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Anti Inflammatory Activity Of Chitosan Nanoparticles With Chlorhexidine- An Invitro Study

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ABSTRACT

Introduction: One of the primary goals of root canal therapy is to relieve the discomfort associated with permanently injured pulps. Root canal therapy is the sole option for saving a tooth with irreversible pulpitis. Nonsteroidal anti-inflammatory medications can be used to treat inflammation (NSAIDs).All NSAIDs have the potential to induce severe adverse effects such as stomach ulcers, gastrointestinal bleeding, renal failure, heart attacks, and strokes.The aim of this study is to evaluate the anti-inflammatory activity of chitosan nanoparticles with chlorhexidine.

Materials and Methods: The chitosan was obtained and plain chitosan with chlorhexidine, chitosan nanoparticles with chlorhexidine was prepared. The anti-inflammatory activity for Chitosan nanoparticles was tested by the following convention proposed by Muzushima and Kabayashi. Diclofenac Sodium was used as the standard.DMSO is used as a control. Percentage of protein denaturation was determined.

Results: Anti-inflammatory activity of plain chitosan and nano chitosan showed an incremental pattern with increase in concentration, where the percentage of inhibition is high at 50µl. Its activity increases with increase in dosage. Nanochitosan with chlorhexidine shows higher anti-inflammatory activity when compared to plain chitosan.

Conclusion: The use of this novel irrigant in the field of endodontics would reduce the postoperative pain, the use of NSAID's and overall improve the experience of the patients undergoing endodontic treatment in the future.

Keywords: *Endodontic Irrigants, Root Canal Treatment,Chitosan, Chitosan nanoparticles , Natural Irrigant, Anti inflammatory*

INTRODUCTION

One of the primary goals of root canal therapy is to relieve the discomfort associated with permanently injured pulps. Clinically, pain in a tooth with irreversible pulpitis can range from moderate to severe, and it can be spontaneous or intermittent. Root canal therapy is the sole option for saving a tooth with irreversible pulpitis. However, some individuals may have mild to extreme discomfort after root canal therapy. As a result, pain management is an essential component of root canal therapy before, during, and after intervention. In some endodontic procedures, single-visit root canal treatment is prevalent.(1) One of the major problems with this method, however, has been the risk of postoperative discomfort.(2)(3)(4)

Inflammation is a combination of interactions between soluble substances and cells that can occur in any tissue in response to autoimmune, traumatic, viral, toxic, or post-ischemic damage.(5–7) Nonsteroidal anti-inflammatory medications can be used to treat inflammation (NSAIDs).(8–10) The most often used category of medicines are NSAIDs. All NSAIDs have the potential to induce severe adverse effects such as stomach ulcers, gastrointestinal bleeding, renal failure, heart attacks, and strokes.(11–14)

Prophylactic preoperative analgesics and corticosteroids, the use of long-acting local anaesthetics, crown-down root canal preparation, and occlusal reduction are all strategies for post-operative pain treatment.(15–18) The impact of irrigating solutions used during root canal therapy in reducing post-operative discomfort is unknown. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most used drugs to treat pain and inflammatory conditions.(19,20) They do, however, have frequent side effects such as ulcers, bleeding, and renal problems.While some studies have found that specific kinds and concentrations of irrigating solutions reduce post-operative pain, others have found no change in post-operative pain with the varied irrigating solutions(21).(22) The aim of this study is to evaluate the anti-inflammatory activity of chitosan nanoparticles with chlorhexidine.

MATERIALS AND METHODS

Chitosan Synthesis

For this study the chitosan powder was obtained from dried exoskeleton of marine shrimps.

Preparation of Chitosan Nanoparticles

500 mg of chitosan was incorporated in 50 ml of 1% acetic acid solution and agitated for 25 minutes in room temperature at 1000 rpm until the liquid turns clear. The resultant solution was sonicated before being titrated with NaOH or HCL solution calibrated to pH5 and filtered through 0.2 mesh. 5 mL of nano-magnetic solution was added to 75 mL of deionized water and sonicated for 10 minutes for the coating procedure. The solution of chitosan was then added and sonicated for 5 minutes. The solution that presented was clear.

Preparation of Nanochitosan with Chlorhexidine solution

50ml of 2% Chlorhexidiene was added to 50 ml of the prepared nano chitosan solution. The resulting solution was sonicated for 10 mins until the solution was clear.

Synthesis of plain chitosan nanoparticles with chlorhexidine

The amount of 500 mg of chitosan was dissolved in 50 ml of 1% acetic acid solution and agitated for 25 minutes at room temperature at 1000 rpm until the solution turned clear. The resultant solution was sonicated before being titrated with NaOH or HCL solution calibrated to pH5 and filtered through 0.2 mesh.50ml of 2% Chlorhexidiene was added to 50 ml of the prepared chitosan solution. The resulting solution was sonicated for 10 mins until the solution was clear.

