Nat. Volatiles & Essent. Oils, 2021; 8(5): 5950 - 5964

NVEO
Natural Volatiles & Essential Oils

Evaluation of anti-inflammatory and antioxidant activity of Adhatoda vasica zinc nanoparticles

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ABSTRACT:

Adhatoda vasica is a perennial herb with a diverse range of beneficial effects over diseases. The zinc nanoparticle has a number of benefits, including high-yield reaction and anti-inflammatory activity. The combination of these two marvelous materials has the potential to improve antioxidant and anti-inflammatory function.

Aim:

To estimate the antioxidant and anti-inflammatory properties of Adhatoda vasica mediated zinc nanoparticles.

Materials and method:

1.08 g of Adhatoda vasica plant powder and was dissolved in 100ml of distilled water. The solution was boiled for 10 minutes, filtered and then allowed to settle. This freshly prepared plant extract was used for green synthesis. 50 ml of Adhatoda vasica extract is combined with 50 ml of zinc nanoparticles. UV-Beckmann spectrometer was used to analyse the nanoparticle synthesis. Antioxidant and anti-inflammatory efficacy of adhatoda Vasica mediated zinc nanoparticles was evaluated and the results were compared with the standard drugs at various concentrations ranging from 10 µl to 50 µl.

Result:

The zinc nanoparticle mediated by Adhatoda vasica has positive antioxidant and anti-inflammatory properties.

Conclusion:

This study reveals that the anti-inflammatory and antioxidant exhibited by Adhatoda vasica mediated zinc nanoparticles showed increased levels of activity at higher concentration. Hence, this can be used as an effective drug in treating disease by natural medicine.

Keywords:

Adhatoda vasica, zinc nanoparticle, Green synthesis, Antioxidant activity, innovative technique, Anti-inflammatory activity.

INRODUCTION:

Nanotechnology is a rapidly expanding scientific discipline that has enormous potential in a variety of sectors, especially in healthcare, to the alteration of matter on the atomic and molecular levels to build materials with a wide range of startling and novel qualities. It plays an important role in the field of diagnostics, material development, medications and therapeutic levels in various arrays of medicine and dentistry (1). A nanoparticle is a tiny particle that ranges in size from 1 to 100 nanometers. These particles are invisible to the naked eye and can have radically different physical and chemical characteristics with their greater material counterparts. The basic aim of nanotechnology research in drug delivery include more precise drug targeting and distribution, reduced toxicity while preserving therapeutic benefits, increased safety and biocompatibility and rapid creation of newer safe medications (2). The goals of drug entrapment using nanoparticles has either improved delivery to or consumption by target cells or a decrease in the lethality of the free drug to non target areas (3). Nanoparticles are typically manufactured by chemical processes, frequently using toxic reactants that reduce the risk to the environment of harmful by-products (4). The development of eco-friendly alternative chemical processes, based on microorganisms such as bacteria and fungus or biological compounds taken from plants more recently, has attracted remarkable attention in recent years by providing a solution for curbing hazardous by product creation (5). As it is environmentally sustainable, biological synthesis of nanoparticles from microorganisms, enzymes, seeds, and plant extract has now become a standard.

Zinc oxide is a multifunctional substance with a wide range of UV absorption, high photostability, biocompatibility, and biodegradability (6). ZnO comes in a number of particle forms, which influence its use in new materials and future applications in a broad range of fields (7). Different methods were used to make zinc oxide nanoparticles, including wet chemical, sol-gel, green leaf extract, microwave, and hydrothermal methods, and the nanoparticles were characterized using XRD, SEM, EDX, and UV. The crystalline size of the zinc oxide nanoparticles that have been prepared ranges from 25 to 30 nm. The size of different types of nanoparticles were compared with zinc nanoparticles in flowchart 1, hence imply the

