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A PIPER NIGRUM BASED ZINC OXIDE NANOPARTICLES FOR ANTI-ARTHRITIC AND ANTIOXIDANT ACTIVITY

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ABSTRACT
Background: Zinc oxide nanoparticles (ZnONPs) are among the most effective metallic oxide nanoparticles for biological applications. They have potential anti-inflammatory and anti-oxidant properties, desirable biocompatibility, lower toxicity, and minimal cost. **Methodology:** Using diverse plant extracts for an ecologically friendly production of metallic nanoparticles is a better choice than conventional chemical synthesis techniques. The present study is decisive on the ZnONPs synthesis from a *Piper nigrum* extract (Pn-ZnONPs). Morphological characteristics of ZnONPs have been studied using UV-spectroscopy, DLS, SEM, and TEM. Further, it is analyzed for its anti-inflammatory (proteinase, collagenase, lipooxygenase, and elastase) and anti-oxidant properties ($DPPH^{\cdot}$, SOD, NO, H_2O_2, and OH). **Results and discussion:** Synthesis of nanoparticles has been confirmed via visible spectroscopy with maximum absorbance of 350nm, having particle size and zeta potential of 80 nm and +7.4 mV, respectively. SEM and TEM analysis confirmed the nanoparticle's shape to be spherical and arranged compactly. Further, biogenic nanoparticles show desired anti-inflammatory properties by inhibiting the activity of collagenase (68.72%), elastase (65.16%), lipooxygenase (58.098%), and denaturation of protein (65.36%). It also exhibits the capability to suppress Superoxide radicals (64.87%), DPPH (65.46%), Hydrogen Peroxide (64.89%), Hydroxyl radical (68.45%), and Nitric oxide radicals (71.343%), which are responsible for the pathogenesis of many inflammatory disorders. **Conclusion**: This suggests that *P. nigrum* Zinc nanoparticles may be a promising agent in treating various inflammatory disorders, such as cancer, Rheumatoid Arthritis, Psoriasis, and others.

INTRODUCTION

Nanocarriers are employed for the treatment of Rheumatoid Arthritis (RA) by the administration of anti-inflammatory agents to the target area. Nanocarriers possess distinct physical and chemical characteristics that can boost the availability of drugs in the body, specifically target injured joint tissue, and amplify the efficacy of medications. Nanotechnology-enabled drug carriers can be tailored to selectively deliver medications to targeted locations within the synovial joints [1]. The small size and modification of these carriers enable effective transportation of therapeutic substances through passive or active means. Nanocarriers can be manufactured using organic polymers,

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liposomes, or inorganic nanomaterials [2]. Recently, researchers have shown an increasing interest in researching several types of metal nanoparticles, including silver, iron, copper, and zinc [3]. Zinc oxide nanoparticles (ZnONPs) are gaining attention for their potential in treating inflammatory disorders due to their unique properties and biological effects. It exhibits remarkable stability, low toxicity, high thermal resistance, and potent antibacterial and photocatalytic characteristics. These nanoparticles exhibit potent anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, which reduce inflammation at the molecular level [4,5].

In addition to their anti-inflammatory effects, they contain antioxidant properties by removing reactive oxygen species (ROS). Nanocarriers made of zinc oxide nanoparticles (ZnONPs) are ideal for transporting anti-inflammatory medications to specific tissues, where they may be released slowly and with few unwanted side effects. In addition to alleviating inflammation and speeding up the healing process, they influence macrophage activity, promoting the transition from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, which has immunomodulatory effects. When tissue damage is a concern in inflammatory illnesses, ZnONPs are particularly significant because they assist in wound healing and tissue repair [3]. Chemical, physical, and biological methods can produce ZnO nanoparticles. Metal ion concentrations are reduced, and metallic nanoparticles are destabilized during the physical and chemical production of ZnONPs. However, the potential for chemical residues in the final goods would restrict the use of these NPs in sectors including the food, pharmaceutical, and cosmetics industries. To circumvent these drawbacks, researchers have been working tirelessly to develop new, more efficient, less expensive, and more environmentally friendly ways of making metal nanoparticles. Biological synthesis approaches, often known as green methods, have several advantages, including cost-effectiveness, availability of resources, quick synthesis process, single-stage nature, and ease of scalability [6].

Piper nigrum, scientifically called *P. nigrum*, is a tropical vine classified under the Piperaceae family, often known as Black Pepper. Its therapeutic effects are widely recognized, including its ability to combat cancer, fight against microbes, reduce inflammation, and inhibit tumor growth [7]. Various investigations have demonstrated that *P. nigrum* extract contains notable bioactive constituents, such as flavonol, flavone, xanthine, cellulose, free steroids, reducing sugars, and small amounts of copper, zinc, magnesium, and iron. Most of these bioactive compounds have properties that decrease and stabilize, making them very suitable for use in the synthesis of organic nanoparticles.

