



Assessment of anti-inflammatory effect of novel Zinc oxide nanoparticles synthesized through a formulation of coffee bean and xylitol

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ABSTRACT

Aim: The aim of this study is to assess the anti-inflammatory effect of novel zinc oxide nanoparticles synthesized through a formulation of coffee bean and xylitol.

Materials and Method: 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the nanoparticle solution was taken in 5 test tubes respectively. To each test tube 2 ml of 1% Bovine Serum Albumin (BSA) was added. 390 µL, 380 µL, 370 µL, 360 µL and 350 µL of distilled water was then added to the test tube containing nanoparticles to reach a volume of 400 µL in total in each test tube. Similarly, a standard group with 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of Diclofenac Sodium was taken in 5 test tubes. To each test tube 2 mL of 1% Bovine Serum Albumin (BSA) was added. The test tubes were incubated at room temperature for 10 minutes. Then they were incubated in a water bath at 55°C for around 10 minutes. Absorbance was measured at 660 nm in the UV Spectrophotometer.

% Inhibition was calculated using the following formula: = (Control OD-Sample OD) / (Sample OD x 100)

Result: Anti-inflammatory properties of novel zinc oxide nanoparticles were found to be higher than that of the standard values at 40µL and 50 µL concentration. Percentage of inhibition was found to be the highest at 40 µL (67.89%) as well as 50 µL (72.43%) as compared to that of the standard at 40 µL (88.79%) as well as 50 µL (94.51%)

Conclusion: Coffee bean phytochemicals can boost the bioactivity of ZnO NPs, whereas xylitol can lower inflammation even further. Natural ingredients like coffee beans and xylitol, when combined with ZnO NPs, can provide a safe and effective alternative to traditional anti-inflammatory medications with fewer adverse effects.

Keywords: Zinc oxide nanoparticles, coffee bean extract, Xylitol, Anti-inflammatory

INTRODUCTION

As a result of nanotechnology, a new field of research, every field of science has undergone a new revolution. Nanoparticles have grown in significance in the scientific world in recent years. This technique has been used in optics, electronics, biology, as well as materials research. In recent years, one of the most popular applications of nanoparticles has been the transport of medications and potent drugs, as well as antibacterial, anticancer, and antioxidant potential (Makhlouf and Barhoun, 2018; Gang, 2020). Nanotechnology is concerned with nanoparticles, which are atomic or molecular aggregates with a size of less than 100 nm (Reddy *et al.*, 2018). These are in fact fundamental elements that have had their atomic and molecular characteristics modified (Kamath, Nasim and Rajeshkumar, 2020).

Because of the need for effective and safe treatment choices, the utilization of natural compounds for therapeutic reasons has attracted substantial attention. Coffee is one such natural chemical that has been shown to provide a variety of health advantages. Coffee beans contain a variety of phytochemicals, including polyphenols, chlorogenic acids, and caffeine, all of which have antioxidant, anti-inflammatory, and anti-cancer activities. Coffee bean phytochemicals are also known to boost the bioactivity of zinc oxide nanoparticles (ZnO NPs) (Ganesh and Senior Lecturer, White Lab-Material Research Centre, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, Chennai- 77, India., 2021).

Because of their unique physical, chemical, and biological characteristics, zinc oxide nanoparticles (ZnO NPs) have been extensively researched for possible therapeutic uses (Ahmed *et al.*, 2017). They feature a high surface area-to-volume ratio, which improves their interaction with biological substances and cells and makes them useful in a variety of biomedical applications (Shukla and Iravani, 2018; Lakshmi and Dean -International Affairs, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, 2021). ZnO nanoparticles have been proven to have anti-inflammatory characteristics by decreasing the generation of pro-inflammatory cytokines and blocking immune cell activation. Xylitol is a natural sugar alcohol that is often used in culinary

goods as a sugar alternative. It has also been discovered to provide a variety of health advantages, including anti-inflammatory qualities. Xylitol has been demonstrated to diminish the expression of adhesion molecules and suppress the production of pro-inflammatory cytokines, both of which are involved in the recruitment of immune cells to the site of inflammation (LeBel *et al.*, 2020). Because of their synergistic effects, the combination of ZnO NPs produced from coffee beans and xylitol has the potential to be an effective anti-inflammatory drug. Coffee bean phytochemicals can boost the bioactivity of ZnO NPs, whereas xylitol can lower inflammation even further. Natural ingredients like coffee beans and xylitol, when combined with ZnO NPs, can provide a safe and effective alternative to traditional anti-inflammatory medications with fewer adverse effects.

Therefore the aim of this study is to assess the anti-inflammatory effect of novel zinc oxide nanoparticles synthesized through a formulation of coffee bean and xylitol.

