Abies webbiana mediated Zinc oxide nanoparticles and its anti-inflammatory activity

ABSTRACT:

INTRODUCTION: Green nanoparticle synthesis provides a number of advantages, including being environmentally friendly, taking less time, being less expensive, being more stable, and, most significantly, not requiring the use of harmful chemicals.

AIM: The aim of the present study is to evaluate the anti-inflammatory activity of zinc oxide [ZnO] nanoparticles prepared using *Abies webbiana* extract.

MATERIALS AND METHODS: In this study, ZnO nanoparticles were characterised using ultraviolet-visible spectroscopy and inhibition of albumin denaturation assay using *A. webbiana* extract.

RESULTS: The biosynthesised ZnO particles exhibited potent anti-inflammatory activity to inhibit COX activity. ZnO nanoparticles can be developed as a novel medicine and can be used as an alternative to commercially available anti-inflammatory agents, thus reducing the major health problems.

CONCLUSION: Using *A.webbiana* extract, the findings suggest a cost-effective and environmentally friendly production of ZnO nanoparticles. ZnO nanoparticles mediated by *A. webbiana* showed promising properties. More investigations are required to understand the properties of these nanoparticles, which have a wide range of medical and dental applications.

Keywords: Abies webbiana,, anti-inflammatory, innovative technology, zinc oxide nanoparticles

1. INTRODUCTION:

Cells of living organisms are typical across 10 µm. However, the parts of the cells are much smaller, with typical size of just 5 nm. The smaller size contributes to the idea of using nanoparticles to spy on cellular machinery without much interference.[1] The driving force behind the development of nanotechnology was to understand the biological processes on the nanoscale level. [2] Nanotechnology deals with the manufacture and application of materials with a size of up to 100 nm. The use of nanoparticles in various fields such as material science, agriculture, food industry, cosmetic, medical has emerged. [3]

Zinc is an essential trace element for the human system. Body metabolism is maintained by zinc through hematopoiesis, enzyme regulation, maintaining cell redox balance and DNA and protein synthesis machinery regulation.[4]. Zinc oxide nanoparticles exhibit various biomedical applications such as tremendous wound healing, catalytic, bio-imaging, anti-bacterial, anti-inflammatory properties.[5] Zinc oxide nanoparticles act by inhibiting the activation of NF-kB [nuclear factor kappa B cells] in mRNA expression of inflammatory cytokines.[6] Owing to the excellent bio-medical properties of zinc oxide nanoparticles, they are used as potential drug delivery vehicles for standard drugs as it provides synergistic effects for the treatment.[7]

Abies webbiana tree most commonly seen in the Himalayan region from Kashmir to Assam states in India also found in neighbouring areas of Nepal, Tibet and Afghanistan. *A. webbiana* is a tall, large, evergreen tree seen normally at an altitude of 2500-4000 m. *A. webbiana* also known as Talispatra in Bengali and Hindi, Talispatram in Sanskrit and Indian Silver Fir in English. [8]

Leaves of *A. webbiana* has many medicinal uses. Traditionally leaves of this plant have been used for their medicinal properties namely for their carminative, stomachic, expectorant, decongestant, antiseptic, astringent, antihyperglycemic, female antifertility, febrifuge and anti-spasmodic functions. In cases of cough, phthisis, asthma, chronic bronchitis and catarrh of the bladder and other pulmonary infections, decoctions of the leaves are given orally. Apart from these functions, in cases of serious ailments like rheumatism, hoarseness, chronic bronchitis leaves of the plant have been used traditionally for its chemotherapeutic efficacies.[9]

Crude extracts from *A. webbiana* are reported to have antibacterial, mast cell stabilizing, anxiolytic, anti-tumor, anti-inflammatory, antitussive and central nervous system [CNS] depressant actions. Chemical constituents namely monoterpenes [from essential oil], flavonoids, biflavonoid glycosides, phytosterols and diterpene glycosides [taxol like compounds] were isolated from *A. webbiana* leaf. Pinitol which was isolated from leaves of *A.webbiana* was found to have an anti-inflammatory effect.[10,11]. Our team has extensive knowledge and research experience that has translated into high-quality publications [12–31]. The aim of the present study is to evaluate the anti-inflammatory activity of *A.webbiana* mediated zinc oxide nanoparticles.

