Anti-inflammatory and Antioxidant Properties of Green Synthesized Chitosan Nanoparticles -An Invitro Study

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ABSTRACT
Important roles in the pathophysiology of many diseases include the inflammatory response and oxidative stress. This work uses extracts from Azadirachta indica and Solanum xanthocarpum to examine the anti-inflammatory and antioxidant characteristics of green produced chitosan nanoparticles (CNPs). These CNPs were evaluated for their potential to stabilize cellular membranes and inhibit protein denaturation, both of which are markers of inflammation. Additionally, the antioxidant properties were assessed through radical scavenging activities. The study found that CNPs exhibited significant anti-inflammatory and antioxidant effects, suggesting their potential as therapeutic agents in the management of inflammatory conditions and oxidative stress-related disorders.

1. Introduction

The use of medicinal plants is becoming more and more popular in the field of endodontics. The cytotoxic effects of commercially available intracanal medications and their poor ability to eradicate bacteria from dentinal tubules are the primary factors behind this trend [9]. *Solanum xanthocarpum* (Kantakari), a commonly used medicinal plant, contains saponins, sterols, alkaloids, flavonoids, and glycosides. This plant has been extensively explored for its anti-fungal, anti-inflammatory, antimicrobial, and other pharmacological properties [10]. *Azadirachta indica* (Neem) is well-known for its potential effects, which include free radical scavenging, detoxification, DNA repair, cell cycle change, anti-inflammatory, and the ability to affect numerous signalling pathways [11]. Highlighting the therapeutic benefits of these kinds of natural medicines is becoming increasingly necessary.

One of the most significant requirements for intracanal medicament is stability and sustained release of the medicament over an extended period of time. An alternative to traditional drug release is the use of nanoparticles (NPs) as a potential drug carrier [12]. Chitosan nanoparticles are frequently utilized in drug delivery systems because they are biodegradable, biocompatible, cationic, non-toxic, and enable a slow and controlled release of intracanal medicament [13].

The literature provides limited details on the use of herbal compounds as intracanal medicaments. Its effectiveness as a vehicle when combined with chitosan nanoparticles is less well-established.

A number of diseases, including cardiovascular disease, cancer, diabetes, and neurodegenerative disorders, are influenced by the interrelated processes of inflammation and oxidative stress. The body's reaction to pathogens, damaged cells, or irritants is inflammation, which is defined by the release of pro-inflammatory mediators such cytokines and reactive oxygen species (ROS). Chronic inflammation can result in tissue damage and a number of disorders, despite the fact that inflammation is a protective mechanism.

On the other hand, an imbalance between the body's capacity to detoxify these reactive intermediates and the generation of ROS leads to oxidative stress. Overexposure to reactive oxygen species (ROS) can harm lipids, proteins, and DNA in cells, which can result in a number of degenerative diseases. Antioxidants are known to have a crucial function in preventing oxidative damage and neutralizing reactive oxygen species (ROS).

Using nanotechnology to create novel therapeutic molecules that specifically target oxidative stress and inflammation is a promising strategy. The natural polysaccharide chitosan, which is derived from chitin, has gained popularity due to its non-toxic, biodegradable, and biocompatibility characteristics. Numerous biological uses of chitosan nanoparticles (CNPs) have been investigated, including tissue engineering, medication delivery, and wound healing.

Because it is sustainable and environmentally friendly, the green synthesis of nanoparticles utilizing plant extracts has become more and more popular in recent years. The medicinal plants Solanum xanthocarpum (Kantakari) and Azadirachta indica (Neem) are prized for their antioxidant, antibacterial, and anti-inflammatory qualities. Using extracts from these plants, the current work intends to investigate the anti-inflammatory and antioxidant capabilities of green produced CNPs.

2. Materials and Methods

2.1 How Plant Extracts Are Made

Solanum xanthocarpum seeds and fresh bark of Azadirachta indica were milled into a fine powder after being dried in the shade. 10g of fine powder was heated to 80°C for 25 minutes while being constantly stirred in 100 mL of distilled water to prepare aqueous extracts of Azadirachta indica and Solanum xanthocarpum. Whatman No. 1 filter paper was used to filter the produced aqueous extracts after they had been allowed to cool to room temperature. Cold storage was maintained for the sterile extracts.

