Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 7s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Fabrication of a novel wound healing gel using Azadirachta indica and Chamaecostus cuspidatus formulation mediated calcium oxide nanoparticles

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Cite this paper as: T Arun Raaj, Subhashree. R, S Rajeshkumar, Thiyaneswaran N, (2025) Fabrication of a novel wound healing gel using Azadirachta indica and Chamaecostus cuspidatus formulation mediated calcium oxide nanoparticles. *Journal of Neonatal Surgery*, 14 (7s), 437-442.

ABSTRACT

Background: Wound healing has been a major focus of both traditional and modern medicine, with herbal medicines playing an important role in stimulating tissue repair and reducing inflammation. Herbal-based wound healing gels have grown in popularity due to their natural healing capabilities and lower risk of side effects compared to standard synthetic treatments. Usually, a blend of herbs with therapeutic properties, including neem and insulin plant, are used to make these gels. These herbs have anti-inflammatory, antibacterial, and antioxidant qualities that help speed up the healing process, relieve pain, and prevent infection.

Aim: The goal of the current work is to make a gel based on *Azadirachta indica and Chamaecostus cuspidatus* mediated with calcium oxide nanoparticles for the post operative healing after a dental procedure.

Materials and Methods: The solution contains calcium oxide nanoparticles infused in *Azadirachta indica and Chamaecostus cuspidatus* . The gel obtained was subjected to an anti-inflammatory test and membrane stabilisation.

Results: The formed gel exhibited membrane stabilising activity at 50µL (82 %).They also show to be biocompatible and have anti-inflammatory properties.

Conclusion: This novel synthesised wound healing gel may help to reduce the inflammation in the soft tissues and have good membrane stabilisation which in turn helps the patients undergoing dental procedures.

Keywords: *Wound healing gel, Herbal extract, Azadirachta indica, Chamaecostus cuspidatus, calcium oxide nanoparticles.*

1. INTRODUCTION

For the past 50 years, prominent scientists from all around the world have developed an interest in nanomedicine. Working on the development of nanomaterials with a size range of 1–100 nm both in the academic and industrial worlds(1). One of the topics of nanotechnology that is growing is the research into nanomedicine. Due to its unique applicability in various fields, the synthesis of various nanomaterials has received considerable attention.(2) Diabetes is now highly common around the world, and this presents a significant issue for public health.(3) Due to the large number of people who have diabetes worldwide, the disease has a significant financial impact on global health concerns. Diabetes is a serious metabolic condition that used to be regarded as one of the leading killers in the globe.(4) According to reports, this deadly disease would likely afflict 552 million people worldwide by the year 2030.(5) Diabetes is a chronic illness characterised by high blood sugar

levels that are suitable for the loss of pancreatic b-cells, which produce insulin, in type I diabetes and the loss of insulin responsiveness in target tissues, such as muscle and adipose, in type 2 diabetes. Most patients with diabetes have type II diabetes. (6)

The wound healing process in diabetic patients can be slower and more complicated than in non-diabetic patients.(7,8) This is because diabetes can cause damage to the nerves and blood vessels, which can lead to reduced blood flow and sensation in the affected area.(9) As a result, diabetic patients are at higher risk for developing chronic wounds and complications such as infections, delayed healing, and even amputation.

Plant extracts have proven to be a cost-effective method for creating nanoparticles, and their application opens up a wide range of non-toxic nanoparticle synthesis options(10). Nanoparticles of calcium oxide (CaO) have been used in a variety of processes, including adsorption, water filtration, catalysis, and the eradication of bacteria.(11) CaO is of special relevance due to its reputation as a substance that is safe for both humans and animals.(12) Several papers describe chemical processes used to create calcium oxide nanoparticles. There are, however, surprisingly few reports of biogenic syntheses in the literature. The extracts of *Azadirachta indica* and *Chamaecostus cuspidatus* were used in this study to create CaO nanoparticles. The prepared nanoparticles were subjected to anti-inflammatory test and membrane stabilisation.

2. MATERIALS AND METHODS

The in-vitro study was conducted in Gold Lab (Nanobiomedicine lab), Saveetha dental college, Chennai in January 2023. In conventional wound healing Gel, CaO NPs with herbal extract of *Azadirachta indica* and *Chamaecostus cuspidatus* plant were added.

