be essential to the development of therapeutic medicine. India has a vast resource of native minerals and plants that have been widely used for therapeutic purposes. *Cissus quadrangularis* is one such plant that offers enormous advantages (Dhanasekaran S.; 2020). Long used as an antioxidant, pain reliever, edema reducer, infection fighter, anabolic, and aid in weight loss, *Cissus quadrangularis* concentrates and powders have a wide range of beneficial properties. The most significant applications have been in the treatment of fractures and bone regeneration. Numerous kinds of research have been conducted to support its clinical use and demonstrate its pharmacological effects. Due to its high vitamin C content and antioxidant chemicals including carotenoids, tannins, and phenols, the *Cissus quadrangularis* plant has healing capabilities and can repair bone loss (Selimović A., 2023). Typically, *It is well known that Vitex negundo* has the ability to regulate cellular functions, such as cell cycle and apoptosis, which supports the anti-inflammatory process. *Vitex negundo* and *Cissus quadrangularis* plants both have additional antimicrobial effects in their herbal extracts (Cosgarea R., 2023).Varnish can be applied to the exposed dental threads after implantoplasty to speed up the healing process, stop plaque buildup on the surface, and reduce the inflammatory host reaction. It has been demonstrated in vitro that using a time-release varnish containing 1% chlorhexidine diacetate and 1% thymol reduces bacterial leakage from the implant-abutment contact. Treatment for peri-implant mucositis can be adapted from this strategy (Shiba T., 2024). A randomized controlled experiment showed that using such varnish as part of a daily self-care routine dramatically reduced pocketing and bleeding around peri-implant mucositis lesions. Patients with a bad prognosis following implant placement can regularly use the varnish.

# MATERIALS AND METHODS

The in-vitro research was performed at Gold Lab, (Nanobiomedicine lab), The study was approved by the ethical clearance committee. In conventional varnish, TiO2 NPs with herbal extract of Cissus quadrangularis plant and Vitex negundo plant extracts were added.

## Plants and Chemicals used

TiO2 was bought from SRL Chemicals and used in this study. Without additional purification, all of the reagents were utilized. The stem of the Cissus quadrangularis plant and the leaves of the Vitex negundo plant were obtained from Gold Lab.

## Preparation of plant formulation

      3gm of Vitex negundo leaf and 3gm stem of Cissus quadrangularis plant were grinded using a mortar and pestle. After adding 100 mL of distilled water, the mixture was heated to between 60 and 80 degrees Celsius for 15 minutes. Heated till bubbles were visibly seen. The extract was boiled and then filtered through muslin cloth. Nanoparticles were subsequently combined with the filtered extract.



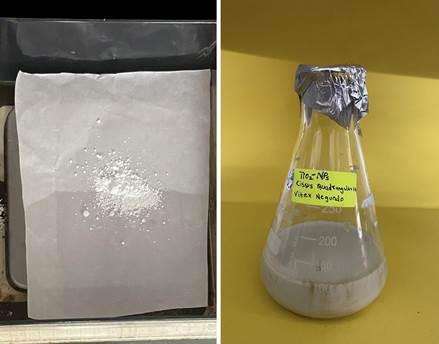
**Figure 1:** Preparation of herbal extract : Leaf of *Vitex Negundo* plant and stem of *Cissus Quadrangularis* plant



**Figure 2:** Filtrated Herbal Extract

# Synthesis of nanoparticles

 An amount of 0.35gm of TiO2 was used to prepare 50mL of titanium solution. 50mL of filtered plant extract was added to the titanium solution to prepare TiO2 NPs.



**Figure 3:** Adding herbal extract to titanium dioxide nanoparticles

## Preparation of Varnish

       500 µL of *Vitex negundo* and *Cissus quadrangularis* with TiO2 NPs and adding 4 mL of ethanol, 0.9 mL of glacial acetic acid and 4.6mL of distilled water.



**Figure 4:** Preparation of Implantoplasty Varnish

## Characterization of  TiO2 nanoparticles

After dissolving by magnetic stirring, the resulting solution was used for characterization(Saadh et al., 2024). Next, using UV-vis spectroscopy, the concentration of silver in the collected solution was recorded at 1, 18, 24, and 36-hour intervals. The evaluated wavelengths ranged from 200 to 600 nm. Additionally, a change in color was noticed and recorded (Almatrafi et al., 2024).