2.2 Chitosan nanoparticles (CNPs) produced by green synthesis

A solution of 1% (w/v) acetic acid was made using chitosan (>90% purity and viscosity 60-300) from BIO BASIC INC.A pH adjustment of 1 N NaOH was applied. The chitosan was completely dissolved by stirring the liquid for twenty-four hours. A mixture of 10 mL of the 1:1 v/v chitosan solution and 10 mL of the prepared extracts of Azadirachta indica and Solanum xanthocarpum was used. CNPs were obtained by shaking the mixture at 110 rpm for 60 minutes at 50 °C. The suspension of CNPs was freeze-dried and centrifuged at 10,000×g for ten minutes after incubation (1).

2.3 Characterization of CNPs

In order to verify the formation and examine the morphology, structure, and elemental composition of the nanoparticles, the synthesized CNPs were characterized using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX).

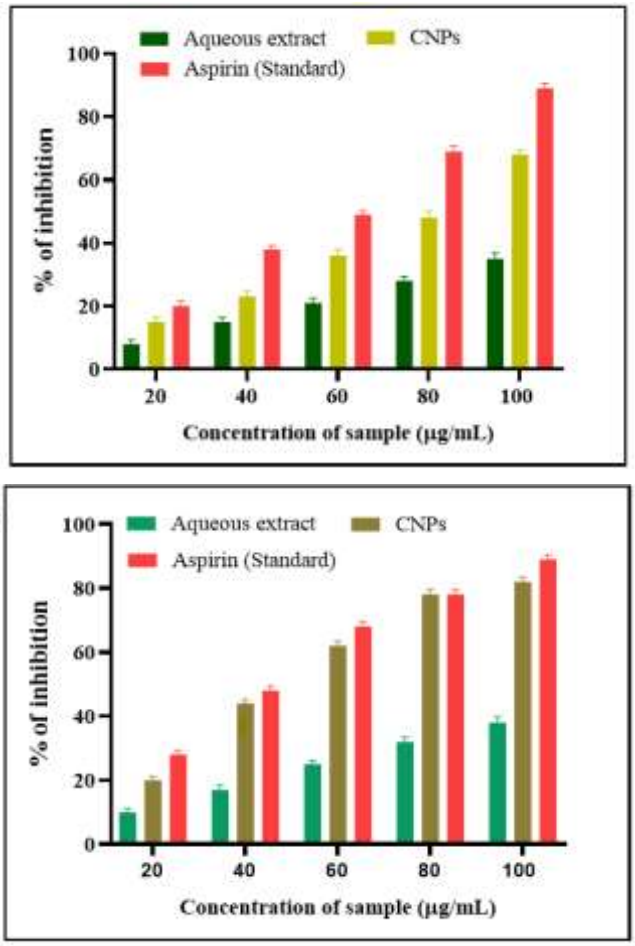
2.4 Anti-inflammatory Activity

2.4.1 Protein Denaturation Assay

An albumin denaturation experiment was used to assess CNPs' capacity to prevent protein denaturation. To a combination of 2.8 mL of phosphate buffer and 0.2 mL of fresh egg albumin, different doses of CNPs (10–50 µg/mL) were added. For thirty minutes, the reaction mixture was incubated at 55°C. Calculating the % inhibition of protein denaturation required measuring the absorbance at 660 nm.

2.4.2 Membrane Stabilization Assay

Human red blood cells (RBCs) were used to measure the membrane stabilizing activity of CNPs. RBCs were treated in a phosphate-buffered saline solution with varying doses of CNPs (20–100 µg/mL)(2). After the combination was centrifuged, the supernatant was taken out and measured at 540 nm using spectrophotometry. Based on the absorbance measurements, the percentage of membrane stabilization was computed.



