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Anti-Inflammatory and Thrombolytic Activity of Calcium Oxide Nanoparticles using Commelinabenghalensis

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Inflammation is the normal response of the body to wounds and foreign objects. However, inflammation that develops in the absence of an injury or intruder can damage healthy body parts and lead to a variety of chronic illnesses. Globally, nanotechnology has gradually but firmly taken over several industries. Nanotechnology is being used worldwide to improve the productivity and structure of their ideas in terms of working, designing and structuring. The aim of the study is to prepare anti-inflammatory and thrombolytic activity of Commelinabenghalensis mediated calcium oxide nanoparticles. UV-Visible spectrometer was done to check their absorption spectrum. The Bovine serum albumin denaturation assay, Membrane Stabilization assay, Egg albumin denaturation assay were done to analyze the anti-inflammatory properties of Commelinabenghalensis mediated CaONPs. The results are graphically represented which shows that the proportion of protein denaturation inhibition stayed close to the standard at all the concentrations. In the BSA assay, the highest concentration of 50µg/ml theCaONPs show 79% inhibition. In the EA assay, the highest concentration of 50 µg/ml calcium oxide nanoparticles shows 80% inhibition. In the Membrane Stabilization assay, the highest concentration of 50 µg/ml the calcium oxide nanoparticle shows 80% inhibition.This study reveals that Commelinabenghalensis mediated CaONPs shows an excellent anti-inflammatory and thrombolytic property. To fully understand the advantages of these plants and the mechanisms underlying their actions, more research is necessary.

Keywords: Anti-inflammatory, Calcium oxide nanoparticles Commelinabenghalensis, thrombolytic.

1. Introduction

Inflammation is a typical complicated (local and systemic) general process of pathology that serves as the foundation for the pathogenesis of illness. An organism reaction to mostly local tissue changes of many kinds is inflammation (Ransom, 1905).Inflammation is a feature of diseases which includes asthma and chronic obstructive pulmonary disease (COPD) (Trevethick et al., 2008).Inflammatory breast cancer is a rare but aggressive subtype of breast cancer, which was historically considered to be uniformly fatal(Giordano &Hortobagyi, 2003).

Nanotechnology is helping in tackling environmental crises which consequently broadens the scope of research for the technical applications (Shafique & Luo, 2019). Nanotechnology facilitates in improving the quality of water, soil and air (Biswas & Wu, 2005). Nanoparticles can more precisely interact with the human body's molecules to target certain cells and tissues (Mitchell et al., 2020). Nanotechnology provides better advancement in diagnosis and treatment of diseases (Laroui et al., 2013). Various fields such as agricultural, biotechnology, environmental, industrial applications of nanoparticles are observed e.g optics, food, electronics, clothes, cosmetics, medicine and engineering (Mohamed et al., 2023). Though Nanoparticles are beneficial for research due to their distinctive properties still there are complications in formation of new nanoparticles (Gunnarsson, 2017).

Calcium oxide nanoparticles (CaO NPs) have a greater biological and medical purpose with acquired characteristics of high porosity, non-toxicity, biocompatibility(Gunnarsson, 2017).Calcium oxide nanoparticles (CaONPs) can be easily accessed and are available at low cost (Gandhi et al., 2021).Calcium oxide nanoparticles (CaONPs) are well known for their antimicrobial activity for decades (Khan et al., 2023).Uses of calcium oxide nanoparticles can be seen in cosmetics, medicine, waste remediation (Jadhav et al., 2022). The Piper betel leaf extract of calcium oxide nanoparticles (CaONPs) has antibiofilm, antioxidant and antibacterial properties (Mazher et al., 2023). It is evident that calcium oxide nanoparticles do not have any disruptions in endocrine function (Roy et al., 2013).

CommelinabenghalensisLinn also called as Benghal day flower or Dew flower is a perennial herb. The appearance of the flower is like bluish- violet flowers. In the system of traditional medicine C. benghalensisis used to treat many diseases. This plant is used in the treatment of snake bite, headache, leprosy, fever, jaundice etc. It is also evident that C. benghalensisis used to treat infertility in women. The plant Commelinabenghalensispossesses antimicrobial property (Admin, 2023). C. benghalensisis also used to treat smolders. For treating the acid reflux the root juice of C. benghalensisis used in ancient time (Roy Orni et al., 2018). The objective of this study was to prepare anti-inflammatory and thrombolytic properties of Commelinabeghalensis mediated calcium oxide nanoparticles.

2. Materials and Methods

2.1. Preparation of Calcium oxide nanoparticle

Fresh Commelinabenghalensis leaves were shade-dried and powdered after being gathered from Saveetha Medical College's herbal garden. 1 gram of powdered Commelinabenghalensiswas mixed with 100 mL of distilled water. The plant extract was then boiled between 50–60°C for 10 minutes using a heating mantle. Later, a muslin cloth was used

to filter the boiled extract. After that, 50 mL of the filtered plant extract was used as a reducing and stabilizing agent for the preparation of calcium oxide nanoparticles.50 mL of distilled water was combined with 30 millimolar calcium hydroxide and used as the precursor solution. 50 mL of C. benghalensiswas mixed with the 50 mL of precursor solution. In order to allow the formation of nanoparticles, the resultant 100 mL solution was placed in a magnetic stirrer for 48 hours. After 24 hours, the change in color of the nanoparticles solution was observed. The nanoparticles solution was centrifuged for 10 minutes at 8000 rpm(rotation per minute) after a 48- hour period. After centrifugation, the pellet was collected and refrigerated for later use.