## Antimicrobial activity

The TiO2 NPs' antibacterial activity was evaluated using strains of *Enterococcus faecalis*, *Streptococcus mutans*, and *Staphylococcus aureus*. MHA agar was utilised in this experiment to determine the zone of inhibition. Muller Hinton Agar was prepared and sterilised at 120 pounds for 45 minutes. The media was poured into sterilised plates and allowed to harden. After the wells were cut with the well cutter, the test organisms were swabbed. The plates were covered with varying amounts of TiO2 NPs, and they were then incubated at 37°C for 24 hours. After the incubation time, the zone of inhibition was evaluated.

## Antifungal activity

Agar well diffusion assays are used to examine *Candida albicans*. The medium is made using Sabouraud's Dextrose Agar. Test organisms were swabbed into the prepared and sterile media, and then titanium dioxide nanoparticles of varying concentrations were added to the wells. The plates were incubated at 28°C for 48–72 hours. After the incubation time, the zone of inhibition was evaluated (Happy A et al., 2019).

## Antioxidant activity

## HYDROXYL RADICAL SCAVENGING ASSAY

A small modification was made to the Halliwell method (Halliwell et al., 1987) in order to perform the experiment. Each solution was created from scratch. The 1.0 mL reaction mixture contained 200 mL of 200 mM Fecl3, 1.04 mM EDTA (1: (1.0 mM)), 500 mL of a solution of different concentrations of *Vitex negundo* and *Cissus quadrangularis* in TiO2 NPs (10 to 80 g), and 100 mL of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer with a pH of 7.4). After an hour of incubation at 37°C, the degree of deoxyribose breakdown was measured using the TBA reaction. Find the absorbance at about 532 nm using a reference blank solution. Vitamin E was used as the positive control.

## Anti-inflammatory activity

## EGG ALBUMIN DENATURATION ASSAY

A 5 mL solution was made with 0.2 mL of hen's egg albumin extraction and 2.8 mL of recently prepared, pH-6.3 phosphate-buffered saline. Different concentrations (10µL, 20µL, 30µL, 40µL, and 50µL) of TiO2 NPs were synthesised for *Vitex negundo* and *Cissus quadrangularis*. We used diclofenac sodium as a positive control. The mixes were thereafter heated to 37°C for 15 minutes in a water bath. The samples' absorbance at 660 nm was measured after they were reduced to room temperature.

## Cytotoxicity activity

## BRINE SHRIMP LETHALITY ASSAY

## Saltwater preparation

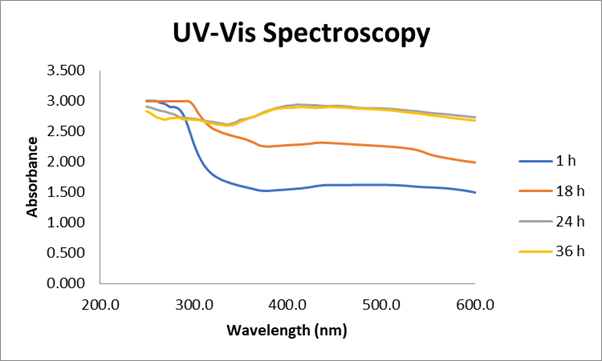
200 mL of filtered water were used to dissolve 2g of iodine-free salt. Salt water containing 10–12 mL was placed into 6 well ELISA plates. Each well received had a gradual addition of 10 nauplii (20μL, 40μL, 60μL, 80μL, and 100μL). After that, *Vitex negundo* and *Cissus quadrangularis* in TiO2 NPs were added in accordance with the concentration level. The plates were incubated for 24 hours. The ELISA plates were checked, numbered, and the presence of living nauplii was calculated using the following method after 24 hours:Number of dead nauplii / Number of dead nauplii + Number of live nauplii x 100