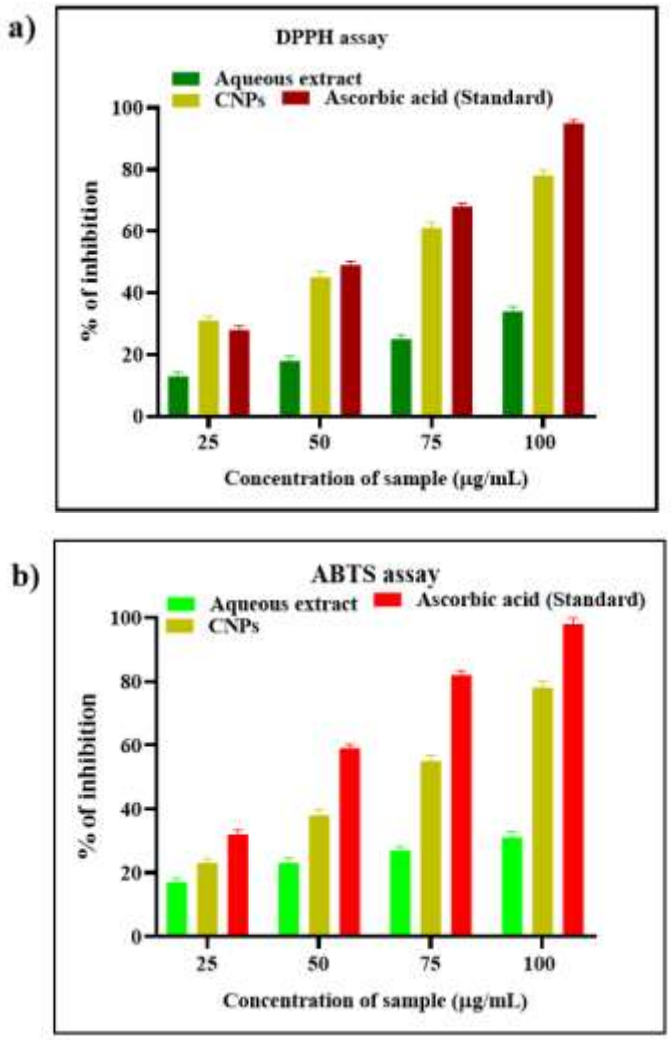
2.5 Antioxidant Activity

2.5.1 DPPH Radical Scavenging Activity

Activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Test samples (2 ml) with different concentrations (20-100µg/ml) were placed in test tubes, and 2 ml of DPPH (2%) was added. The sample concentration that provided 50% inhibition may have been found by charting the percentage of inhibition against sample concentration. Anti-Bis(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Properties Two milliliters of the ABTS radical solution (20–100µg/ml) were mixed with one milliliter of the green synthetic CNPs. Subsequently, the mixture was incubated in the dark at room temperature for exactly 10 minutes. The percentage of ABTS radical inhibition brought about by the green-generated CNPs' scavenging function was ascertained (3).

2.5.2 ABTS Radical Scavenging Activity

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity was evaluated. At different concentrations, CNPs were added to the ABTS radical solution, and the combination was then let to sit at room temperature for ten minutes. At 734 nm, the absorbance was measured, and the % inhibition was computed.



3. Results

3.1 Characterization of CNPs

The FTIR analysis confirmed the presence of functional groups characteristic of chitosan and plant extracts, indicating successful synthesis. The XRD pattern showed peaks corresponding to the crystalline structure of CNPs, while SEM-EDX analysis revealed the presence of elements such as oxygen, carbon, and nitrogen, confirming the composition of the nanoparticles.

One of the most important methods for describing the structural characteristics of green synthesized CNPs is XRD. Using a Ni-filtered Cu Kα radiation source and a Bruker D2 Phaser second Gen diffractometer, X-ray diffraction studies were performed at room temperature. The generator was using 30 mA of current at 10 kV. Data were gathered between 0 and 90 at a scanning rate of 2°/min for 2θ.