There is a growing interest in exploring ways to synthesize ZnONPs via a biogenic method, especially with the help of plants. The benefits of environmental sustainability, simplicity, and affordability endorse this. Producing ZnO nanoparticles with morphology suitable for the selected application is essential. Synthesis of ZnONPs from plant extracts offers a higher control over the size and form of the resultant NPs compared with physical and chemical methods [8]. The primary objective of this work was to synthesize biogenic ZnONPs using black pepper (Pn-ZnONPs) as the reducing agent and chitosan as the stabilizing agent. This choice was motivated by several advantages, such as its nontoxicity to humans and the environment and its suitability for large-scale applications. In addition, the synthesized Pn-ZnONPs were analyzed to determine their morphological properties. The Pn@ ZnO NPs were also examined for their antioxidant and anti-inflammatory characteristics.

MATERIAL AND METHOD

Materials

All analytical-grade chemicals have been purchased from Sigma Aldrich. A commercial-grade Black Pepper has been purchased from the local market in Bhilai, Chhattisgarh.

Methods

Synthesis of biogenic ZnO NPs

10 g of ground Black Pepper was added to 50 ml of Milli-Q water and boiled at 100°C for 2 h. Subsequently, the solution was cooled overnight and double purified with the help of Whatman filter paper no. 1. The resultant extract was subsequently utilized as a solvent in producing ZnO nanoparticles. A solution of 0.1% chitosan in 1% acetic acid was added with zinc acetate dihydrate with a concentration of 0.01 M and heated on a magnetic hot plate at 70°C for 30 minutes. 10 ml of freshly made extract was added, stirring the solution for 10 minutes. The pH of the reaction mixture was adjusted by incrementally adding 2M NaOH. The synthesis procedure

involved the optimization of temperature, pH, and incubation period. The change in the fluid's hue from colorless to yellowish brown after extended churning signified the synthesis of ZnONPs. The synthesized ZnONPs underwent centrifugation at 10,000 revolutions per minute for 5 minutes. Subsequently, they were washed twice using deionized water. The production mechanism and reactions of ZnO nanoparticles (ZnONPs) have been investigated. The colloidal stability of nanoparticles was assessed one month later using a UV-visible spectrophotometer [9,10].

Morphological characterization

To confirm the formation, the surface Plasmon resonance peak was measured using a UV–Vis spectrophotometer (Shimadzu 2000) between 300 and 700 nm in wavelength to determine the maximum absorbance of Pn@ZnONPs. Further, the formulation's particle size, zeta potential, and polydispersity Index (PDI) were determined by the Malvern zeta sizer (Malvern, UK). Detailed morphological structure and arrangement of the molecules have been confirmed through SEM and TEM analysis. The presence of functional groups and metallic ions was determined via Fourier transform infrared (FTIR).

Anti-inflammatory property

Inhibition of protein denaturation

1 mL aqueous solution of bovine albumin fraction (1%, w/v) was mixed with the extract of *Piper nigrum* and Pn@ZnONPs solutions (25-100 µg mL-1) independently to prepare the reaction mixture. Afterward, for 30 min, the reaction mixture was heated at 51 ºC and incubated for 20 minutes at 37 ºC, with the pH then adjusted to 6.3. The substance was examined for absorbance at 660 nm utilizing a UV-Vis spectrophotometer upon cooling to room temperature (RT). Dexamethasone sodium phosphate (DSP) is a reference [11]. The percentage inhibition of protein denaturation has been determined via equation (1).

$$\%\ of\ inhibition = \left(\frac{Ac-As}{As}\right) * 100 \text{..... Equation (1)}$$

Where Ac and As represents the absorbance of the control and standard sample, respectively

Inhibition of lipooxygenase activity

The anti-inflammatory properties of *Piper nigrum* extract and Pn@ZnONPs extract were evaluated by lipooxygenase (LOX) inhibition [11]. The substrate was 0.2 M linoleic acid in a 0.2 M borate buffer at pH 9. Synthesized formulations were incubated at ambient temperature for 5 minutes before exposure to LOX enzyme at different concentrations (25-100 µg mL-1). Subsequently, absorbance was quantified at 243 nm utilizing a UV-vis spectrophotometer after adding the formulations (*Piper nigrum* extract and Pn-ZnONPs) to the substrate (750 µL) with the enzyme mixture. DSP served as the positive control. The inhibition of LOX activity was determined using Equation 1.