## Zebrafish Embryotoxicity

Zebrafish (*Danio rerio*) were received and held in separate tanks at 28 ± 2°C, with 14 hours of light and 10 hours of dark. The pH was kept between 6.8- 8.5, and the fish were fed twice daily with shrimp and dry flake. To enable the fish reproduce, the males and females were manually divided using a clear block, and they were taken out the next morning. By mating one female with two male males, embryos were obtained. E3 medium (5 mmol/L sodium chloride, 0.18 mmol/L potassium chloride, 0.33 mmol/L calcium chloride, and 0.33 mmol/L magnesium sulphate) was used to rinse viable eggs.The culture plate received the fertilised eggs. Groups that were experimental and controlled were separated. The fertilized embryos were incubated for 24 to 96 hours at varnish concentrations of 5, 10, 20, 40, and 80μL. The experimental group of 5 fishes was exposed to various varnish concentrations for 96 hours. Every 24 hours, the viability and hatching rate were recorded. Five fish were kept as control groups in addition. Fish that were dead were noted and taken out of the aquarium. Under a stereo microscope, the zebrafish embryo's development was tracked periodically throughout. The developing embryos were captured on camera with a stereomicroscope.

# RESULTS AND DISCUSSION

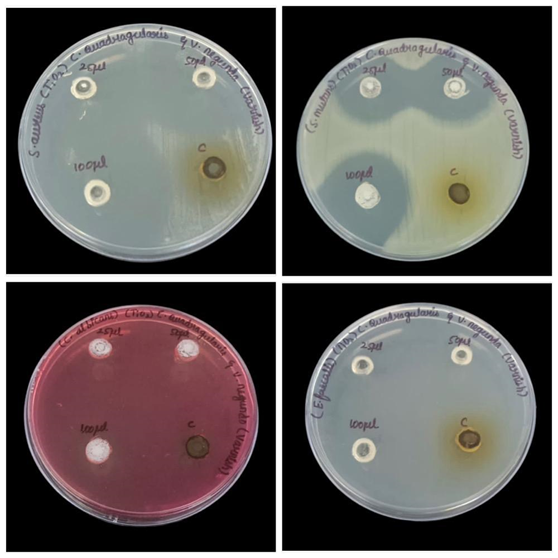
## UV-Vis spectra analysis

UV-Vis spectroscopic analysis was used to investigate the optical characteristics of the produced TiO2 NPs. Surface plasmon resonance of the TiO2 NPs created by *A. paniculata* is depicted in Figure 1. The spectra showed the characteristic of the TiO2 NPs SPR band between 300 nm, showing the initial confirmation of the NPs presence.

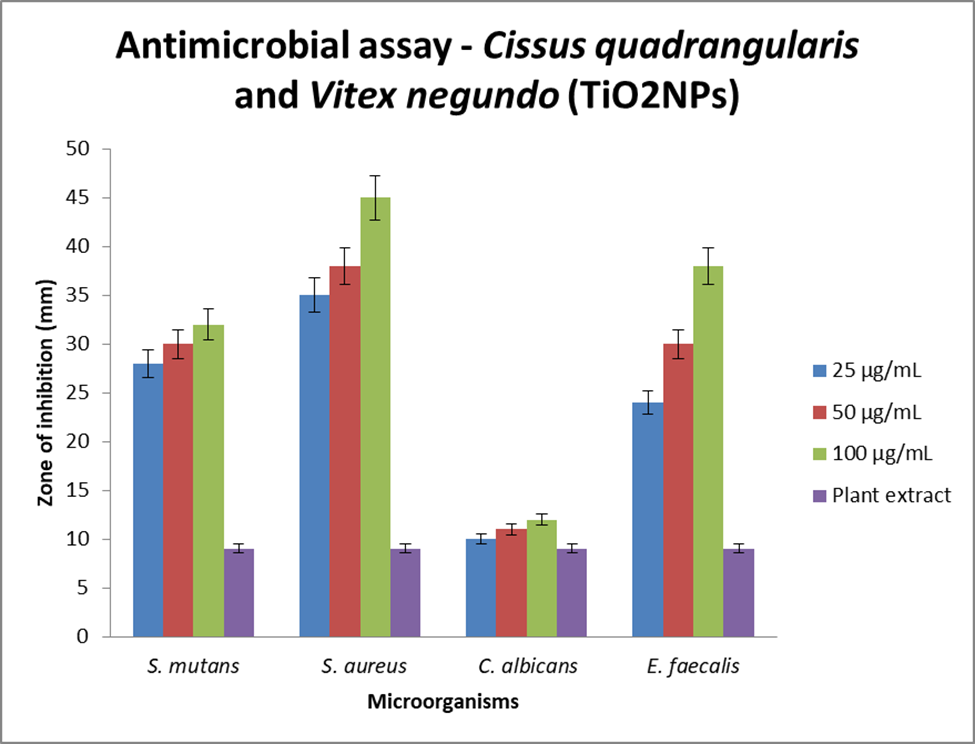
**Figure 5:** Result of UV – vis spectroscopy of TiO2 NPs