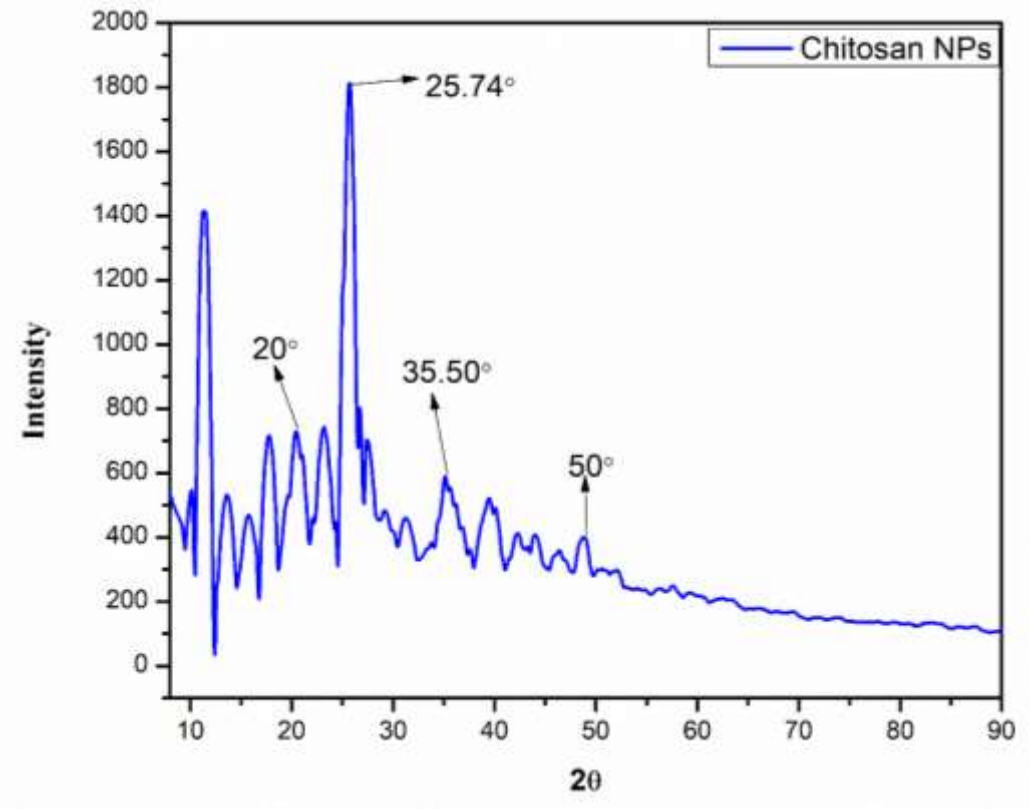
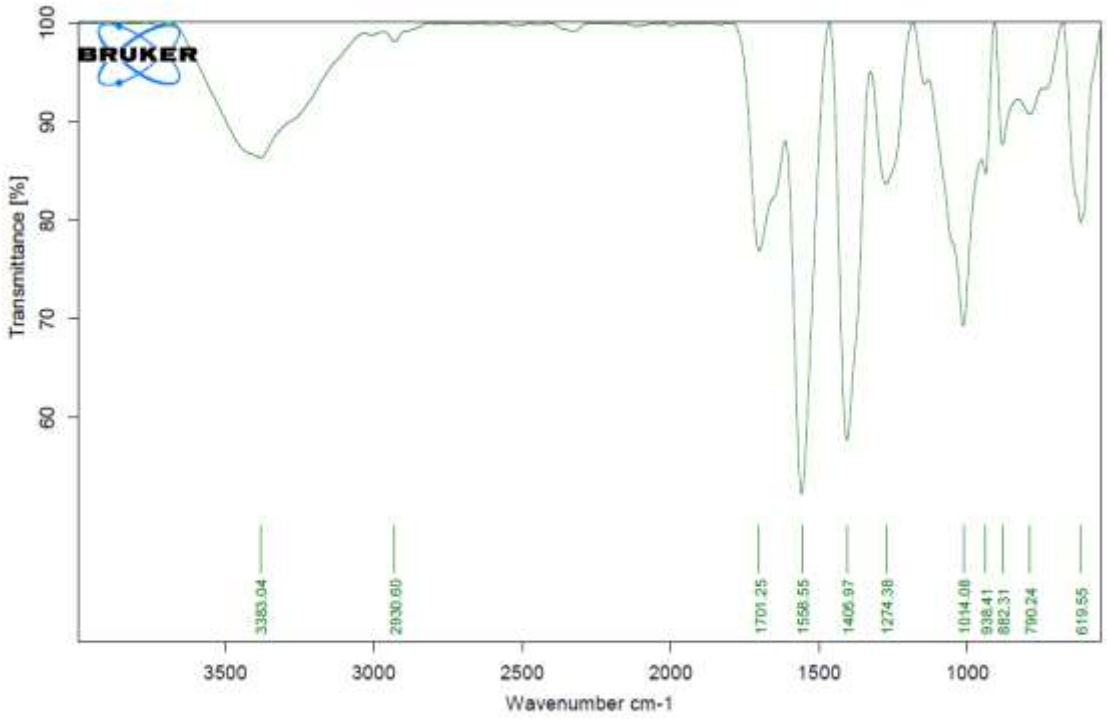