tiny particle size of nano - ZnO makes zinc more easily absorbed by the body (8). Many enzymes, such as carbonic anhydrase, carboxypeptidase, and alcohol dehydrogenase, become inactive without zinc, whereas the other two members of the same group of elements of the same electronic structure, cadmium and mercury, are poisonous. It's essential for eukaryotes because it controls a variety of physiological functions. Bamboo salt, which contains zinc, is used as a herbal remedy to relieve inflammation by inhibiting caspase-1 activity (9). Zinc oxide nanoparticles have been shown to suppress inflammatory cytokine mRNA expression by inhibiting NF-kB activation (nuclear factor kappa B cells) (10). Zinc oxide was used in many ointments for the treatment of injuries and boils during the reign of the Pharaohs, and historical accounts indicate that it was used in many ointments for the treatment of injuries and boils as early as 2000 BC (11). Despite the fact that a large volume of ZnO is created per year, only a limited amount is used in medicine (12).

Nanomedicine refers to the use of nanotechnology for the treatment, diagnosis, monitoring, and control of biological systems (13). The therapeutic effect of herbal medicines is based on the overall function of a range of active elements, since all ingredients work together to generate synergistic effects. Each active ingredient has a critical job to perform, and they are all interconnected (14). Most herbal origin drugs, on the other hand, have an insoluble nature, which leads to lower bioavailability and increased systemic clearance, necessitating repetitive administration or a higher dose, making them a weak candidate for therapeutic usages (15). The phytoformulatory research has a wide array of benefits for herbal medicines including advancement of solubility and bio disposability, protection against toxicity, increased pharmacological activity, stabilization improvements, improved stability and better nanospheres [polymeric nanoparticles, liposomes, proliposomes [SLNs], nanoemulsions, etc.] (16). As a result, nano-sized drug delivery systems for herbal treatments may have a bright future in terms of improving effectiveness and eliminating issues linked with plant medicines (17).

The herb Adhatoda vasica, also known as vasaka, is a well-known indigenous medicine with beneficial effects. It belongs to the Acanthaceae family. Adosa is another name for it. This plant's entire medicinal importance in curing the disease can be found in all of its components. It's a popular plant in Ayurveda and Unani medicine, especially for respiratory problems, including bronchitis and tuberculosis (18). This plant's leaf extract has antibacterial properties, especially against bacillus subtilis and vibrio cholerae. These plants' alkaloids are antiasthmatics and bronchodilators. It also works as an expectorant (antitussive), relieving irritation and loosening phlegm. Adhatoda vasica has a ton of potential for treating gastric ulcers as an antiulcer agent (compared with aspirin) (19). When compared to hydrocortisone, the major alkaloid from Adhatoda vasica demonstrated potent anti-inflammatory activity (20). The plant has an abortifacient effect by increasing prostaglandin synthesis and release. It also causes the myometrium to contract in a rhythmic pattern in both pregnant and non-pregnant women. The heart depressant activity of a mixture of vasicine and vasicinone was significantly reduced. This plant's extract had a radio protective function, preventing chromosomal damage. Adhatoda vasica is present in the formulations of two commonly used tuberculosis products, bromhexine and ambroxol (21). Anti-cholinesterase activity was observed in extracts from the plant's root. Methanolic extract from the plant leaves had a strong sucrose inhibitory effect, suggesting that it could be used as a natural anti-diabetic drug. As a result, Adhatoda vasica has a wide range of medicinal properties.