## Antibacterial and antifungal activity

The antibacterial activity of TiO2 NPs is shown in Figure 6, and the high zone of inhibition was observed in *Staphylococcus aureus*. The zone of inhibition for *Enterococcus faecalis* and *Streptococcus mutans* was less, which shows that the zone inhibition was increased with the TiO2 NPs. The zone of inhibition was measured in mm (Rajeshkumar C et al., 2015). The antifungal activity was tested against *Candida albicans*. It shows that the zone of inhibition was greater at 100 μL. Another study showed that silver nanoparticles have been shown to impact the inhibition of the growth of *Candida albicans* (Mollabashi V., 2023).



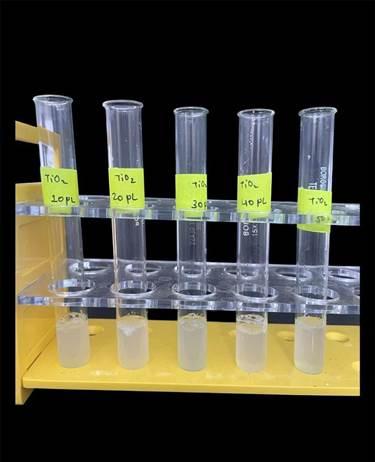
**Figure 6:** Antimicrobial and anti - fungal activity of green synthesized TiO2 NPs mediated implantoplasty varnish



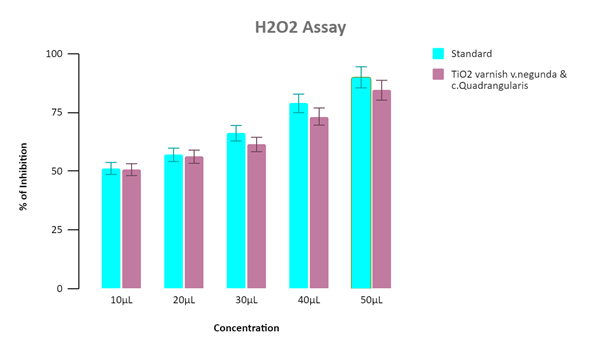
**Figure 7:** Result of Antimicrobial and Antifungal activity of green synthesized TiO2 NPs mediated implantoplasty varnish

## Anti-inflammatory activity

The fabricated TiO2 NPs have comparable anti-inflammatory activity to that of the chemical analgesic standard diclofenac sodium at a concentration of 50 mL. The earlier research demonstrates that TiO2 NPs have effective anti-inflammatory effects. This proves that the newly fabricated varnish also exhibits anti-inflammatory effects (Sunny NE., 2022).



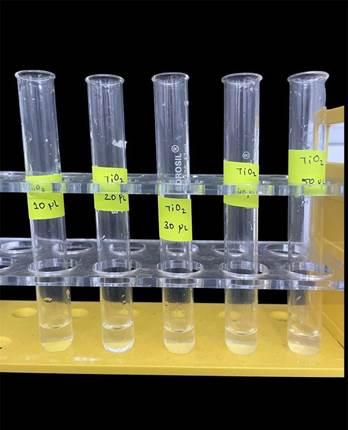
**Figure 8:** Anti-inflammatory activity of green synthesized TiO2 NPs mediated implantoplasty varnish



