Assessment of Cytotoxicity, Anti-Inflammatory and Antioxidant Activity of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation - An In-Vitro Study

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ABSTRACT

BACKGROUND

Zinc oxide nanoparticles play a vital role in diagnostics, biomolecular detection, and microelectronics. Several conventional methods are used for synthesis of zinc oxide nanoparticles. But, toxic chemicals are required as capping agents to maintain stability, thus leading to toxicity in the environment. Thus, we need to shift to "green synthesis". Hence, this study was conducted to assess the cytotoxicity, antiinflammatory, and antioxidant activity of zinc oxide nanoparticles reinforced with clove and cinnamon.

METHODS

Cytotoxic effect, anti-inflammatory activity, antioxidant activity of zinc oxide nanoparticles reinforced with clove and cinnamon extract were assessed using Brine Shrimp Assay, Bovine Serum Albumin (BSA) and DPPH Assay respectively at 5 µL, 10 µL, 20 µL, 30 µL, 50 µL.

RESULTS

As the concentration increased, the cytotoxicity of the nanoparticles increased. Values for anti-inflammatory property of nanoparticles was higher than the standard values at all concentrations. Percentage of inhibition was highest at 40 µL (91.1%) and 50 µL (90.5%). The values for antioxidant property of nanoparticles was found to be higher than the standard values at all concentrations except at 50 µL. Percentage of inhibition was highest at 20 µL (86.2%).

CONCLUSIONS

Zinc oxide nanoparticles reinforced with clove and cinnamon extract have a potential as an anti-cancer, anti-inflammatory and antioxidant agent and can be used as an alternative to commercially available products.

KEY WORDS

Zinc Oxide Nanoparticles, Clove, Cinnamon, Antioxidant, Anti-Inflammatory, Cytotoxicity

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BACKGROUND

Nanotechnology is an emerging technology and has led to a new revolution in every field of science.[1] This technology has been used in the fields of optics, electronics, and biomedical and materials sciences. Nanotechnology deals with nanoparticles that are atomic or molecular aggregates characterized by size less than 100 nm. These are actually modified form of basic elements derived by altering their atomic as well as molecular properties of elements.[2]

Among the various inorganic nanoparticles available, Zinc Oxide (ZnO) has easy processing methods, is inexpensive, has wide range of applications and is a safe material. Due to these properties, Zn O pulls a particular interest among researchers.

Zinc Oxide nanoparticles play a vital role in diagnostics, biomolecular detection, microelectronics.[3] Several conventional methods are used for synthesis of zinc oxide nanoparticles like chemical reduction,[4] laser ablation,[5] solvothermal,[6] inert gas condensation,[7] sol-gel method.[8] Even though less time is utilized for synthesizing large quantities of nanoparticles using conventional physical and chemical methods, toxic chemicals are required as capping agents to maintain stability, thus leading to toxicity in the environment.

Thus, we need to shift to "green synthesis" that offers numerous benefits of eco friendliness and compatibility for biomedical applications, where toxic chemicals are not used for the synthesis protocol. The use of agricultural wastes [9] or plants and their parts,[10] has emerged as an alternative to chemical synthetic procedures because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures.[11]

In biological system, over production of highly reactive radical species or their precursor. leads to oxidative stress which has been observed in various disease such as cancer, cardiovascular disease, diabetes and arthritis.[12] In few studies antioxidant and free radical scavenging activities of Zinc Oxide nanoparticles in biological system has been described.[13]

Many studies have shown that Zinc Oxide nanoparticles induce various toxic effects, including cytotoxicity, genotoxicity, inflammation, and oxidative stress.[14] Zinc Oxide nanoparticles are also known to have anti-inflammatory properties by blocking pro-inflammatory cytokines, inhibiting mast cell proliferation and suppressing LPS induced COX-2 expression.[15]

Syzygium aromaticum commonly known as clove, is a median size tree (8-12 m) belonging to the *Mirtaceae* family native from the Maluku islands in east Indonesia. For many years the trade of clove and the search of this valuable spice has caused the economic development of this region.[16] This plant plays a vital role as a spice, but it's essential oils and other constituents also have important activities like including antimicrobial, antifungal, antioxidant, and antidiabetic.[17]

Cinnamon is a spice obtained from the inner bark of several tree species from the genus Cinnamomum. Cinnamon has many health benefits like-it has antioxidant [18], anti-inflammatory properties,[19] may reduce the risk of heart disease, lowers blood sugar level, protective against cancer, and anti-microbial properties.