MATERIALS AND METHOD

Preparation of Coffee bean and Xylitol formulation

Coffee bean extract was prepared by mixing 1 g of freshly grounded coffee bean powder with 100 mL of distilled water. The mixture was boiled for 30 mins at 60 °C on a heating mantle. The mixture was then cooled down to room temperature and double filtered using the Whatman no.1 filter paper. 20m M Zinc nitrate was used as precursor and 50 mL of coffee bean extract was used as a reducing agent. The mixture was kept on a magnetic stirrer for uniform dispersion of all the contents at 600-800 rpm for 48hrs. 50mg of Xylitol powder was mixed with 10 mL of distilled water. This mixture was then mixed with the previously prepared coffee bean-zinc nitrate extract. The mixture was stirred for 2 h and UV-Visible readings were recorded, wherein a strong peak was observed at the end of 3 h. The mixture was then centrifuged at 8000 rpm for 10 min.

The sedimented pellet was double washed with distilled water and dried in a hot air oven operating at 80 °C. The brown colored powder that was obtained was then used for characterization.

Characterisation of Zinc Oxide nanoparticle

Crystalline nature and particle morphology of zinc oxide nanoparticles was analyzed through the Scanning Electron microscope.

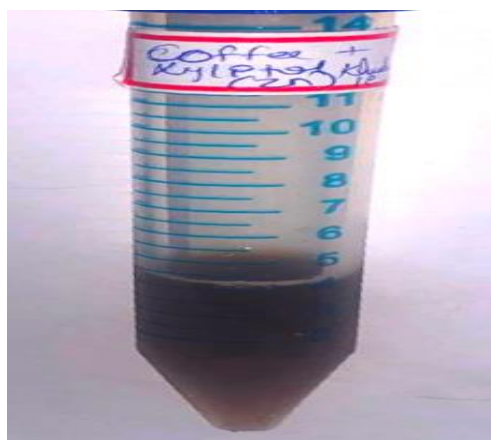


FIGURE 1: Coffee bean-Zinc nitrate-Xylitol formulation.

Anti-Inflammatory Activity

Test Group

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the nanoparticle solution was taken in 5 test tubes respectively. To each test tube 2 ml of 1% Bovine Serum Albumin (BSA) was added. 390 µL, 380 µL, 370 µL, 360 µL and 350 µL of distilled water was then added to the test tube containing nanoparticles to reach a volume of 400 µL in total in each test tube.

Control Group

2 mL of zinc chloride was added to 2 mL of BSA solution.

Standard Group

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of Diclofenac Sodium was taken in 5 test tubes. To each test tube 2 mL of 1% Bovine Serum Albumin (BSA) was added. The test tubes were incubated at room temperature for 10 minutes. Then they were incubated in a water bath at 55°C for around 10 minutes. Absorbance was measured at 660 nm in the UV Spectrophotometer.

% Inhibition was calculated using the following formula:

$$\% \text{ of inhibition} = (\text{Control OD} - \text{Sample OD}) / (\text{Sample OD} \times 100)$$

RESULTS

Anti-inflammatory properties of novel zinc oxide nanoparticles was found to be at higher than that of the standard values at 40µL and 50 µL concentration. Percentage of inhibition was found to be the highest at 40 µL (67.89%) as well as 50 µL (72.43%) as compared to that of the standard at 40 µL (88.79%) as well as 50 µL (94.51%) . (Table 1)

TABLE 1: Percentage of inhibition as recorded with Standard sample and Nanoparticle laced sample.

S.No	Concentration (µl)	Nanoparticle (% Of Inhibition)	Standard (% Of Inhibition)
1	10	76.59	46.71
2	20	78.95	56.78
3	30	85.76	61.92
4	40	88.79	67.89
5	50	94.51	72.43

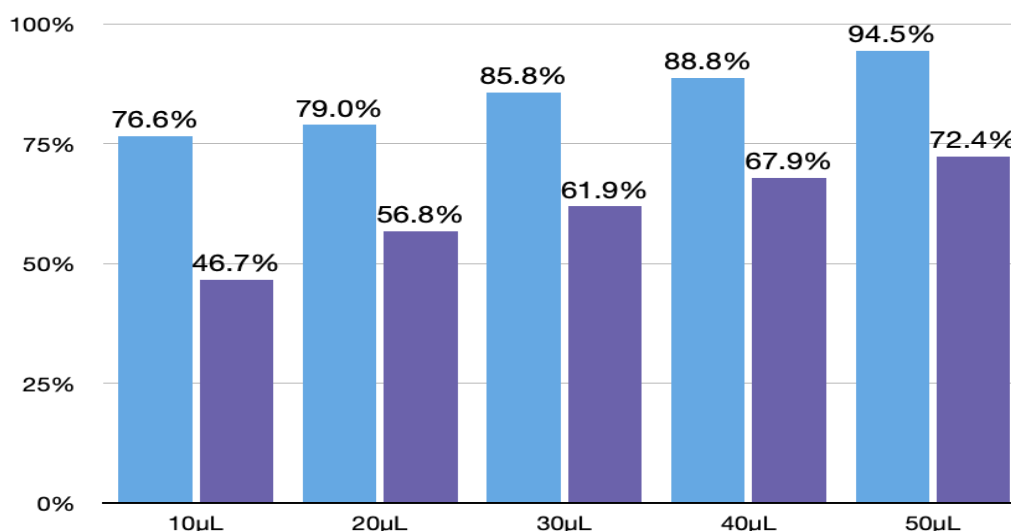


FIGURE 2: Bar graph showing the percentage of inhibition of both the standard as well as test group-ZNO NP.