Inhibition of elastase activity

With minor adjustments, the anti-elastase activity of the synthesized materials (*Piper nigrum* extract and Pn@ZnONPs) was assessed by Chiocchio et al.[12]. Porcine pancreatic elastase (3.33 mg mL^{-1}; 25 µL), 20 µL of various sample concentrations (25-100 µg mL^{-1}), and 20 µL of tiliroside solutions were incubated at 29ºC (10 min) in Tris-buffer (0.2 mM, pH 8.0). The reaction was started by adding the substrate N-succinyl-Ala-Ala-Ala-p-nitroanilide (2 mM; 125 µL). After incubation for 15 minutes, absorbance at 420 nm was measured. The positive control in this experiment was DSP. Using equation (1), the degree of elastase activity inhibition was determined.

Inhibition of collagenase activity

To measure the anti-collagenase activity, N-[3-(2-furyl)-acryloyl]-Leu-Gly-Pro-Ala (FALGPA) was utilized as the substrate. Using 0.05 M tricine buffer containing 0.4 M NaCl and 0.01 M calcium chloride (pH 7.5), *Clostridium histolyticum* collagenase (20 mU) was dissolved in the samples (20 µL) of different concentrations (25-100 µg mL-1) and tiliroside solutions (20 µL) and incubated for 10 min at 29 ºC. The reaction was initiated by adding 1 mM FALGPA, and after 15 minutes of incubation, the absorbance was measured at 340 nm. DSP was used as a positive control [11]. Equation (1) was used to determine the amount of suppressed collagenase activity.

Inhibition of proteinase activity

1 mL of test substances, which involves *Piper nigrum* extract and Pn@ZnONPs (at concentrations ranging from 25 to 100 µg mL-1), were amalgamated with a reaction mixture containing 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 0.06 mg of trypsin. After incubating the mixture at 37 ºC for 5 minutes, 1 mL of 0.7% (w/v) casein was included. After 20 minutes of incubation, 2 mL of 70% (v/v) perchloric acid was introduced to terminate the reaction. The reaction mixture underwent centrifugation at 4ºC and 6000 RPM for 10 minutes to get the supernatant [13].

The spectrophotometer was used to measure the absorbance of the supernatant at a wavelength of 210 nm. The DSP was chosen as the positive control. The % inhibition of proteinase was determined using Equation (1).

Anti-Oxidant property

1 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) scavenging assay

The DPPH˙ free radical assay is commonly employed to determine a substance's antioxidant capacity as a percentage. The DPPH˙ scavenging activity was measured using the method described by Yesmin et al. [14]. At first, a 3 mL DPPH˙ nanoparticle in methanol at a concentration of 0.004% (w/v) was mixed with *Piper nigrum* extract and Pn@ZnONPs at 25-100 µg/mL concentrations. Subsequently, the samples were placed in total darkness and incubated at room temperature for 30 minutes. Subsequently, the samples were analyzed for absorbance using a UV-Vis spectrophotometer at 517 nm. Ascorbic acid was the positive control (standard), and methanol was utilized as the blank. The DPPH radical scavenging % was assessed using Equation (1).

Superoxide radical scavenging assay

The method described by Wang et al. [15] was employed to determine the superoxide anion ($O2^{\cdot-}$) radical scavenging activity of *Piper nigrum* extract and Pn@ZnONPs. In summary, the generation of $O2^{\cdot-}$ was triggered by the non-enzymatic reduction of phenazine methosulfate/ reduced nicotinamide adenine dinucleotide (PMS/NADH) using nitroblue tetrazolium (NBT).

This reaction led to synthesizing a purple formazan compound, which was then measured using a spectrophotometer. The experimental setup involved combining PBS (pH 6.5), NADH (468 µM), NBT (156 µM), PMS (60 µM), and different quantities (25-100 µg mL-1) of *Piper nigrum* extract and Pn@ZnONPs to create a 2 mL reaction mixture. Following a 5-minute incubation period at room temperature, the absorbance at 560 nm was measured to quantify the formazan produced, utilizing a suitable blank. Ascorbic acid served as the positive control in this test. The % inhibition was determined by applying Equation (1).

Hydrogen peroxide scavenging assay

The hydrogen peroxide (H_2O_2) scavenging activity of extract of *Piper nigrum* extract and Pn@ZnONPs was assessed using the method described by Wang et al. [15], with minor adjustments. PBS (pH 6.5) was utilized in this experiment to create a solution of H_2O_2 with a concentration of 100 mM. Next, 600 µl of hydrogen peroxide (100 mM) and 300 microliters of test samples containing various concentrations of Black Pepper extract and Pn@ZnONPs (25-100 mg/ml) were added. The volume was then adjusted to 2 ml using PBS. Following a 10-minute incubation period, the samples were examined using spectrophotometry by measuring the absorbance at 230 nm compared to appropriate blank samples. The inhibition percentages of all the samples and ascorbic acid were determined using Equation (1).