2. MATERIALS AND METHODS:

2.1 Preparation of *Abies webbiana* extract:

1gm of powdered *A.webbiana* extract was mixed with 100 mL of distilled water [Figure 1]. The solution was boiled under 60-70 degrees celsius in the heating mantle for 10-15 minutes. And the solution was filtered using Whatman No. 1 filter paper. The filtered extract was collected and stored at 4° C for further use.

2.2 Synthesis of ZnO Nanoparticles:

20 millimolar [0.574g] of zinc sulphate was dissolved in 60 mL of distilled water. 40 mL of filtered *A.webbiana* extract was added with the metal solution. A total of 100 mL of solution was obtained. Colour changes were observed visually and recorded by photographs. The solution is kept in a magnetic stirrer or orbital shaker for nanoparticle synthesis.

2.3 Characterization of ZnO Nanoparticles:

The synthesized zinc oxide nanoparticles solution was preliminarily characterized using ultraviolet [UV]-visible spectroscopy; 3 mL of the solution is taken in the cuvette and scanned in a double-beam UV-visible spectrophotometer from 300 nm to 700 nm wavelength. The results were recorded for the graphical analysis.

2.4 Preparation of Nanoparticles Powder:

The nanoparticles solution is centrifuged using Lark refrigerated centrifuge. The ZnO nanoparticles solution was centrifuged at 8000 rpm for 10 mins and the pellet was collected and washed with distilled water twice. The final purified pellet is collected and dried at 100–150°C for 24 h and finally, the nanoparticles powder was collected and stored in an airtight Eppendorf tube.

2.5 Inhibition of Albumin Denaturation Assay:

Bovine serum albumin [BSA] was used as a reagent for the assay. BSA makes up approximately 60% of all proteins in animal serum. It is commonly used in culture, particularly when protein supplementation is necessary and the other components of serum are unwanted. BSA undergoes denaturation on heating and starts expressing antigens associated with Type III hypersensitivity reaction which is related to diseases such as rheumatoid arthritis, glomerulonephritis, serum sickness, and systemic lupus erythematosus. Two milliliters of 1% bovine albumin fraction was mixed with 400 µl of plant crude extract in different concentrations [500–100 µg/mL] and the pH of the reaction mixture was adjusted to 6.8 using 1 N HCl. The reaction mixture was incubated at room temperature for 20 min and then heated at 55°C for 20 min in a water bath. The mixture was cooled to room temperature and the absorbance value was recorded at 660 nm. An equal amount of plant extract was replaced with DMSO for control. Diclofenac sodium in different concentrations was used as standard. The experiment was performed in triplicate.

% Inhibition was calculated using the formulae:

$$\% \text{ Inhibition} = \frac{\text{Control O.D} - \text{sample O.D}}{\text{Control O.D}}$$

3. RESULTS AND DISCUSSION:

3.1 Visual observation:

The present study shows the synthesis of Zinc oxide nanoparticles by using *A. webbiana.* Reduction of Zinc sulfate to zinc ion process was observed by colour change through direct visual observation of the solution. At various stages of incubation, the colour changes in the reaction mixture were examined continuously. Color change revealed that the zinc oxide had been converted into ZnO nanoparticles. After incubating for 1 hour, the colour of the solution had changed to brown. After 24 hours of incubation, the colour turned from light brown to dark brown [Figure 2 and 3]. After 24 hours, no colour change was observed indicating that the creation of Zinc oxide nanoparticles was complete. Bio- based synthesis of ZnO nanoparticles prepared from using amla fruits observed colour change from yellow to dark brown.[32] Similar colour change was observed in the present study.