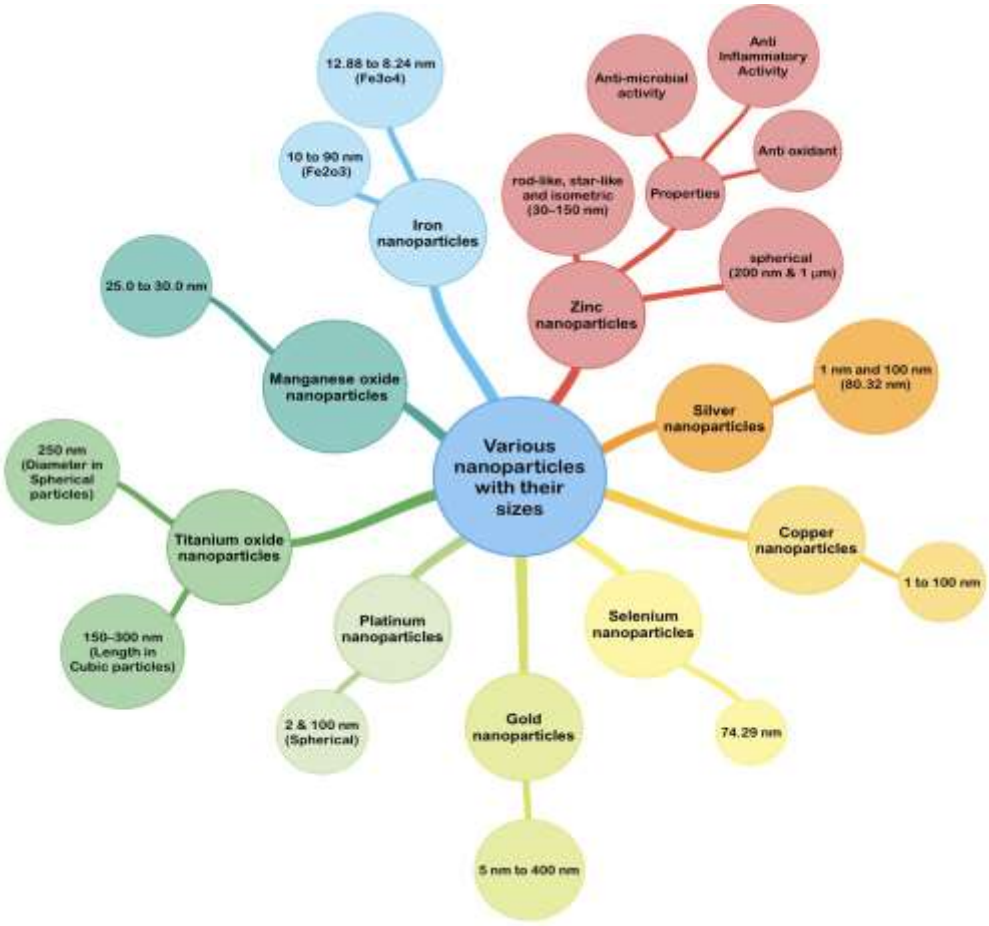
Oxidation processes are involved in all of our body's metabolisms. It is a chemical reaction in which free radicals are released as a result of oxidative stress, resulting in a chain reaction that damages an

organism's cell membrane (22). Antioxidants are substances that prevent or hinder free radical activity. Organisms have an antioxidant system that includes enzymes including superoxide dismutase and catalase, which are generated internally or obtained from a vitamin C and vitamin E-rich diet. Some diseases may develop as a result of oxidative stress. Adhatoda vasica contains vitamin C and can tolerate oxidative stress, according to research (23). Inflammation is a dynamic biological reaction characterized by redness, discomfort, swelling, heat, and loss of sensation as a result of a pathogen, irritants, or cell injury. It is responsible for removing cell injury, tissue destruction, tissue repair and it is the most common symptom of most diseases. If this condition persists, it can progress to a more severe condition like autoimmune disorders (24). Adhatoda vasica also helps to relieve inflammation and avoids future problems.

Our team has extensive knowledge and research experience that has translated into high-quality publications.(25–37),(38–42) (43) (44). ZnO NPs allows Zinc to be easily absorbed through the biological membranes due to its size in the nano-scale range. Previous literature suggests potent anti-inflammatory activity of zinc oxide nanoparticles (45). Above studies confirm a potent anti-inflammatory and antioxidant property in Adhatoda vasica and hence combining Adhatoda vasica with zinc nanoparticles can yield better results. Many studies have been done with the same plant but in combination with different nanoparticles like copper, selenium etc other than zinc nanoparticles (46). Due to the various clinically beneficial activities, mainly anti inflammatory properties in both Adhatoda vasica as well as in zinc nanoparticles which have inflammatory cytokines suppression characteristics, we would like to evaluate their combined synergistic action in order to develop a potent herbal formulation.