Hydroxyl radical scavenging assay

The scavenging activity of the hydroxyl radical (˙OH) was evaluated using the method described [16]. To summarize, a reaction mixture of 2 mL volume was prepared by adding sodium phosphate buffer (200 mM, pH 7.0), 0.15 mL deoxyribose (10 mM), 0.15 mL H_2O_2 (10 mM), 0.150 mL FeSO4-EDTA (10 mM), 0.075 mL of Black Pepper extract and Pn-ZnONPs (25-100 µg mL^{-1}), and deionized water. Subsequently, the reaction tubes were incubated for 4 hours in the digital incubator. Next, 0.75 mL of trichloroacetic acid (2.8% weight/volume) and 0.75 mL of thiobarbituric acid (1% weight/volume, in 50 mM NaOH) were added one after the other to prevent the reaction. The tubes were immersed in a water bath at boiling temperature for 10 minutes and then cooled to room temperature. The absorbance at 520 nm was subsequently measured against a suitable blank. Ascorbic acid served as the positive control. The percentage of ˙OH scavenging was determined by applying Equation (1).

Nitric oxide radical scavenging assay

The scavenging capacity of Black Pepper extract and Pn@ZnONPs against nitric oxide (NO) radicals was assessed using the Griess Illosvoy reaction approach [17]. In this system, sodium nitroprusside generates NO radicals that interact with oxygen molecules to produce nitrite ions. A 3 mL reaction mixture was formed, consisting of sodium nitroprusside (10 mM), PBS (pH 6.5), and several treatment samples with their corresponding quantities [Black Pepper extract, and Pn@ZnONPs, (25-100 µg mL^{-1})].

The combination was then placed in a dark environment at room temperature for 90 minutes. Subsequently, 0.5 mL of the reaction mixture was combined with 1 mL of sulfanilamide

(0.33% in 22% v/v glacial acetic acid), followed by the addition of 1 mL of naphthyl ethylenediamine dihydrochloride (0.1% w/v). This created a pink chromophore, which was then analyzed using spectrophotometry at 540 nm. The percentage of radical scavenging was determined using Equation (1).

RESULT AND DISCUSSION

Morphological characteristics

The synthesis of zinc nanoparticles was confirmed via visible analysis, changing from colorless to whitish brown precipitate. The surface plasma resonance of Pn@ZnONPs was determined with the help of UV-visible spectroscopy at a max wavelength of 350nm (Figure 1(A)). Prepared biogenic Zinc nanoparticles (Pn@ZnONPs) were characterized for the particle size (figure1 (A)) and zeta potential with the help of Malvern zetasizer. It has been found Pn-ZnONPs have a particle size of 80nm with a zeta potential of +7.4Mv (figure 1(B)) and a PDI value of 0.285. Further, the exact shape of the nanoparticles has been analyzed via SEM and TEM images, which are found to be spherical and arranged in a compact manner each other. FTIR has been used to analyze the functional group present in the formulation, and peaks were represented in Figure 1(C).

The peaks have been shown at 1564 cm^{-1} and 1441 cm^{-1}, corresponding to the asymmetric and symmetric stretching vibrations of COO−, respectively. A prominent band at 3291-3612 cm^{-1} indicates N-H and O-H stretching and intramolecular hydrogen bonding. Further bands at 2921 and 2877 cm^{-1} correspond to C-H symmetric and asymmetric stretching, respectively, representing chitosan bending. Figure 1.C illustrates the absorption peaks of DSP at 1707, 1666, and 1624 cm^{-1}, which correspond to -C=O bonds.