3.2 UV-visible Spectroscopy:

The UV-visible analysis of the ZnO nanoparticles was analyzed in the absorbency range of 250–500 nm. The peak was found to be maximum at 350 nm [Figure 4]. Reduction of aqueous metal ions with the *A. webbiana* extract indicates the formation and synthesis of the ZnO nanoparticles. ZnO nanoparticles synthesized from Amla fruit showed plasmon resonance at 350nm.[32]

3.3 Anti-inflammatory Activity:

The anti-inflammatory activity of the *A. webbiana* extract was measured by the inhibition of albumin denaturation assay. The synthesized zinc oxide nanoparticles showed the quality of anti-inflammatory activity in the range of 80–90%, respectively (Figure 5). The extract showed a greater percentage of inhibition when compared to the standard solution.

Fig. 1. Dried *A. webbiana* powder and 100 mL of distilled water.

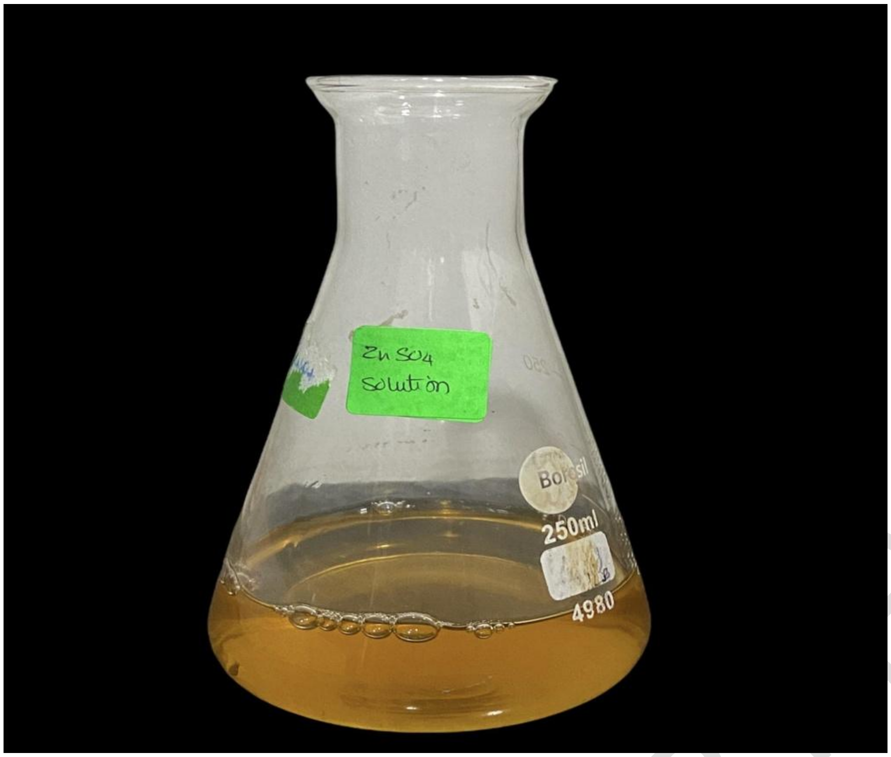

Fig. 2. ZnO nanoparticles solution

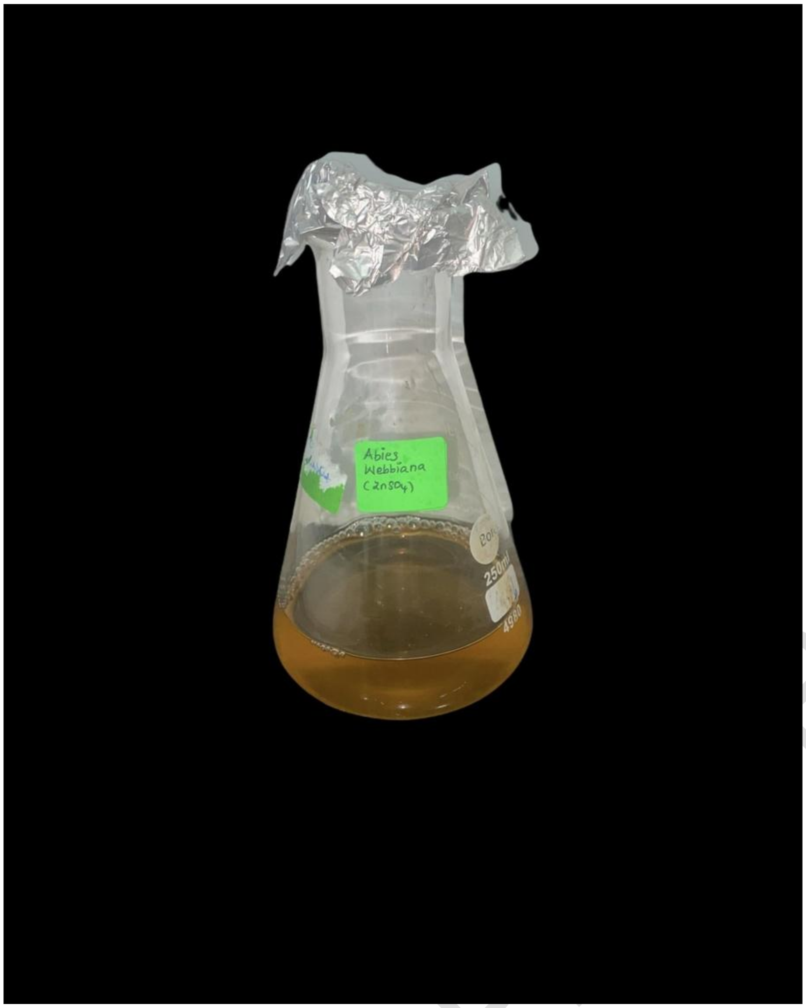

Fig. 3. Visual observation of *A. webbiana* and ZnO nanoparticles

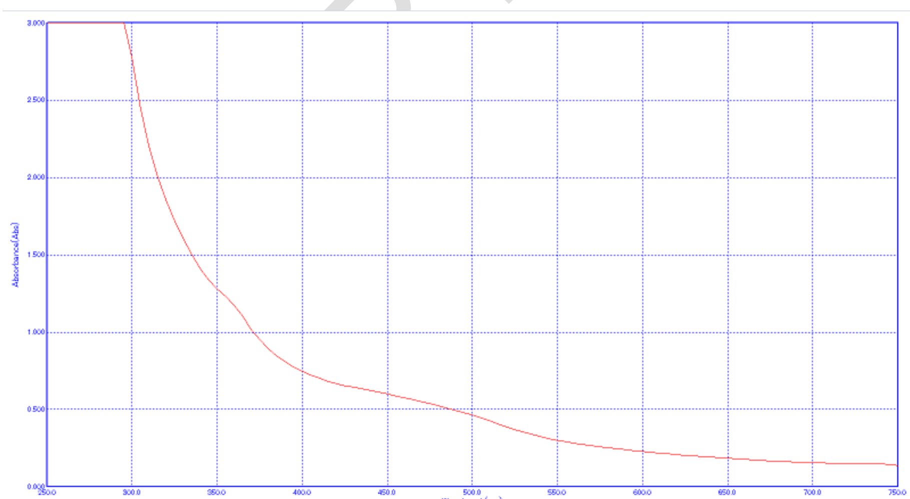

Fig. 4. UV vis spectroscopy of ZnO nanoparticles synthesized using *A. webbiana* recorded as function of time