The rationale of this study is that no study has been conducted so far in which the properties of Zinc Oxide nanoparticles reinforced with clove and cinnamon have been assessed. Hence the aim of the study was to assess the cytotoxicity, anti –inflammatory and antioxidant activity of Zinc Oxide nanoparticles reinforced with clove and cinnamon.

METHODS

Study Design

In vitro study.

Preparation of Plant Extract

Clove buds and cinnamon bark sticks were purchased from the market of South India and were powdered using a mixer grinder. 5 mg of clove powder and 5 mg of cinnamon powder was dissolved in 100 mL of distilled water. The solution was boiled in a heating mantle at 60 ^{o}C for 10 minutes until the bubbles appeared. The solution was then filtered using a funnel and a Whatman filter paper and collected in a conical flask to obtain the plant extract. Then the plant extract was transferred to an airtight container and refrigerated overnight.

Synthesis of Zinc Oxide Nanoparticles Using Clove and Cinnamon Extract

20 mM of Zinc Sulphate was prepared using 60 ml of distilled water and mixed thoroughly. 40 ml of the plant extract was added to this solution and was placed in the orbital shaker. Colour change of the solution was noted every 2 h. Readings were recorded every 2 h in U V Spectrophotometer and after around 36 hours, centrifugation was done at 7000 rpm for 10 minutes. Zinc Oxide nanoparticles pellets reinforced with clove and cinnamon were obtained after centrifugation.

Cytotoxic Effect

The cytotoxicity of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract was assessed using Brine shrimp assay. 12 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. Zinc oxide nanoparticles reinforced with clove and cinnamon was added to each well at different concentrations (5 µL, 10 µL, 20 µL, 30 µL, 50 µL) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked.

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} - \text{number of live nauplii}} \times 100$$

Anti-Inflammatory Activity

Test Group

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the nanoparticles 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 added to the test tube containing 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of nanoparticles respectively.

Control Group
2 mL of Dimethyl Sulphoxide (DMSO) 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 respectively. 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 water bath at 55 ^{o}C for around 10 minutes. Absorbance was measured at 660 nm in UV Spectrophotometer.

% Inhibition was calculated using the following formula:

$$\% \text{ of inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Antioxidant Activity

Test Group
10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the nanoparticle was taken in 5 test tubes respectively. To each test tube 1 ml of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was added. 1990 µL, 1980 µL, 1970 µL, 1960 µL and 1950 µL of 50% methanol solution was added to the test tube containing 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of nanoparticles respectively.

Control Group
1 mL of DPPH was added to 2 mL of methanol solution.

Standard Group
Ascorbic acid was used as standard
The test tubes were incubated in a dark cupboard for around 20 minutes. Absorbance was measured at 517 nm in UV Spectrophotometer.

% Inhibition was calculated using the following formula:

$$\% \text{ of inhibition} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{CControl Absorbance}} \times 100$$

RESULTS

Table 1 depicts the cytotoxicity of Zinc Oxide Nanoparticles reinforced with and cinnamon extract. At 5 µL concentration there was a death of 20% of nauplii, at 10 and 20 µL there was a death of 30% of nauplii, at 30 µL there was a death of 40% of nauplii and at 50 µL there was a death of 50% of nauplii. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased.

Concentration (µL)	Viable Nauplii	% Death
5	8	20
10	7	30
20	7	30
30	6	40
50	5	50

Table 1. Cytotoxicity of Zinc Oxide Nanoparticles Reinforced with Clove and Cinnamon Extract