Figure 2: FTIR spectrum analyses of green synthesized CNPs



SEM-EDX

SEM-EDAX was performed with an accelerating voltage of 15 KeV on green synthesized CNPs. The results showed the presence of a total of 41.5 wt.% (o) oxygen, 40.5 wt.% (C) carbon, 17.5 wt.% (Na) sodium, 0.2 wt.% (Ca) calcium, 0.2 wt.% (k) potassium, and 0.1 wt.% (Cl) chlorine. Figure 2 confirms the presence of CNPs.

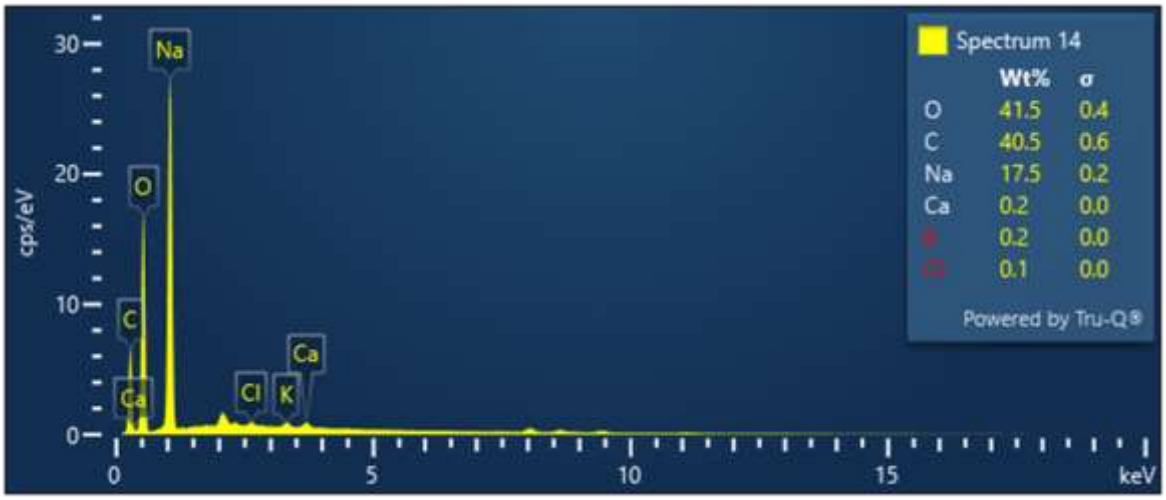


Figure 3: Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDAX) of green synthesized CNPs.

3.2 Anti-inflammatory Activity

3.2.1 Protein Denaturation Assay

CNPs exhibited a dose-dependent inhibition of protein denaturation. At a concentration of 100 µg/mL, CNPs showed 78% inhibition, which was comparable to the standard drug aspirin, which showed 86% inhibition.

3.2.2 Membrane Stabilization Assay

The membrane stabilization assay revealed that CNPs stabilized RBC membranes effectively. At a concentration of 100 µg/mL, CNPs demonstrated an 87% inhibition, similar to the standard drug aspirin (91%).