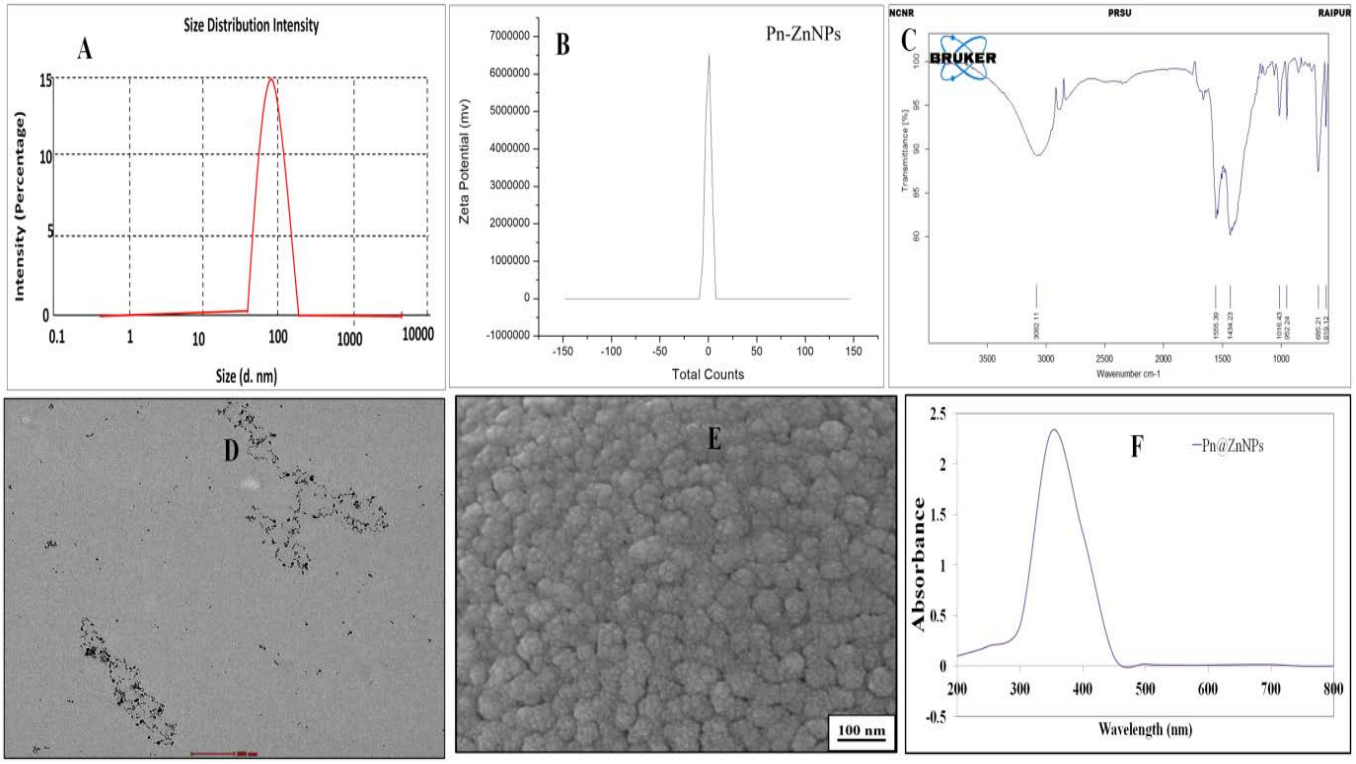

Figure 1: Morphological Characteristics of *Piper nigrum* based Zinc Oxide nanoparticles (A) Particle Size, (B) Zeta Potential, (C) FTIR analysis, (D) TEM image, (E) SEM image, (F) UV spectrum analysis

Anti-inflammatory property

The synthesized nanoparticles have been characterized for their anti-inflammatory potential, inhibiting certain anti-inflammatory agents such as proteinase, collagenase, elastase, lipooxygenase, and protein denaturation. At the highest concentration, the Pn@ZnONPs show the highest inhibition for proteinase activity (70.56%), Protein denaturation (65.36%), Lipoxygenase (58.098%), Inhibition of elastase activity (65.16%), and collagenase activity (68.72%). All these inhibitory percentages are lower than the standard agent (DSP) but higher than the *Piper nigrum* extract. In this analysis, DSP has been used as a standard compound. The differential

inhibition of enzymes by Pn@ZnONPs can be attributed to several factors, including enzyme specificity, structural interaction between the nanoparticles and the enzyme active sites, and differences in enzyme mechanisms. Enzymes like elastase and lipooxygenase have distinct three-dimensional structures and active sites, and the effectiveness of enzyme inhibition by nanoparticles depends on how well these nanoparticles can bind to or block the active site.

Pn@ZnONPs may exhibit a higher affinity for elastase's active site due to structural complementarity, charge interactions, or hydrophobic/hydrophilic balance, which enhances their inhibitory effect on elastase. The catalytic mechanisms of these enzymes differ, with elastase being a serine protease that breaks down proteins and lipooxygenase involved in the oxidation of polyunsaturated fatty acids to produce inflammatory mediators. ZnONPs may interfere more effectively with elastase's protease activity through binding or denaturing effects. At the same time, lipooxygenase, an oxidoreductase, may require a different mechanism of inhibition that ZnONPs are less effective at targeting. The inhibitory percentage for each analysis is shown in Figure 2 and Table 1.