Anti Inflammatory Activity

Albumin Denaturation Assay

The anti-inflammatory effect of Chitosan nanoparticles was evaluated using the Muzushima and Kabayashi protocol, with minor modifications. 0.05 mL Chitosan nanoparticles of varied fixation (10L,20L,30L,40L,50L) were added to 0.45 mL bovine serum albumin (1% aqueous solution), and the pH of the mixture was adjusted to 6.3 with a little amount of 1N hydrochloric acid. These samples were incubated at room temperature for 20 minutes before being heated in a water bath at 55 °C for 30 minutes. After cooling the samples, the absorbance at 660 nm was measured spectrophotometrically. The standard was diclofenac sodium. As a control, DMSO is employed. The percentage of protein

denaturation was calculated using the following equation: % inhibition= Absorbance of control-Absorbance of sample×100 Absorbance of control

RESULTS

Anti-inflammatory activity of plain chitosan and nano chitosan showed an incremental pattern with increase in concentration, where the percentage of inhibition is high at 50µl. Its activity increases with increase in dosage. Nanochitosan with chlorhexidine shows higher anti-inflammatory activity when compared to plain chitosan.

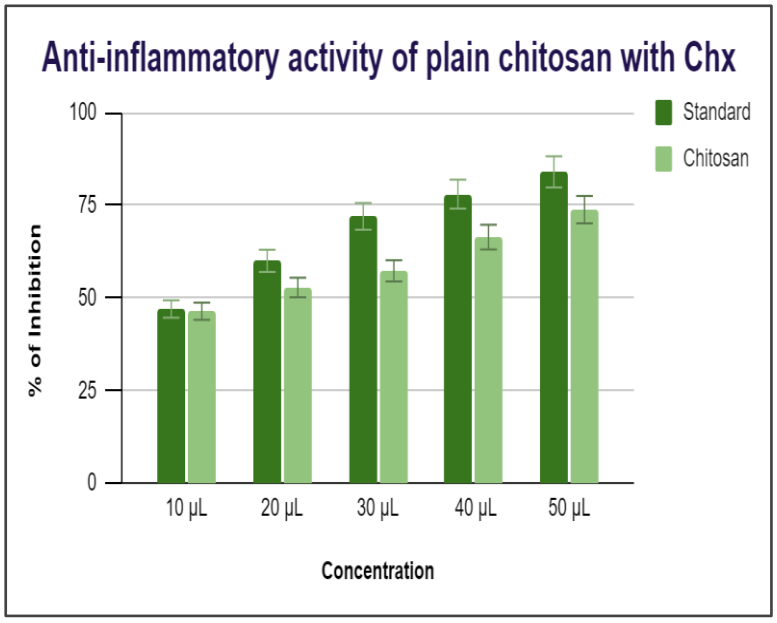

FIG 1: Anti-inflammatory activity of plain chitosan showing an incremental pattern with increase in concentration, where the percentage of inhibition is high at 50µl. Its activity increases with increase in dosage.

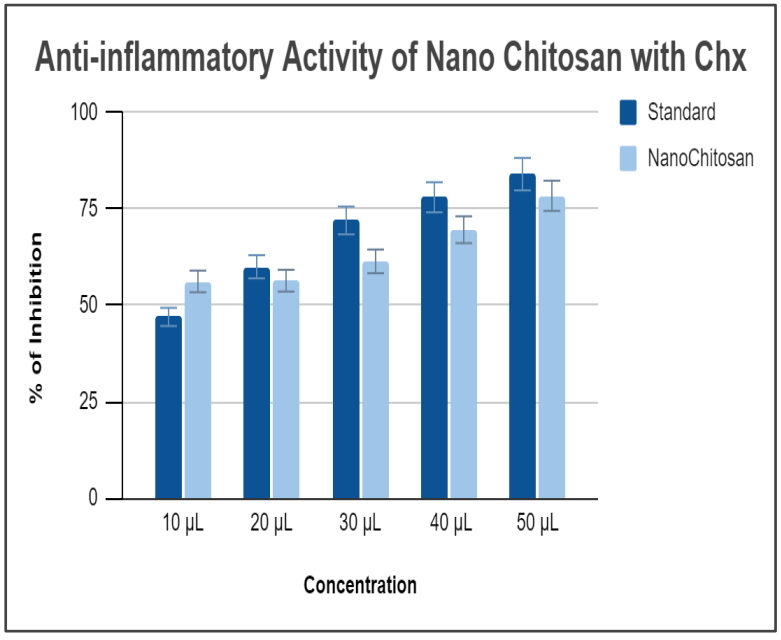

FIG 2: Anti-inflammatory activity of chitosan nanoparticles showing an incremental pattern with increase in concentration, where the percentage of inhibition is high at 50µl. Its activity increases with increase in dosage

J Popul Ther Clin Pharmacol Vol 30(14):e26–e32; 04 May 2023.