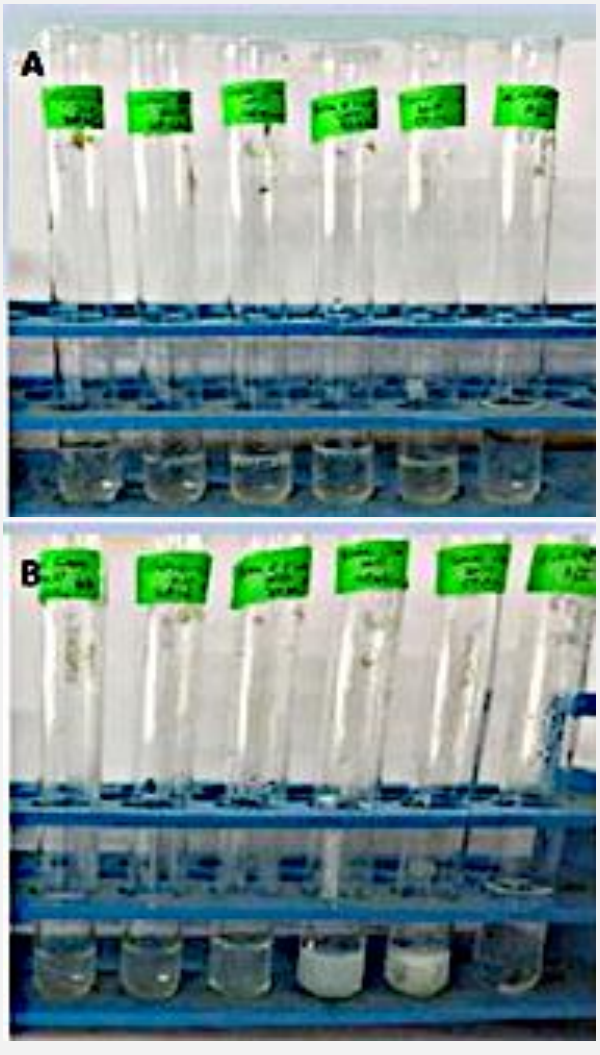

Figure 1. Anti–Inflammatory Property of Zinc Oxide Nanoparticles Reinforced with Clove and Cinnamon Extract
A-Pre- Incubation B- Post-Incubation C- Various Concentrations Compared with Standard Values

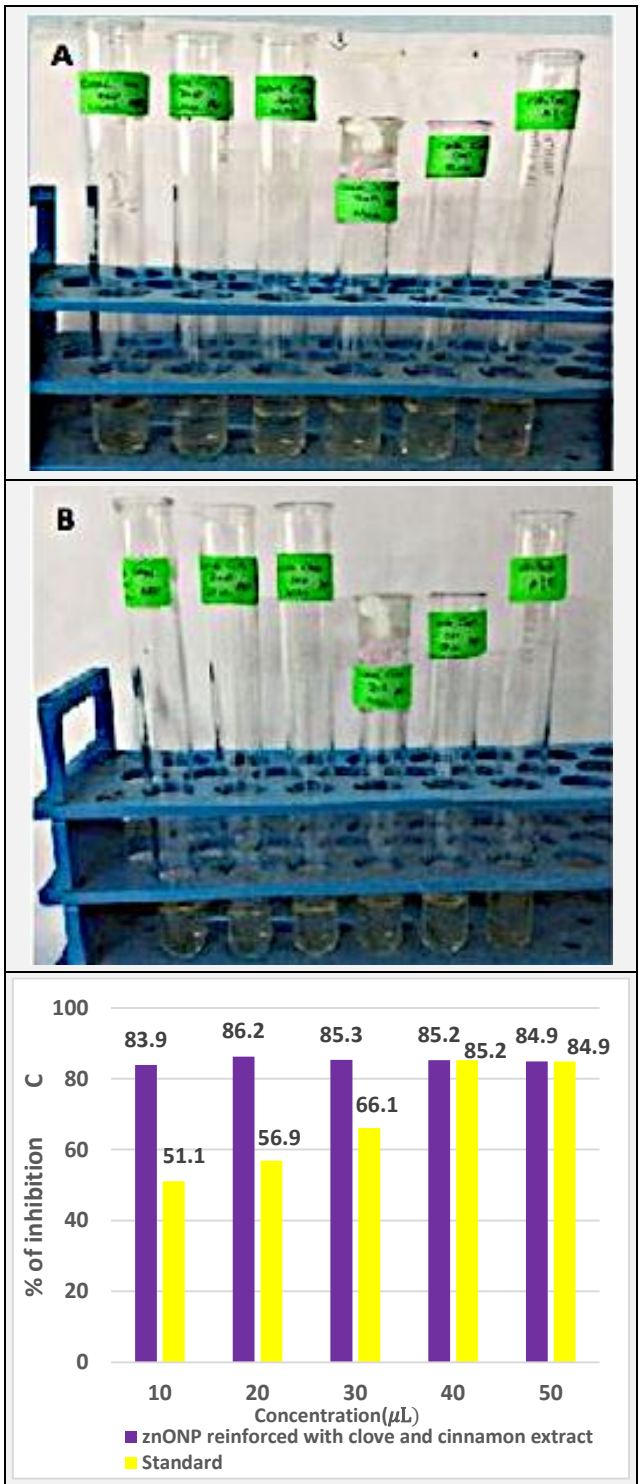

Figure 2. Antioxidant Property of Zinc Oxide Nanoparticles Reinforced with Clove and Cinnamon Extract

A-Pre -Incubation, B- Post- Incubation, C- Various Concentrations Compared with Standard Values

Figure 2 A depicts the anti- inflammatory property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract pre-incubation. Figure 2 B depicts the anti-inflammatory property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract post-incubation. Figure 2 C depicts the anti- inflammatory property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract at various concentrations compared with the standard values.