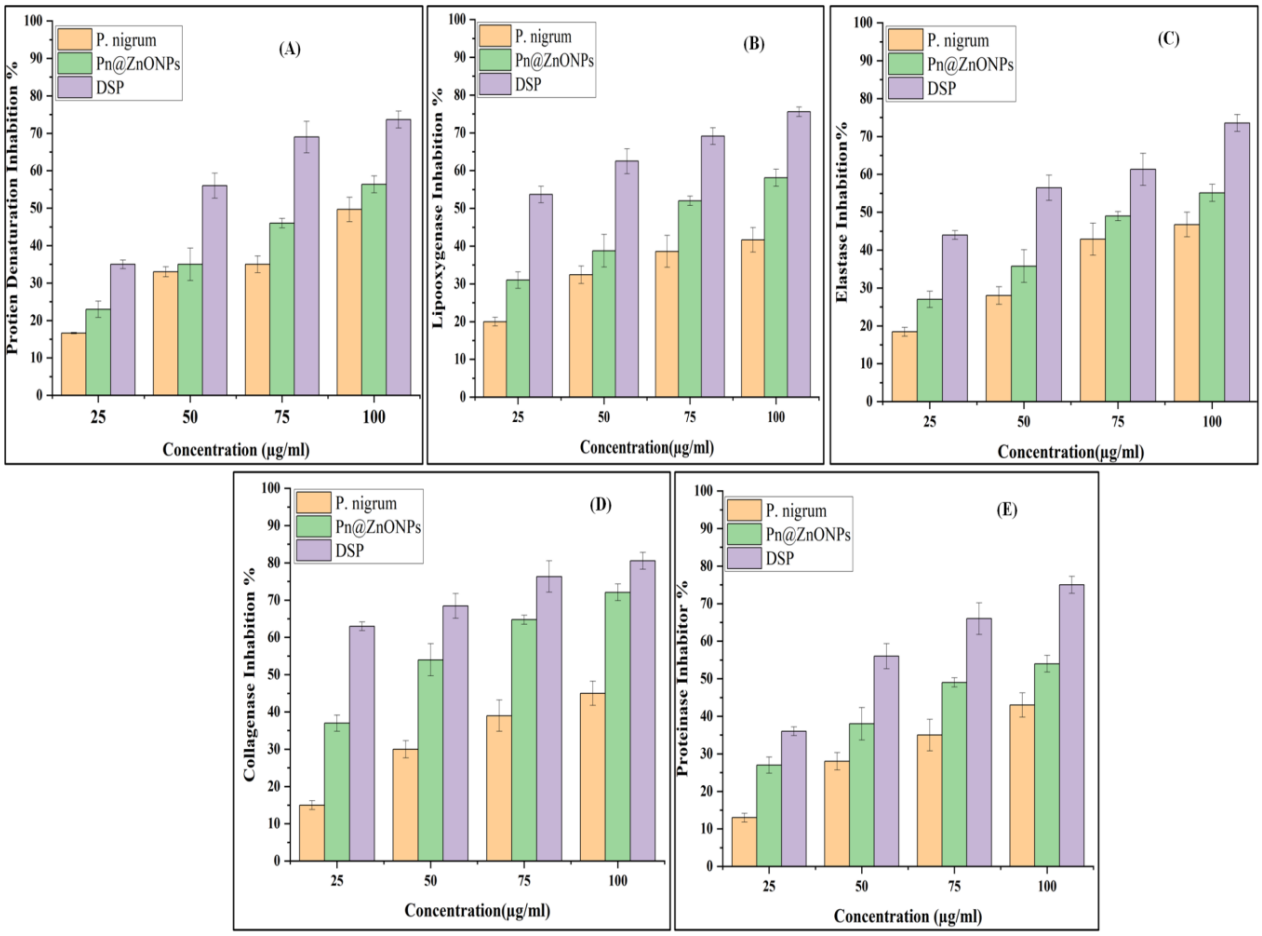

Figure 2: The anti-inflammatory properties of the prepared formulation, *Piper nigrum* extract, and dexamethasone sodium phosphate have been analyzed for (A) Protein denaturation assay, (B) Lipoxygenase inhibition assay, (C)Inhibition of elastase activity, (D) Inhibition of collagenase activity, and (E) proteinase inhibition assay.

Anti-oxidant property

Antioxidant processes can be complex, mainly when nanoparticles are present, since they may interact with free radicals differently than plant extracts. Examining these mechanistic elements, including the differential interaction of Pn@ZnONPs with radicals compared to the extract and their potential role in facilitating or enhancing antioxidant pathways. Oxidative reduction activities, color change, and UV-absorption spectra were used to determine the Black Paper extract and Pn@ZnONPs. Pn@ZnONPs exhibits the highest anti-oxidant potential at 100 µgmL^{-1} by suppressing the DDPH, Hydrogen peroxide, Hydroxyl ions, Nitric Oxide, and Superoxide radicals.