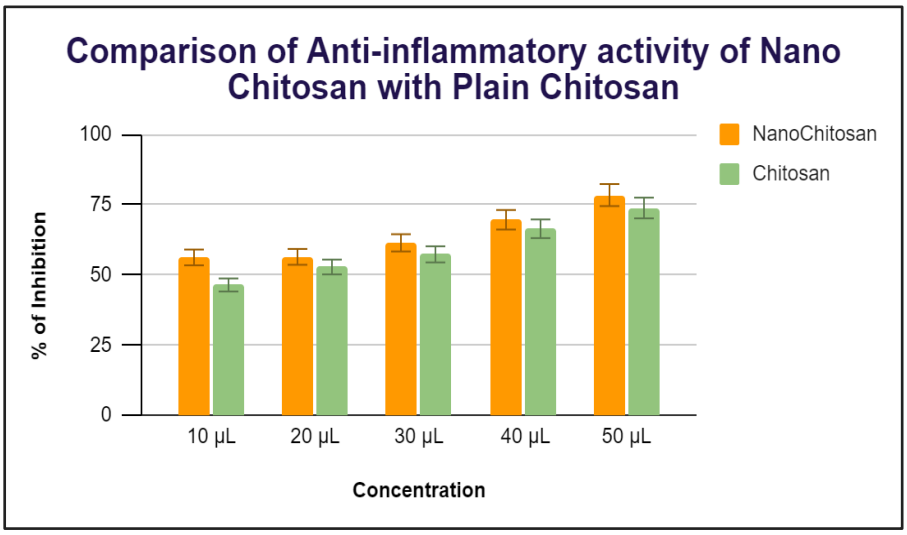

FIG 3: Nanochitosan with chlorhexidine shows higher anti-inflammatory activity when compared to plain chitosan. Its activity increases with increase in dosage.

DISCUSSION

The main objective of this study was to assess the anti-inflammatory activity of a novel irrigant plain chitosan with chlorhexidine and nano chitosan with chlorhexidine. The results of the study demonstrated that both t plain chitosan with chlorhexidine and nano chitosan with chlorhexidine showed increased anti-inflammatory activity when compared to the control group.Nanochitosan with chlorhexidine shows higher anti-inflammatory activity when compared to plain chitosan. The anti-inflammatory activity increased with the increase in dosage.

Inflammation is the initial defensive reaction of the human body to infection or damage, driven in a tissue compartment by a specialised collection of immune and inflammatory cells with the goal of restoring structural and functional integrity following exposure to an unfavourable stimulus.(23) Numerous studies on the anti-inflammatory and pro-inflammatory activities of chitosan and its derivatives have been conducted. Davydova and colleagues investigated the anti-inflammatory activity of chitosan with high (MW: 115 kDa) and low (MW: 5.2 kDa) molecular weights, and both chitosan samples showed an increased induction of anti-inflammatory IL-10 cytokine in animal blood as well as suppression of colitis progression.(24,25) The scientists determined that the predominant contribution of chitosan to anti-inflammatory action was driven by structural features inside its molecule.(24,26) Oliveira et al. investigated the anti-inflammatory and pro-inflammatory effects of chitosan film.(27)

Many studies have proved that chitosan has anti-inflammatory properties, despite the fact that the specific mechanism is not entirely known. Chung et al. examined the anti-inflammatory efficacy of two kinds of chitosan with high (70 kDa) and low (MW: 1 kDa) molecular weight. The strong inhibitory impact of low molecular weight chitosan on IL-4, IL-13, and TNF-cytokines was discovered, indicating the possibility for reducing allergic inflammation in vivo. Li et al. postulated a mechanism for lipopolysaccharide-induced NF-B-dependent inflammatory gene expression in COS, which was linked to decreased NF-B nucleus translocation.(28)(29) NF-B is a transcription factor that has a role in mediating proinflammatory responses. Ma et al. (30) conducted a similar investigation, and the favourable impact of chitosan pretreatment on the reduction of LPS-induced NF-B and AP-1 activation in macrophages was explained. The findings demonstrated that chitosan is a potential inhibitor of NF-B and AP-1-mediated inflammatory responses in macrophages by suppressing LPS-induced c-fos (proto-oncogene) production in macrophages in a concentration-dependent approach.(31) (32) Li et al. observed proinflammatory and inflammatory actions of COS (obtained by enzymatic hydrolysis with chitosanase) on cytokines.(33) The researchers looked at the levels of proinflammatory cytokines like IL-1, IL-6, and TNF- and anti-inflammatory cytokines like IL-2 in a mouse

osteoarthritis (OA) model. The serum expression of proinflammatory cytokines was reduced, while anti-inflammatory activity was increased.(34)(35)

Chitosan and its metabolites are being researched intensively for medicinal and pharmacological purposes. In situ and in vitro tests have demonstrated their distinct and appealing bioactivities.(36) They are easily produced in nature by low-cost alkaline deacetylation of chitin processes.(12) Given their numerous advantages, the curiosity in chitosan and its derivatives may continue to grow. Commercial exploitation of materials that contain chitosan and its derivatives is not yet widespread or simple to come by (37). Our team has extensive knowledge and research experience that has translated into high quality publications (38–47). More research investigations on natural polysaccharides with beneficial bioactivities, including the mechanism of bioactivities of chitosan molecules, may be completed in the future.

CONCLUSION

The use of this novel irrigant in the field of endodontics would reduce the postoperative pain, the use of NSAID's and overall improve the experience of the patients undergoing endodontic treatment in the future.

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