It was found that the values for anti-inflammatory property of nanoparticles was higher than the standard values at all concentrations. Percentage of inhibition was 68.2% at 10 µL concentration, 89.8% at 20 µL, 88.5% at 30 µL and highest at 40 µL (91.1%) and 50 µL (90.5%)

Figure 3 A depicts the antioxidant property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract pre-incubation. Figure 3 B depicts the antioxidant property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract post-incubation. Figure 3 C depicts the antioxidant property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract at various concentrations compared with the standard values.

The values for antioxidant property of nanoparticles was found to be higher than the standard values at all concentrations except at 50 µL. Percentage of inhibition was 83.9% at 10 µL concentration, 86.2% at 20 µL, 85.3% at 30 µL, 85.2% at 40 µL and 84.9% at 50 µL.

DISCUSSION

There has been a rapid evolution of nanoparticle synthesis recently as compared to the early part of the century.[20] Earlier, physio-chemical methods were involved in nanoparticle synthesis. Even though less time is utilized for synthesizing large quantities of nanoparticles using conventional physical and chemical methods, toxic chemicals are required as capping agents to maintain stability, thus leading to toxicity in the environment. Keeping this in consideration, green nanotechnology using plants is emerging as an eco-friendly alternative, as plant extract mediated biosynthesis of nanoparticles is cost-effective [21]. We therefore undertook this study to evaluate the cytotoxicity, anti-inflammatory and antioxidant property of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract.

In the present study it was seen that at 5 µL concentration there was a death of 20% of nauplii, at 10 and 20 µL there was a death of 30% of nauplii, at 30 µL there was a death of 40% of nauplii and at 50 µL there was a death of 50% of nauplii. Percentage of inhibition of protein denaturation (anti-inflammatory activity) was 68.2% at 10 µL concentration, 89.8% at 20 µL, 88.5% at 30 µL and highest at 40 µL (91.1%) and 50 µL (90.5%). Percentage of inhibition of DPPH free radicals (antioxidant activity) was 83.9% at 10 µL concentration, 86.2% at 20 µL, 85.3% at 30 µL, 85.2% at 40 µL (85.2%) and 84.9% at 50 µL.

As the concentration increased the cytotoxicity, anti-inflammatory and antioxidant activity of the nanoparticles increased. The highest percentage of death of nauplii was at 50 µL concentration of Zinc Oxide nanoparticles reinforced with clove and cinnamon extract. The values for anti-inflammatory property of Zinc Oxide nanoparticles was higher than the standard values at all concentrations. Percentage of Inhibition was highest at 50 µL (90.5%; IC_{50}=0.095 µg/mL). The values for antioxidant property of nanoparticles was found to be higher than the standard values at all concentrations except at 50 µL.

Many studies have been conducted to assess the cytotoxicity of Zinc Oxide nanoparticles, clove and cinnamon.[22-27] The mechanisms of cytotoxicity from Zinc

Oxide nanoparticles are not yet entirely understood, but the generation of hydroxyl radicals (OH•), superoxide anion, and perhydroxyl radicals from the surface of Zinc Oxide are believed to be major components.[28] High eugenol content in clove makes it cytotoxic.[29] Hence they can be used against cancer cells.

Studies have also revealed that Zinc Oxide nanoparticles, clove and cinnamon have anti-inflammatory and antioxidant properties.[30-41] Zinc Oxide nanoparticles are known to have anti-inflammatory properties by blocking pro-inflammatory cytokines, inhibiting mast cell proliferation and suppressing LPS induced COX-2 expression. Antioxidant Zinc Oxide nanoparticles is due to release of hydrogen which reduces DPPH free radical easily.[42]

Anti-inflammatory and antioxidant of clove is due to its high eugenol content. Eugenol is a natural phenolic compound which reduces DPPH free radical by easily donating hydrogen.[43] Different flavonoids isolated from cinnamon have anti-inflammatory free-radical-scavenging activities.[44]

Based on the findings of the study we can say that reinforcing zinc oxide nanoparticles with clove and cinnamon has a synergistic effect and can be used as an alternative to commercially available anti-inflammatory and antioxidant agents.

Limitations

The study was conducted in vitro, so it cannot be assumed that the results of cytotoxicity, anti-inflammatory activity and antioxidant activity could be translated into clinical effectiveness.

Recommendations

- This product can be given to the patients in the form of a mouthwash.
- In further studies, in vivo studies are recommended with people' acceptance values as well.

CONCLUSIONS

Findings from this study suggests that zinc oxide nanoparticles reinforced with clove and cinnamon extract have a potential as an anti-cancer, anti-inflammatory, and antioxidant agent and can be used as an alternative to commercially available products.

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