1. Chemical and Plant Materials :

CaO was acquired from SRL Chemicals and used in this study. Without additional purification, all of the reagents were utilised. The powder of the *Azadirachta indica* and *Chamaecostus cuspidatus* plant were prepared at a gold lab in saveetha dental college in Chennai, Tamil Nadu, India.

2. Preparation of plant formulation :

1mg each of *Azadirachta indica* leaf powder and *Chamaecostus cuspidatus* extract powder is combined with 100 ml of distilled water. On a heating mandrel, this solution was heated for 10 to 15 minutes at 50 to 60 degree Celsius. Filtration was used to purify the solution using Whatman filter paper no. 1. The supernatant was collected in a conical flask, and the residue that had been gathered in the filter paper was discarded.

3. Synthesis of nanoparticles:

In the process of making nanoparticles, 50 ml of distilled water was combined with 0.511 grammes of calcium carbonate. 50 ml of filtered plant extract was added to this solution and stirred on an orbital shaker for 24hrs to ensure that all the particles were mixed thoroughly. On a magnetic stirrer, the reaction mixture was constantly swirled until a colour change was visible. By using UV-vis spectroscopic spectroscopy, the formation of nanoparticles was tracked every hour. Calcium oxide nanoparticle's colour change on a UV-vis spectrophotometer indicated that they formed at a certain wavelength. The mixture was collected in 5 test tubes after spectroscopic analysis, and the calcium oxide nanoparticles were separated from the solution using centrifugation for 20 minutes.

4. Preparation of Carbopol Gel :

5g Carbopol was mixed with 50mL of distilled water. It was mixed very well without any air bubbles.

5. Preparation of Titanium dioxide gel :

5mL of carbopol gel was added in calcium oxide nanoparticles and it was mixed very well.

6. Anti-inflammatory activity

Egg Albumin denaturation assay:

A 5 mL solution was created by mixing 0.2 mL of egg albumin that was isolated from a hen's egg with 2.8 mL of freshly manufactured phosphate-buffered saline (pH - 6.3). Different quantities of calcium oxide nanoparticles (10µL, 20µL, 30µL, 40µL, and 50µL) were synthesised for Azadirachta indica and Chamaecostus cuspidatus. Sodium diclofenac served as the positive control. Subsequently, the combinations were cooked for 15 minutes at 37ºC in a water bath. The samples were then allowed to cool to room temperature, and the absorbance at 660 nm was determined.

7. Human red blood cells Membrane stabilisation assay:

The in vitro anti-inflammatory action of the extract is examined using a technique for stabilising the membrane of human red blood cells (HRBCs), in accordance with Gandhidasan et al. The quantity of RBC lysis was utilised to gauge the anti-inflammatory activity of standard NSAIDs. HRBC membrane 207 functions similarly to the lysosomal membrane. In the

event that the extract causes it to solidify, the lysosomal membrane will follow suit. Using a spectrophotometer with a 560 m range, the suspension's haemoglobin content was estimated. The major criterion for exclusion is the use of NSAIDs during the two weeks before the study; blood was provided by healthy volunteers. The use of Na-Oxalate halted the haemorrhage. All blood samples were stored for 24 hours at 4 °C before being used. At 5:00 p.m., the supernatant was removed by centrifugation for five minutes. After washing in sterile saline solution (0.9% w/NaCI), centrifugation was done for five minutes at 2500 rpm. After the three rounds of supernatant washing, the packed cell volume was calculated. A 40% solution (w/v) of phosphate-buffered saline (10 mM, pH 7.4) was mixed with 1 L of distilled water, 0.26 g of NaHPO4.2H,O, 1.15 g of Na,HPO4, and 9 g of NaCl to facilitate the reconstitution of cellular components.

Heat-induced Haemolysis:

A portion of the isotonic buffer containing an ethanol solution of the crude extract at 50 g/mI, 100 g/mI, 200 g/mI, 400 pg/ml, and 800 g/ml was added to two sets of centrifuge tubes in duplicate. The same number of cars were added to another tube, which served as a control. 50 pl of RBC suspension were added to each test tube, and the test tubes were gently mixed by inverting them. One pair of tubes was incubated in a water bath at 54 °C for 20 minutes. The other pair was maintained at 5 °C in an ice bath. A spectrophotometer at 560 mm was used to measure the absorbance after the mixture was centrifuged for five minutes at 5000 rpm at 540 nm. The reference standard was 200 g/ml of acetyl salicylic acid (ASA).