Hence the main aim of this analysis is to determine the antioxidant and anti-inflammatory activity of Adhatoda vasica plant extract through zinc nanoparticles.

Flowchart 1: Different type of nanoparticles with their sizes



MATERIALS AND METHODS:

Preparation of Adhatoda vasica plant extract:

Adhatoda vasica plant powder was obtained from Annai Arvind herbal shop - the approved shop in Chennai and 1.08 g of the powder was dissolved in 100 ml of distilled water. The solution was boiled for 10 minutes, then filtered using Whitman filter paper and allowed to settle. This set-up was left alone for 20 minutes. The filtrate mixture was then weighed, and it was found to be 50ml. This freshly prepared plant extract was used for green synthesis.

A. 

B. 

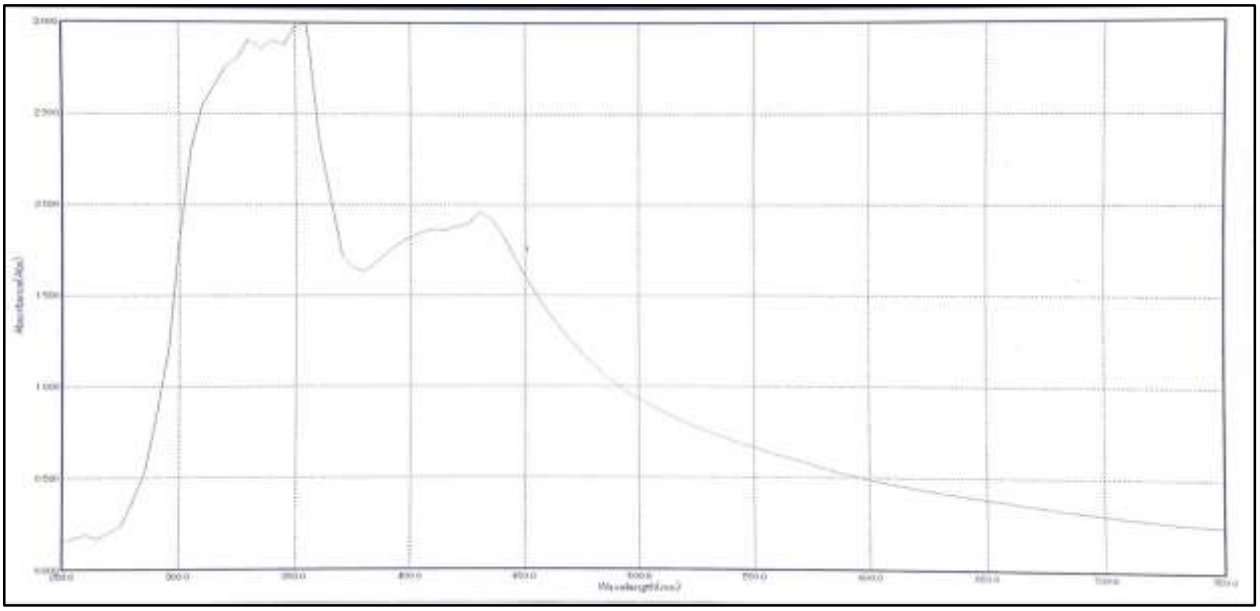
Figure 1: **A.** Preparation of Adhatoda vasica plant extract by boiling 1.08 g of Adhatoda vasica powder with 100ml of distilled water, **B.** filtration of the prepared plant extract using Whitman filter paper.