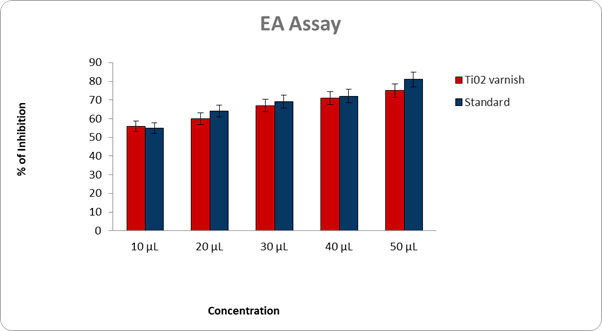
**Figure 9:** Result of Anti-inflammatory activity of green synthesized TiO2 NPs mediated implantoplasty varnish

## Antioxidant Activity

Figure 10 indicates the enhanced concentration of antioxidant activity in the synthesized TiO2 NPs. The previous study demonstrates that TiO2 NPs have strong antioxidant action. Thus, it is proven that the TiO2 NPs made from Vitex negundo and Cissus quadrangularis likewise possess antioxidant activity. (Li Q., 2021). The use of nanoparticles for antioxidant activity in today's biomedical applications is very common. The green synthesis of silver nanoparticles is highly safe for biomedical applications, as demonstrated by the antioxidant potential of S. anacardium, G. lanceolarium, and B. retusa. This finding is compatible with another study that also supports the same conclusion (Anupong W., 2019).



**Figure 10:** Antioxidant activity of green synthesized TiO2 NPs mediated implantoplasty varnish



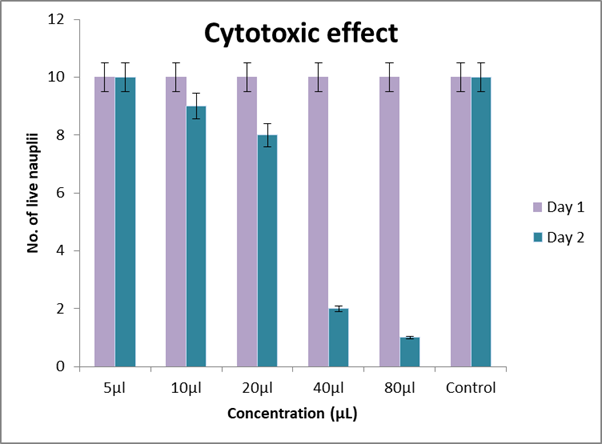
**Figure 11:** Result of Antioxidant activity of green synthesized TiO2 NPs mediated implantoplasty varnish

## Cytotoxic effect

According to the data, there were 10 living nauplii at 5μL and just 1 at the maximum concentration of 80μL. This demonstrates that the cytotoxicity increased as the varnish's concentration did. The number of viable nauplii decreased as concentration was raised in a dose-dependent manner (Fei J., 2022). Titanium dioxide nanoparticles mediated by Cissus quadrangularis and Vitex negundo formulation showed varying levels of inhibition. For example, 5μL inhibited 0% of live nauplii, 10μL inhibited 10% of live nauplii, 20μL inhibited 20% of live nauplii, 40μL inhibited 80% of live nauplii, and 80mL inhibited 90% of live nauplii. Individuals exposed to TiO2 NPs demonstrated cytotoxicity that was dose- and time-dependent and was related to changes in cell viability and morphology, elevated levels of intracellular reactive oxygen species, and decreased levels of intracellular glutathione when compared to the nanoparticle-free control (Rajeshkumar S et al., 2018).