The following equation was used to compute the % inhibition of hemolysis:

$$\%\text{inhibition of hemolysis} = 100 \times \frac{1 - OD2 - OD1}{OD3 - OD1}$$

Where, OD, - Iest Sample Unheated; OD, - Test Sample Heated and OD - Control Sample Heated.

3. RESULTS AND DISCUSSION

Figure 1: Preparation of herbal extract: powder of *Azadirachta indica* and *Chamaecostus cuspidatus* plant and calcium carbonate powder

Figure 2: 100 ml of distilled water mixed with plant extract. This solution is then heated for 10-15 mins on a heating mandrel.

Figure 3: filtration of plant extract

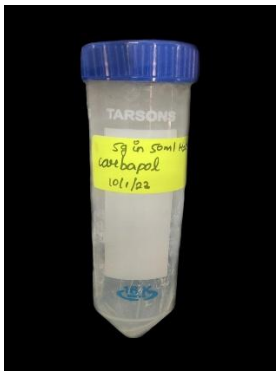

Figure 4: Preparation of newly formulated wound healing gel

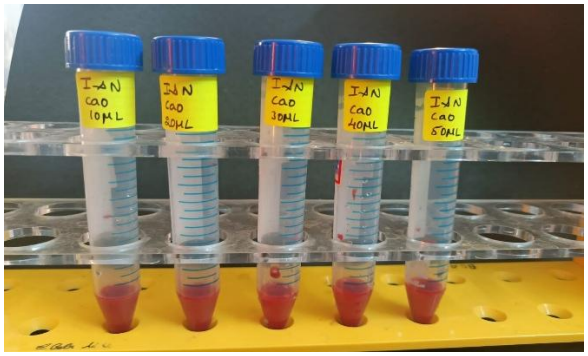

Figure 5: Membrane stabilisation assay activity of green synthesised CaO NPs mediated wound healing gel.

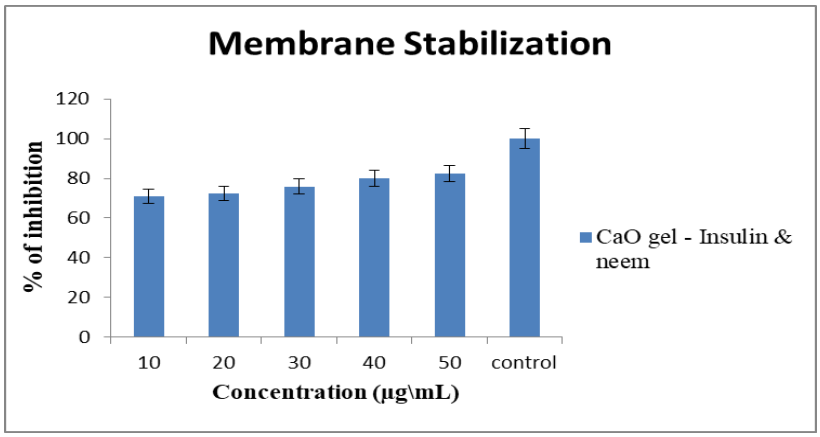

Figure 6: Result of Membrane stabilisation of green synthesised CaO NPs mediated wound healing gel

Membrane stabilisation activity :

The 50µL (80%) showed the highest amount of membrane stabilising action. Figure 6 illustrates the moderate membrane-protecting activity observed at all concentrations (i.e., >70% suppression of hemolysis). These biosynthesized nanoparticles demonstrated membrane stabilising action without impairing the integrity of red blood cells membrane by not causing denaturation of the protein bovine serum albumin (BSA), hemolysis, or heat-induced hemolysis. There was no hemolytic impact demonstrated by the quercetin-CaONPs. Similar to a normal medicine, they demonstrated 82.6% suppression of bovine serum albumin (BSA) denaturation and 95.3% red blood cells (RBCs) membrane stabilisation activity, proving their anti-inflammatory actions.