Synthesis of zinc nanoparticles:

A total of 0.573 g of zinc sulphate was combined with 50 ml of water. 50 ml of Adhatoda vasica extract is combined with 50 ml of zinc nanoparticles. This solution was then mounted in a laboratory shaker for nanoparticle synthesis. The UV-Beckmann spectrometer was used to analyse the nanoparticle synthesis. The solution was removed from the shaker every 2 hours to record the reading and color change (the reading was taken 5 times). This was done on a regular basis before the zinc nanoparticles were properly synthesized. With the passage of time, the colour of the solution gradually changed, becoming darker than it had been at the beginning. Synthesis of nanoparticles was later confirmed using UV–Visible spectroscopy. UV–Visible readings were recorded in the wavelength range of 250 – 750 nm. Maximum absorption was found in the range of 350 – 400 nm and a strong peak was found at 355 nm (Graph 1). This was in agreement with previous studies where ZnO NP was synthesized using Cassia alata (47). Just after synthesis, the samples were centrifuged for a few minutes. The pellets were extracted separately after the procedure. The antioxidant and anti-inflammatory efficacy of adhatoda vasica mediated zinc nanoparticles was evaluated using this extract with properly synthesized nanoparticles.

Figure 2: Prepared solution of Adhatoda vasica mediated zinc nanoparticles

Figure 3: Centrifugation of Adhatoda vasica mediated zinc nanoparticles for pellet collection



Graph 1: UV–Visible absorption spectra of ZnO NP synthesized using Adhatoda vasica.

Estimation of anti-inflammatory activity

ALBUMIN DENATURATION ASSAY:

The anti-inflammatory activity for Adhatoda vasica ZnNP was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of Adhatoda vasica ZnNP of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated

spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation of antioxidant activity

DPPH METHOD:

DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles. Diverse concentrations (2-10 µg/ml) of Justicia adhatoda leaf extract interceded zinc oxide nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control.

The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULTS:

The current study evaluated the antioxidant and anti-inflammatory activity of Adhatoda vasica zinc nanoparticles. The first set of data estimated the anti-inflammatory activity of Adhatoda vasica mediated zinc nanoparticles at various concentrations ranging from 10 µl to 50 µl (figure 4) which was then compared with activity of standard (Diclofenac sodium). The results showed 67%, 70% and 89% of anti-inflammatory activity at 10 µl, 20 µl and 50 µl respectively which indicates higher percentage than the standard diclofenac**.** According to figure 5, Adhatoda vasica mediated zinc nanoparticles at a concentration of 50 µl displayed 89 % equipotent inhibition against conventional Diclofenac sodium, indicating that they have potent anti-inflammatory effect.

The second set of data estimated the antioxidant activity of Adhatoda vasica mediated zinc nanoparticles at various concentrations ranging from 10 µl to 50 µl (figure 6) which was then compared with activity of standard (Ascorbic acid). According to figure 7, the antioxidant activity of the extract increased as the concentration of the extract increased, with roughly 77 % inhibition at 50 µl, which is substantially equivalent to the amount of inhibition shown by conventional ascorbic acid.

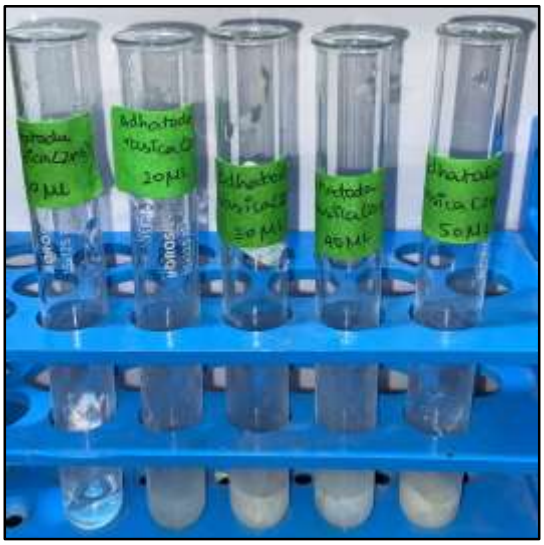


Figure 4: Anti-inflammatory activity of Adhatoda vasica mediated zinc nanoparticles at various concentrations ranging from 10 µl to 50 µl.