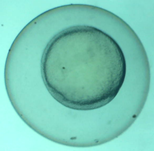
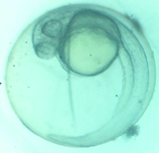
**Figure 12:** Cytotoxicity activity of green synthesized TiO2 NPs mediated implantoplasty varnish



**Figure 13:** Result of cytotoxic activity of green synthesized TiO2 NPs mediated implantoplasty varnish

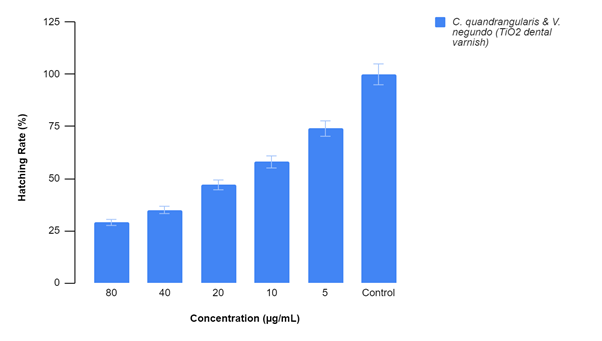
## Embryonic toxicology

The rate at which fertilized eggs in a medium containing varnish hatched is shown in Figure 15. 75–85% of eggs hatched at a concentration of 5μL, whereas 25–35% of eggs hatched at a concentration of 80μL, indicating embryonic hatchability. The hatching rate was marginally lower but remained greater than 50% in concentrations of 10 and 20μL. The hatching rate was lowered to 30% even at a 40μL hatching rate. A visual depiction of the vitality of zebrafish embryos is shown in Figure 14. At all concentrations, it was demonstrated that the viability was greater than 50%. The viability of the embryos was 80% at 1μL concentration. The viability was reduced to less than 60 at the maximum concentration measured (16μL). In Figure 16, stereo microscope photographs taken at regular intervals demonstrate the growth of the embryo in the control and TiO2 NPs-mediated implantoplasty varnish. In the various developmental stages, no abnormalities were seen. This showed that the varnish used in the test sample had no negative effects on how zebrafish embryos developed. were exposed to zebrafish embryos at a low concentration (1 mg/L), however despite the fact that several studies have demonstrated that TiO2 NPs cause premature hatching in a dose-dependent manner, this did not result in major developmental defects.

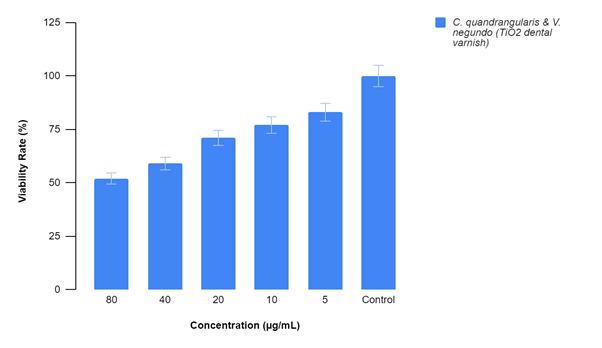
  

Day 1 Day 2 Day 3

**Figure 14:** Embryo development in different time interval with green synthesized TiO2 NPs mediated implantoplasty varnish



**Figure 15:** Hatching rate of zebrafish embryos in green synthesized TiO2 NPs mediated implantoplasty varnish



**Figure 16**: Viability rate of zebrafish embryos in green synthesized TiO2 NPs mediated implantoplasty varnish

# CONCLUSION

A decrease in the number of pathognomonic micro-organisms is the most important aspect followed by the anti-inflammatory properties of varnish when applied to the implant surface after the implantoplasty procedure is done. TiO2 NPs that have been photosynthesized from the stem of a *Cissus quadrangularis* plant and a *Vitex negundo* leaf are made of affordable, environmentally beneficial plant material. The formation and characterisation of TiO2 NPs in varnish were seen in a dose-dependent manner, similar to antibacterial activity, which would lessen harmful pathogenic microorganisms like *Candida albicans* and fungi like *Enterococcus faecalis* and *Streptococcus mutans* and *Staphylococcus aureus* that are important for medicinal purposes. Additionally, they have antioxidant and anti-inflammatory properties that demonstrate their biocompatibility. The surface of the embryo was significantly affected by TiO2 NPs. The green synthesis of TiO2 NPs revealed a modest level of possible toxicity for zebrafish and low deformities found in zebrafish toxicology when made from the stem and leaf of the *Vitex Negundo* plant. This development in the field of implant dentistry will aid the adjacent soft tissues around the implant and the surface of the implant to restore to their initial condition after implantoplasty (Samaee SM., 2015).

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