4. Discussion

Some plants, such Solanum xanthocarpum and Azadirachta indica, include a range of physiologically active chemicals that contain antibacterial properties. Green manufactured nanoparticles' biological compatibility is derived from their phytochemicals. Because they have a higher surface to volume ratio than their parent chitosan material, chitosan nanoparticles (CNPs) exhibit strong antibacterial activity [19]. We examined the effects of a herbal intra-canal medication based on chitosan on the survival and viability of dental pulp stem cells in this work.

The past few decades have seen an evolution in antimicrobial treatment resistance as microbial diseases grow more resistant to commercially available antimicrobial drugs. E. faecalis [36.6%] was the most common microbe in teeth with root canal therapy, followed by C. albicans (20%), according to Pourhajibagher M et al.'s culture-dependent methods [20].

Intracanal chitosan had good antibacterial effectiveness against biofilms of Candida albicans and Escherichia faecalis, as reported by Thienngern P and colleagues [21]. The chitosan + propylene glycol (PG) paste was discovered to be substantially more effective than calcium hydroxide at killing E. faecalis (4).

According to Aliasghari A et al.'s investigation of chitosan and chitosan nanoparticles [22], nano-chitosan considerably reduced S. mutans development more effectively than chitosan (P>0.05). He added that the growth of S.mutans was suppressed by 5 mg/ml of Ch-NPs, with an inhibition zone measuring 15 mm in diameter. In a related investigation, Parolia A et al. [23] found that while chitosan by itself was ineffective against E faecalis, it was incredibly powerful when mixed with CPN.

Similarly, in the current study green synthesized CNPs also exhibited considerable activity against *S. mutans*, *E. faecalis*, and *C. albicans* and a maximum zone of inhibition against *E. faecalis*. The results were in accordance with Del Carpio-Perochena et al [29], who also found that the incorporation of nanoparticles into intracanal medications could potentially be beneficial since they have the ability to kill bacteria over time(5).

The viability of green synthesized CNPs was evaluated on DPSCs, which showed a significantly higher percentage at various concentrations of 10 mg/ml, 20 mg/ml, 40 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml.

Similarly, the results of the current study are in agreement with the findings of Gendly et al [30].

Using SEM, the size, shape, and morphology of green synthesized CNPs were visualized in the current study, showing agglomerated particles in contrast to Ducret et al finding rounded aggregates in chitosan hydrogels [26].

EDX spectrum analysis of biosynthesized chitosan nanoparticles by El-Naggar et al revealed that carbon, oxygen, and nitrogen are the main elements of chitosan nanoparticles [27]. Oxygen, carbon, sodium, calcium, potassium, and chlorine were found in the current study, which is similar to observations made by other researchers [28].

The results of this study show that green synthesized CNPs exhibit dose-dependent anti-oxidant, anti-inflammatory, and cytotoxic properties. These results are in line with studies by Lee et al and Feuangthit et al [29-30].

This study was limited by the fact that nanoparticles may cause unanticipated problems and it failed to examine NP coating stability. Additionally, future clinical trials will be needed to examine the efficacy of these unique intracanal green synthesized CNPs in a clinical setting.

According to the study, green synthesized chitosan nanoparticles made using extracts from Azadirachta indica and Solanum xanthocarpum have strong anti-inflammatory and antioxidant qualities. CNPs are interesting candidates for therapeutic uses in inflammatory situations because of their ability to significantly reduce inflammation, as evidenced by their prevention of protein denaturation and stabilization of RBC membranes.

According to the antioxidant tests, CNPs have the ability to effectively scavenge free radicals, suggesting a potential protective role against damage caused by oxidative stress. Since oxidative stress is a major factor in the management of chronic inflammatory illnesses, the anti-inflammatory and antioxidant qualities of CNPs may be helpful.

5. Conclusion

Significant anti-inflammatory and antioxidant properties are shown by green manufactured chitosan nanoparticles made with extracts from Azadirachta indica and Solanum xanthocarpum. According to these results, CNPs may one day be created as therapeutic agents to treat conditions linked to oxidative stress and inflammation. To investigate the therapeutic uses of these nanoparticles, more in vivo research is necessary.

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