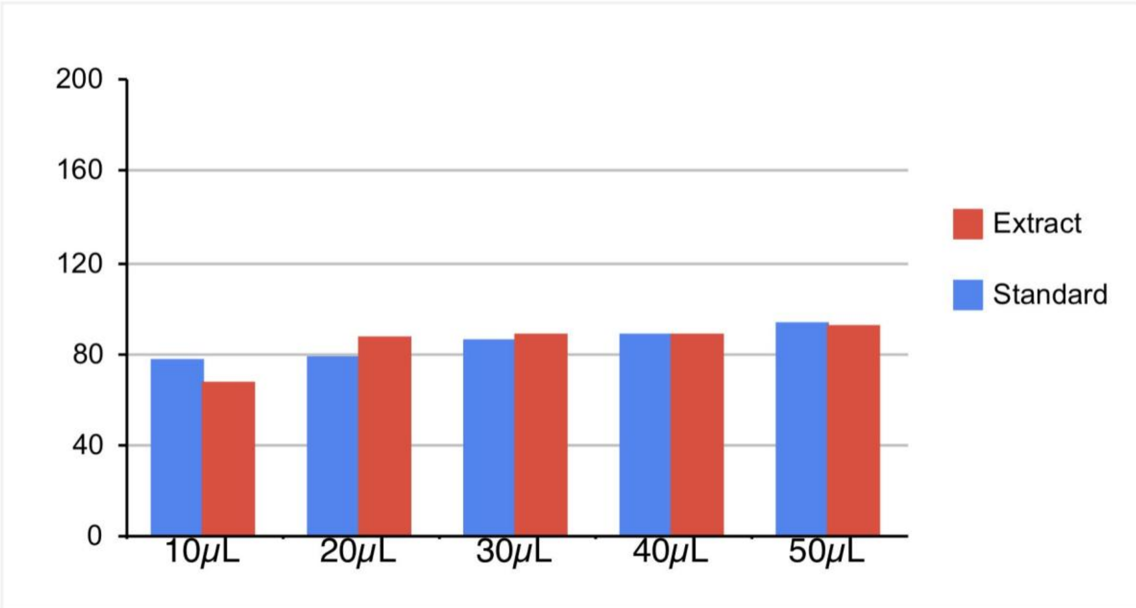

Fig. 5. The quality of anti-inflammatory activity ZnO nanoparticles synthesized using *A. webbiana* which is compared to the standard substance

Inflammation occurs as a response of our immune system to harmful stimuli which represents pathological conditions. Various chronic diseases are associated with inflammation process e.g., cancer, diabetes, inflammatory bowel disease and rheumatoid arthritis. Several cell types of neutrophils, basophils, eosinophils, and mononuclear cells are involved in the inflammatory process. [33–35]

Inflammation can be of two types as either acute or chronic inflammation. Cyclooxygenase [COX] is the key causative factor/ enzyme in the synthesis of prostaglandins, prostacyclins and thromboxanes which are responsible for inflammation, pain, and platelet aggregation.[32,36]

Non-steroidal anti-inflammatory drugs and immunosuppressants are currently most widely used in the treatment of acute inflammatory disorders. However there are many undesired side effects compared to the effectiveness of the drugs which deepens the needs and discovery of new anti-inflammatory drugs.[37,38]

Some researchers have reported that ZnO nanoparticles exhibit potent anti-inflammatory action by inhibiting COX activity. ZnO nanoparticles extracted from *Polygala tenuifolia* root, exhibited excellent anti-inflammatory activity by dose-dependently inhibiting the LPS-induced protein expression of COX-2 and iNOS.[39]

The most commonly used anti-inflammatory group of drugs includes aspirin, diclofenac, and ibuprofen. The drugs have adverse side effects on the liver and gastrointestinal tract. Based on in-vitro studies, ZnO nanoparticles showed anti-inflammatory activity and can be considered as a potential candidate as an anti-inflammatory agent.

4. CONCLUSION:

The present study has demonstrated an eco-friendly and cost-effective synthesis of ZnO nanoparticles using *A.webbiana* extract. ZnO synthesis was identified initially by direct visual observation of colour change to dark brown colour. UV-visible analysis of the ZnO nanoparticles was analyzed in the absorbency range of 250–500 nm. The peak was found to be maximum at 350 nm. The ZnO nanoparticles showed potent anti-inflammatory activity by inhibiting COX. Assuring its effectiveness in the therapeutic application for various diseases. The development of new classes of analgesics and anti-inflammatory drugs from *A. webbiana* is still under further investigation.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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