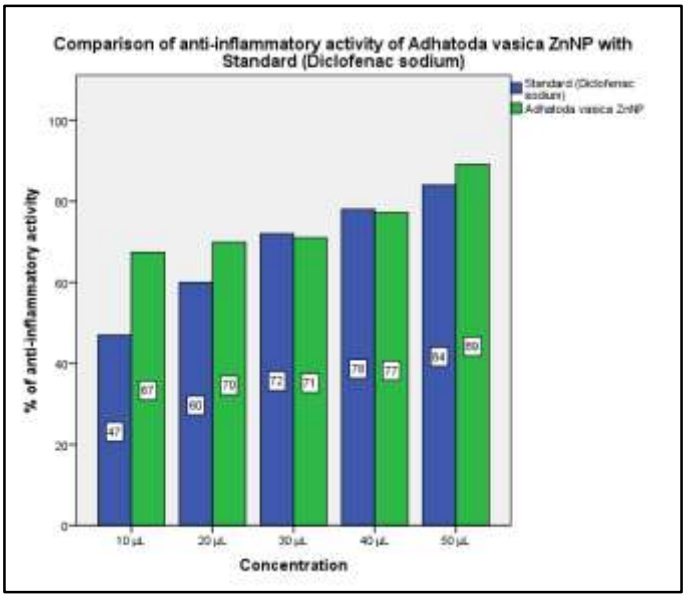


Figure 5: This bar graph represents the comparison of anti-inflammatory activity of Adhatoda vasica mediated zinc nanoparticles with the standard (Diclofenac sodium). X-axis represents the different concentration of standard and Adhatoda vasica mediated zinc nanoparticle in microlitres (10µl, 20µl, 30µl, 40µl and 50µl), Y- axis represents the percentage of anti-inflammatory activity shown by standard (blue) and Adhatoda vasica mediated zinc nanoparticle (green). Out of five different concentrations, at 10 µl, 20 µl and 50 µl Adhatoda vasica mediated zinc nanoparticle exhibited 67%, 70% and 89% respectively which showed higher percentage of anti-inflammatory activity than the standard diclofenac (Mean = 74.92; Standard deviation = 8.70). From the graph we can interpret that the Adhatoda vasica mediated zinc nanoparticles at the concentration of 50 µl showed 89% of equipotent inhibition against the standard Diclofenac sodium exhibiting a potent anti inflammatory activity.

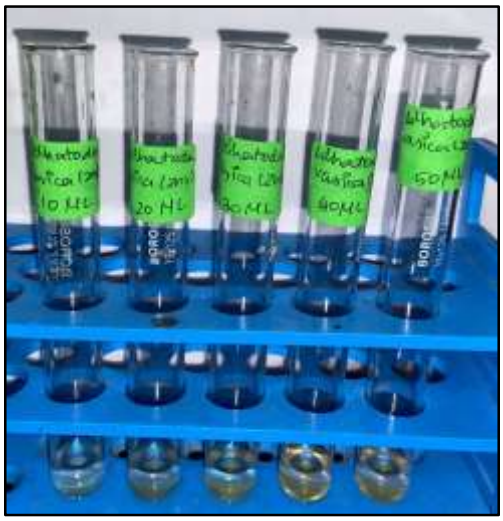


Figure 6: Antioxidant activity of Adhatoda vasica mediated zinc nanoparticles at various concentrations ranging from 10 µl to 50 µl.