Pn@ZnONPs have been compared with *Piper nigrum* and Ascorbic acid (Standard). Figure 3 shows that compared to conventional ascorbic acid, Pn@ZnONPs significantly inhibited activity. The different numbers obtained showed a notable disparity. A dose-dependent increase in the oxidative scavenging levels was observed. Table 1 and Figure 2 display the different percentages of antioxidant suppression for each test.

It is well-known that Pn@ZnONPs may catalyze the production of ROS, leading to oxidative stress and the potential damage it might do to DNA, proteins, and cell membranes. Since many diseases and cancer cells are more vulnerable to oxidative damage, this stress may explain why Pn@ZnONPs are often thought to have antibacterial and anticancer effects. Pn@ZnONPs may provide more active surface sites for radical interactions than the compounds in the extract alone because of their higher surface-to-volume ratio and specific physicochemical properties. Thanks to their nanoscale size, Pn@ZnONPs can neutralize reactive species, including hydroxyl, superoxide, and DPPH radicals, by donating or accepting electrons. Compared to the extract's bigger molecular structures, this could cause it to have more antioxidant action. In addition, the bioactive components in the *Piper nigrum* extract may interact synergistically with Pn@ZnONPs.

The extract's radical scavenging efficiency may be enhanced because the nanoparticles stabilize or increase the activity of the phenolic compounds and alkaloids. Surface catalysis, in which the nanoparticle surface participates in redox reactions, is another way that Pn@ZnONPs may interact with radicals directly, which might lead to better neutralization of free radicals. It is crucial to comprehend these interactions to understand the observed increased antioxidant activity and to guide future medical or commercial applications of Pn@ZnONPs.