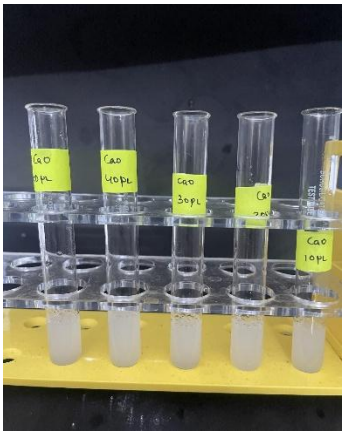

Figure 7 : Anti-inflammatory activity of green synthesised CaO NPs mediated wound healing gel

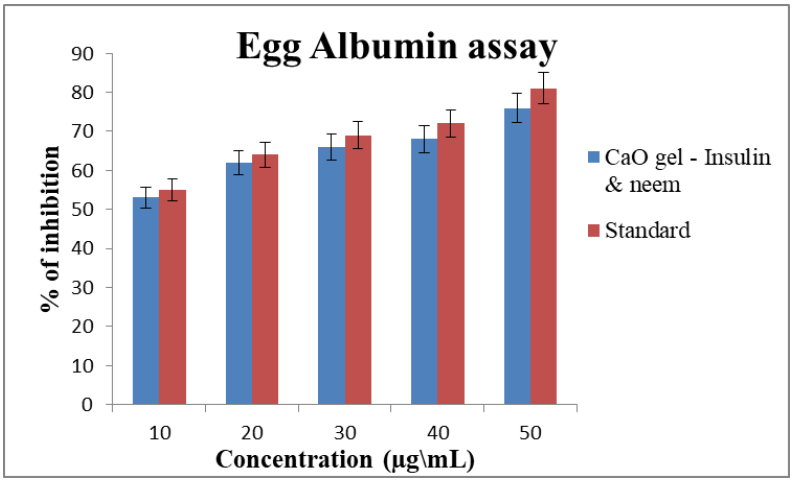

Figure 8 : Result of Anti-inflammatory activity of green synthesised CaO NPs mediated wound healing gel

Anti-inflammatory activity:

The synthesised CaO NPs show comparable anti-inflammatory activity with standard diclofenac sodium which is a chemical analgesic at 50 µL concentration. Prior studies have demonstrated that CaO NPs exhibit strong anti-inflammatory properties. Hence, it is confirmed that the CaO NPs synthesised using *Azadirachta indica* and *Chamaecostus cuspidatus* also have anti-inflammatory activity. The anti-inflammatory activity of *Azadirachta indica* and *Chamaecostus cuspidatus* mediated CaO NPs increases with an increase in the concentration from 10 µl, 20 µl, 30 µl, 40 µl to 50 µl.).

4. DISCUSSION

It has been demonstrated that calcium oxide nanoparticles possess antibacterial, anti-inflammatory, and pro-regenerative qualities. The coated sutures have the potential to mitigate tissue repair, lower inflammation, and prevent infection by dispersing these nanoparticles into the wound site.(13)

The development of ecologically acceptable techniques for nanoparticle synthesis is a fundamental component in the field of nanotechnology. Herbs like *Chamaecostus cuspidatus* and *Azadirachta indica* will work together to benefit the green synthesis of nanoparticles utilising eco-friendly components, which is an economically and environmentally stable

process.(14) Studies on these herbs claim to have demonstrated their antibacterial, antioxidant, cytotoxic, and anti-inflammatory properties. A notable number of these plant constituents have been employed in the production of nanoparticles.

5. CONCLUSION

Within the limits of the study we were able to successfully synthesise calcium oxide nanoparticles and infuse them with plant extract and analyse the characteristics of plant extract of *Azadirachta indica* and *Chamaecostus cuspidatus* green synthesised CaO NPs mediated wound healing gel and found that the synthesised product has anti-inflammatory properties, non-toxic after which the concentrate was made into gel form.

The membrane stabilisation activity was seen to be in the range of 70-90 % of inhibition. Prior to utilising this innovative product as the gel in people with diabetes, more research needs to be conducted.

Data Availability:

The data used to support the findings of this study are included in the article.

Conflicts of Interest:

The authors declare that they have no conflicts of interest.

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