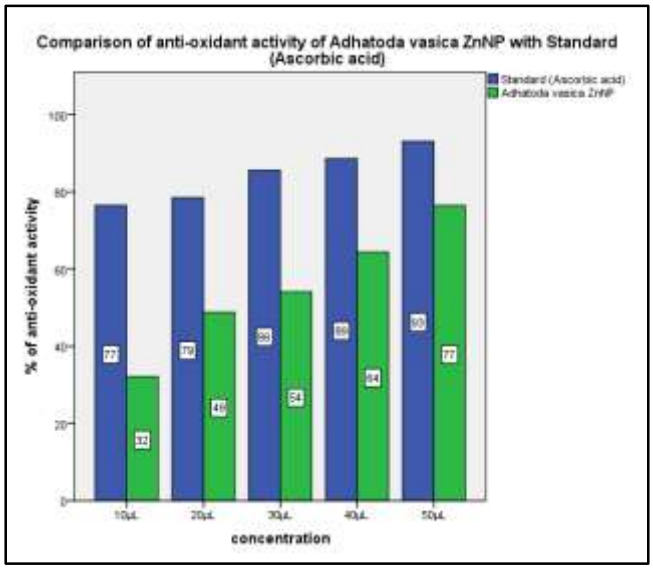


Figure 7: This bar graph represents the comparison of antioxidant activity of Adhatoda vasica mediated zinc nanoparticles with the standard (Ascorbic acid). X-axis represents the different concentration of standard and Adhatoda vasica mediated zinc nanoparticle in microlitres (10µl, 20µl, 30µl, 40µl and 50µl), Y- axis represents the percentage of antioxidant activity shown by standard (blue) and Adhatoda vasica mediated zinc nanoparticle (green). From the graph we can interpret that with the increasing concentration of the extract their antioxidant activity was also found to be increasing, showing about 77% of inhibition at 50µl which is nearly equal to the level of inhibition exhibited by the standard ascorbic acid (Mean = 55.20; Standard deviation = 16.65).

DISCUSSION:

Both analyses yielded positive findings in all concentrations of the extract controlled by zinc nanoparticles, according to the results of the two tests. From figure 5, we can conclude that 10 µl, 20 µl and 50 µl concentrations of Adhatoda vasica induced zinc nanoparticles exhibited a comparatively higher percentage of anti-inflammatory activity than the standard. Particularly, Adhatoda vasica mediated zinc nanoparticles at the concentration of 50 µl showed 89% of equipotent inhibition against the standard Diclofenac sodium exhibiting a potent anti inflammatory activity. Adhatoda vasica has been shown to have the similar anti-inflammatory properties in other few studies done with copper nanoparticles also (48). Another study in which Carrageenan and a CFA-model mediated paw edema were used to assess the anti-inflammatory efficacy of Adhatoda vasica phytochemicals. At 6 hours after carrageenan injection, vasicine had the most potent anti-inflammatory activity (59.51 %) at a dosage of 20.0 mg/kg (49). The modified hen's egg chorioallantoic membrane test was used in another experiment to assess the anti-inflammatory efficacy of Adhatoda vasica methanol extract (50). At a dosage of 50 g/pellet, equal to hydrocortisone, the alkaloid fraction displayed potent activity (51). The uniqueness in our study is the use of zinc nanoparticles which has the advantages of being cost-effective, having a high yield in reactions, and demanding less time. Proteins with fundamental structural stability and functional damage may be modified or denatured. Inflammation shows the sequence of denaturation, which must be watched closely (52). Diclofenac sodium or aspirin, is a commonly used anti-inflammatory medication that has the capacity to regulate thermally mediated protein denaturation in a dose-dependent manner. The capacity of different concentrations of Adhatoda vasica to suppress protein degradation was investigated as part of our research into the assessment of potent anti-inflammatory action (53).

From the figure 7, we can say that with the increasing concentration of the extract their antioxidant activity was also found to be increasing, showing about 77% of inhibition at 50µl which is nearly equal to the level of inhibition exhibited by the standard ascorbic acid. A similar study done by Jahagir et al, illustrated that Adhatoda vasica has antioxidant activity against two agents, cadmium and ferric nitrilotriacetate (Fe-NTA) (54). Another study done with cadmium intoxicated Swiss albino mice, prophylactic pretreatment of Adhatoda vasica extract inhibited lipid peroxidation (LPO) and xanthine oxidase activity (55). Jahagir et al. also confirmed Adhatoda vasica's anti mutagenic effectiveness, which can be attributed to its antioxidant restoring effects and suppression of malondialdehyde production (56). The antioxidative and therefore chemopreventive effects of the other assay were observed against Fe-NTA-induced renal oxidative stress, hyperproliferative reaction, and two-stage renal carcinogenesis (57).