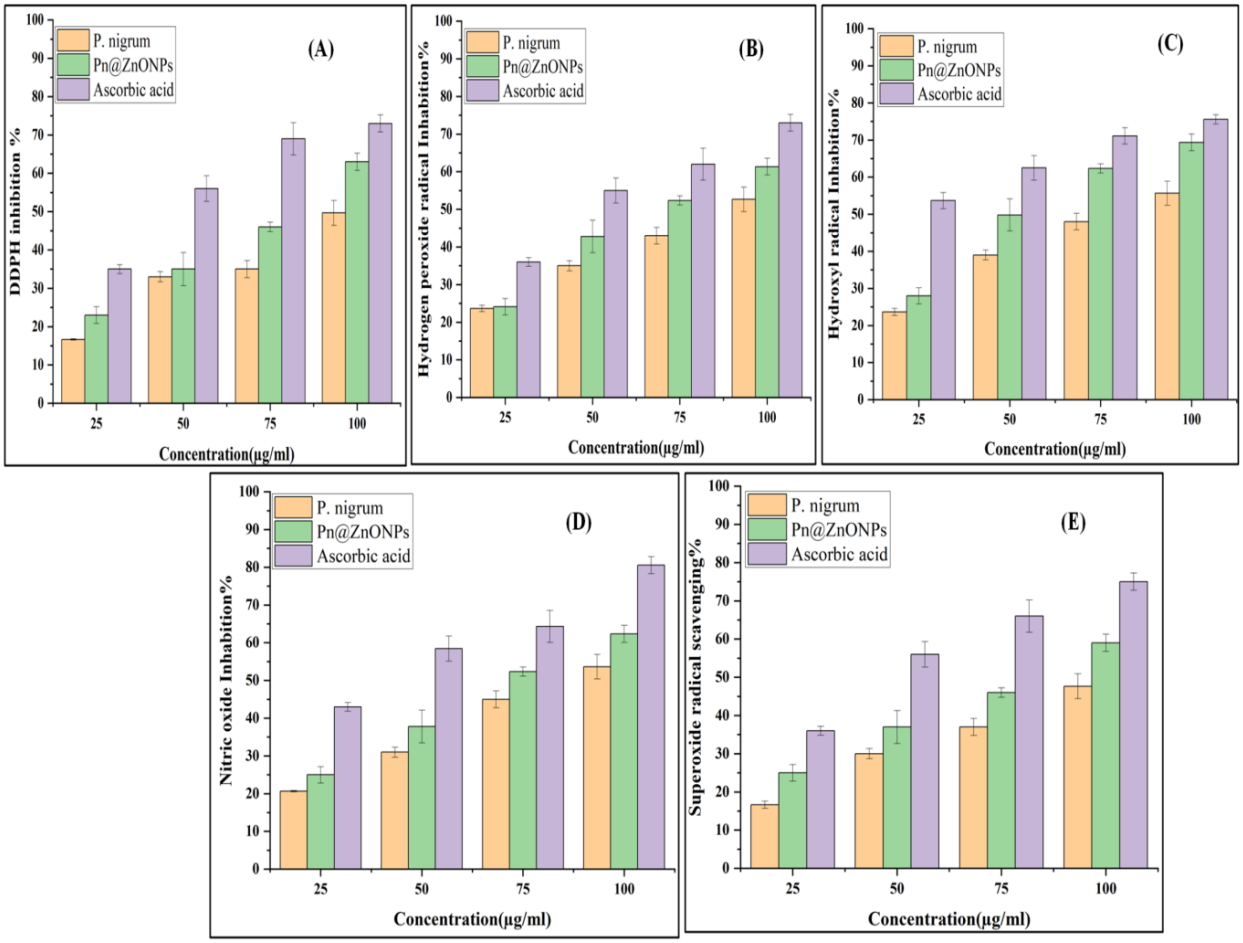

Figure 3: Antioxidant activity of Black Pepper, Pn@ZnONPs, and Ascorbic acid (Standard) determined by (A) DDPH inhibition, (B) Hydrogen peroxide inhibition, (C) Hydroxyl inhibition, (D) Nitric Oxide inhibition assay, (D) Superoxide radical inhibition assay

Table 1: Comparative analysis of Synthesized Biogenic Zinc Oxide Nanoparticles for its Antioxidant and anti-inflammatory Inhibitory property

S No.	Examination	*P. nigrum*	Pn@ZnONPs	DSP	Ascorbic Acid
Anti-inflammatory Inhibitory					
1.	**Protein denaturation inhibition**	49.66 %	65.36%	73.66%	
2.	**Lipooxygenase inhibition**	41.66%	58.098%	75.599%	
3.	**Elastase inhibition**	55.098%	65.16%	73.56 %	
4.	**Collagenase inhibition**	45.34%	68.72%	72.25%	
5.	**Proteinase inhibition**	48.2%	70.56%	72.69%	
Anti-oxidant property					
6.	**DPPH**	49.66%	65.46%		73.48%,
7.	**Superoxide radical inhibition**	41.32%	64.87%		75.66 %
8.	**Hydrogen peroxide inhibition**	52.66%,	64.89%		73.66%
9.	**Hydroxyl radical inhibition**	55.6%	68.45%,		75.599%
10.	**Nitric oxide inhibition**	53.66%	71.343%		80.56%

CONCLUSION

Zinc oxide metallic nanoparticles (ZnONPs) from biological black pepper or *Piper nigrum* have shown promise as an anti-inflammatory and antioxidant therapy for rheumatoid arthritis (RA). These nanoparticles, which were synthesized using black pepper extract, use the bioactive elements present in the spice to improve their medicinal properties. Green synthesized nanoparticles are preferred for their eco-friendly, cost-effective, and sustainable production methods, unlike chemically synthesized nanoparticles that often involve toxic reagents and harmful by-products. A comparative analysis of factors such as bioavailability, antioxidant capacity, and enzyme inhibition strength of Pn@ZnONPs versus other green-synthesized nanoparticles would be critical. When comparing Pn@ZnONPs to other green-synthesized nanoparticles and conventional medicines, it is crucial to look at their safety profiles. To compare their possible cytotoxicity or long-term consequences to those of normal therapies is necessary, even though Pn@ZnONPs is often thought to be harmless. To know how biocompatible, safe, and likely to accumulate in tissues Pn-ZnONPs are, we need to compare them to other green-synthesized nanoparticles from diverse sources.

By regulating the immunological response, Pn@ZnONPs can reduce inflammatory cytokines associated with RA, reducing joint inflammation and pain. They provide an alternative to conventional RA drugs that is safer and more effective because of their biocompatibility and ability to target specific sites of inflammation. Black pepper extract is utilized in manufacturing these nanoparticles, enhancing their therapeutic capabilities due to its bioactive components. Zinc oxide nanoparticles (ZnONPs) have strong anti-inflammatory properties via regulating immune responses and decreasing the levels of pro-inflammatory cytokines.

Moreover, their ability to serve as antioxidants aids in removing harmful free radicals, thereby reducing oxidative stress that contributes to the development of rheumatoid arthritis. The combinations of these two actions make black pepper ZnONPs an up-and-coming option for developing novel and efficient treatments for RA. This approach offers advantages over conventional medications, perhaps resulting in fewer adverse effects. In summary, expanding the discussion to compare Pn@ZnONPs with other green-synthesized nanoparticles and traditional therapies would place this research in a broader context and underscore these nanoparticles' unique benefits and potential.

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NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Shradha Devi Dwivedi conducted the experiments, collected data, and wrote the original manuscript draft. Manju Rawat Singh conceptualized the work, arranged resources, and supervised and interpreted the results. Deependra Singh led the team, oversaw the work, and contributed to the final draft. All the authors reviewed the draft.

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