DISCUSSION

Coffee beans include phytochemicals such as polyphenols, chlorogenic acids, and caffeine, all of which have anti-inflammatory benefits ('Coffee by-products in topical formulations: A review', 2021). These phytochemicals have the potential to increase the bioactivity of ZnO NPs, which have distinct physical, chemical, and biological features that make them attractive for therapeutic applications (Pergolizzi *et al.*, 2020). ZnO NPs have been demonstrated to diminish the expression of adhesion molecules and suppress the production of pro-inflammatory cytokines, both of which are important in the recruitment of immune cells to the site of inflammation. Anti-inflammatory qualities have also been discovered for xylitol, a natural sugar alcohol often used as a sugar substitute. Xylitol, like ZnO NPs, has been found to block the production of pro-inflammatory cytokines and diminish the expression of adhesion molecules. The combination of ZnO NPs generated from coffee beans with xylitol can boost both drugs' anti-inflammatory action, resulting in a powerful therapeutic impact.

Zinc oxide nanoparticles (ZnO NPs) derived from coffee beans and xylitol as mentioned previously, have been individually shown to have potent anti-inflammatory activity previously (Reddy *et al.*, 2018; LeBel *et al.*, 2020). Therefore the combination of these natural

substances has the potential to provide a safe and effective alternative to conventional anti-inflammatory drugs, which tend to have adverse side effects ('Green synthesis of copper nanoparticles using green coffee bean and their applications for efficient reduction of organic dyes', 2021; Raghu and Reader, Department of Conservative Dentistry and Endodontics, Saveetha Dental college and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 2021). ZnO nanoparticles are an inorganic substance that is multifunctional, basic, and nontoxic. Because of its versatility, it is affordable and has a wide range of applications in a variety of sectors, including drug delivery and antimicrobial agents, as well as bio-imaging, anti-inflammatory agents, and wound healing (Zahoor *et al.*, 2023). The green synthesized NPs have the added benefit of being safe to utilize in a variety of biological applications such as anti-inflammatory actions of metal oxides (Willander, 2014).

The results of this study suggest that the anti-inflammatory properties of the novel zinc oxide nanoparticles derived from coffee beans and xylitol are promising. The percentage of inhibition of inflammation was found to be high at both 40µL and 50µL concentrations, with the highest percentage of inhibition observed at 40µL (67.89%) and 50µL (72.43%). Although the percentage of inhibition is lower than the standard values, which were observed to be

88.79% and 94.51% at 40 μ L and 50 μ L respectively, the results are still encouraging. It is important to note that the percentage of inhibition observed in this study is concentration-dependent, meaning that higher concentrations of the nanoparticles may lead to greater inhibition of inflammation. Future studies may explore the use of higher concentrations of the nanoparticles to determine their optimal anti-inflammatory activity.

Zinc oxide nanoparticles can limit immune cell migration to inflammatory areas by inhibiting the expression of pro-inflammatory cytokines (Lakshmi and Dean -International Affairs, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, 2021; Abdel Razak, AlJoofy and Zaki, 2022). The presence of polyphenols and other bioactive components in coffee beans, as well as the anti-inflammatory qualities of xylitol, may increase the anti-inflammatory effects of zinc oxide nanoparticles combined with coffee beans and xylitol ('Caffeine-loaded gold nanoparticles conjugated with PLA-PEG-PLA copolymer for in vitro cytotoxicity and anti-inflammatory activity', 2017).

CONCLUSION

While there is very scarce and limited literature in terms of the anti-inflammatory activity exhibited by nanoparticles extracted from either coffee extract or xylitol, there are no evidences focusing light on zinc oxide nanoparticle extracted and showing anti-inflammatory activity from a formulation of coffee bean extract and xylitol.

With the current study, coffee bean and xylitol formulation not only showed the presence of zinc oxide nanoparticles, but also showed that with an increase in testing concentration of the formulation, there was a direct increase in the percentage of inhibition. According to the findings of this study, the new zinc oxide nanoparticles produced from coffee beans and xylitol have a promising anti-inflammatory effect. Green synthesis which includes the use of natural compounds combined with nanoparticles have shown to offer a viable alternative to conventional anti-inflammatory medications, with fewer side effects and the ability to deliver tailored therapy.

Further research and focus into the safety and efficacy of these nanoparticles in animal models of inflammation, as well as human clinical trials, is imperative to be certain of the same.

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