Our study findings showed positive outcome using combination of Adhatoda vasica mediated zinc nanoparticles in expressing its potent anti inflammatory activity and also a near equal antioxidant activity with standards, hence may lead to the prevention of disease by further drugs preparation using this combination which will have comparatively less adverse effects than the chemical drugs now in use.

CONCLUSION:

From this study we can conclude that Adhatoda vasica mediated zinc nanoparticles were effective as anti-inflammatory and antioxidant. This study reveals that the anti-inflammatory and antioxidant activity exhibited by Adhatoda vasica mediated zinc nanoparticles showed increased levels of activity at higher concentration when compared to standards particularly the anti-inflammatory activity of Adhatoda vasica

ZnNP was more effective than the standard. The limitations of the study can be the specificity of nanoparticles selected for the analysis. Further research is needed to determine the active components in Adhatoda vasica as well as the signaling pathway that underpins its anti-inflammatory efficacy.

LEGENDS:

Figure 1: Preparation of Adhatoda vasica plant extract by boiling 1.08 g of Adhatoda vasica powder with 100ml of distilled water and then filtering it using Whitman filter paper.

Figure 2: Prepared solution of Adhatoda vasica mediated zinc nanoparticles.

Figure 3: Centrifugation of Adhatoda vasica mediated zinc nanoparticles for pellet collection

Graph 1: UV–Visible absorption spectra of ZnO NP synthesized using Adhatoda vasica.

Figure 4: Anti-inflammatory activity of Adhatoda vasica mediated zinc nanoparticles at various concentrations ranging from 10 µl to 50 µl.

Figure 5: This bar graph represents the comparison of anti-inflammatory activity of Adhatoda vasica mediated zinc nanoparticles with the standard (Diclofenac sodium). X-axis represents the different concentration of standard and Adhatoda vasica mediated zinc nanoparticle in microlitres (10µl, 20µl, 30µl, 40µl and 50µl), Y- axis represents the percentage of anti-inflammatory activity shown by standard (blue) and Adhatoda vasica mediated zinc nanoparticle (green). Out of five different concentrations, at 10 µl, 20 µl and 50 µl Adhatoda vasica mediated zinc nanoparticle exhibited 67%, 70% and 89% respectively which showed higher percentage of anti-inflammatory activity than the standard diclofenac. From the graph we can interpret that the Adhatoda vasica mediated zinc nanoparticles at the concentration of 50 µl showed 89% of equipotent inhibition against the standard Diclofenac sodium exhibiting a potent anti inflammatory activity.

Figure 6: Antioxidant activity of Adhatoda vasica mediated zinc nanoparticles at various concentrations ranging from 10 µl to 50 µl.

Figure 7: This bar graph represents the comparison of antioxidant activity of Adhatoda vasica mediated zinc nanoparticles with the standard (Ascorbic acid). X-axis represents the different concentration of standard and Adhatoda vasica mediated zinc nanoparticle in microlitres (10µl, 20µl, 30µl, 40µl and 50µl), Y- axis represents the percentage of antioxidant activity shown by standard (blue) and Adhatoda vasica mediated zinc nanoparticle (green). From the graph we can interpret that with the increasing concentration of the extract their antioxidant activity was also found to be increasing, showing about 77% of inhibition at 50µl which is nearly equal to the level of inhibition exhibited by the standard ascorbic acid.

ACKNOWLEDGEMENT:

The authors are thankful to Saveetha Dental College for providing a platform to express our knowledge.

CONFLICT OF INTEREST:

The author declares no conflict of interest.

SOURCE OF FUNDING:

The present study was supported by the following agencies

- Saveetha Dental College,
- Saveetha Institute of Medical and Technical Science,
- Saveetha University
- Dhivyasree beauty parlour, Thiruverkadu, Chennai-77. (Reference number: DBP3305)

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