2.2. Anti-inflammatory activity

2.2.1. Bovine serum albumin denaturation assay (BSA assay)

The BSA assay was used to evaluate the anti-inflammatory properties of the green synthesized calcium oxide nanoparticles. Commelinabenghalensismediated calcium oxide nanoparticles in various concentrations (10, 20, 30, 40, and 50 µg/mL) were combined with 0.45 mL of bovine serum albumin. A pH of 6.3 was achieved. After being left at room temperature for ten minutes, it was incubated for thirty minutes at 55°C in a water bath. The standard group was diclofenac sodium, and the control group was dimethyl sulphoxide. The samples were then subjected to spectrophotometric measurements at 660 nm.

2.2.2. Egg Albumin denaturation assay (EA assay)

The EA assay was performed by 2.8 mL of 1X phosphate buffer and 0.2 mL of fresh egg albumin were combined to carry out the egg albumin denaturation test. The reaction mixture was mixed with various amounts of calcium oxide nanoparticles mediated by Commelinabenghalensis(10, 20, 30, 40, and 50 µg/mL). A pH of 6.3 was achieved. After being left at room temperature for ten minutes, it was incubated for thirty minutes at 55°C in a water bath. The standard group was diclofenac sodium, and the control group was dimethyl sulphoxide. The samples were then subjected to spectrophotometric measurements at 660 nm.

2.3.3. Membrane stabilization assay

One method that is frequently used to assess the ability of both natural and synthetic substances to stabilize membranes is the in vitro membrane stabilization assay. This experiment assesses a compound's capacity to maintain the integrity of the cell membrane and stop intracellular materials from leaking out by stopping membrane disruption. Human red blood cells (RBCs), phosphate-buffered saline (PBS), Tris-HCl buffer (50 mM, pH 7.4), various calcium nanoparticle concentrations (10, 20, 30, 40, and 50 µg/mL), centrifuge tubes, and UV-Vis spectrophotometer are among the components.To assess a substance's ability to stabilize membranes, one common method is the in vitro membrane stabilization assay, both natural and synthetic. This experiment evaluates a compound's ability to prevent membrane disruption, preserve the integrity of the cell membrane, and block intracellular components from leaking out. Components include centrifuge tubes, UV-Vis spectrophotometer, phosphate-buffered saline (PBS), calcium nanoparticle concentrations (10, 20, 30, 40, and 50 µg/mL), and Tris-HCl buffer (50 mM, pH 7.4). Each centrifuge tube was filled with 1 mL of the RBC suspension using a pipette. Subsequently, varying quantities of calcium oxide nanoparticles (10, 20, 30, 40, and 50 µg/mL) were introduced into every tube. After a gentle

mixing, incubate the tubes for half an hour at 37°C. To pellet the RBCs, centrifuge the tubes at 2500 RPM for 5 minutes at room temperature. Using a UV-Vis spectrophotometer, find the supernatant's absorbance at 560 nm.The RBC suspension absorption without the test compound(s) is known as the OD control, and the absorbance of the RBC suspension with the test is known as the OD sample.

2.4. Thrombolytic activity

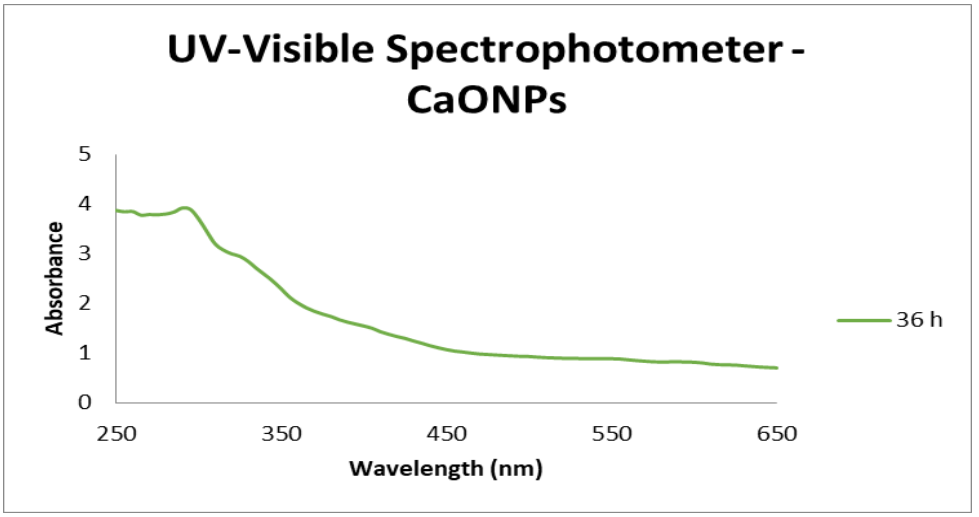
One drop of blood was taken and incubated for 45 minutes at room temperature on a sterile glass slide. Once the blood has coagulated, add the 10, 20, 30, 40, and 50 µg/ml concentrations of calcium oxide nanoparticles. Then the sample was compared with the control (without addition of nanoparticles). The glass slide was incubated for ninety minutes at room temperature; the hours of incubation were recorded in order to track the lysis of the clot.

3. Results:

3.1 UV- Visible spectroscopy:

The calcium oxide nanoparticles solution was analyzed for UV-visible spectra using 1 - 4 mL of the nanoparticles solution. The absorption spectra of surface plasmon absorbance resonance of CaONPs were observed at 295 nm as shown in figure 1.

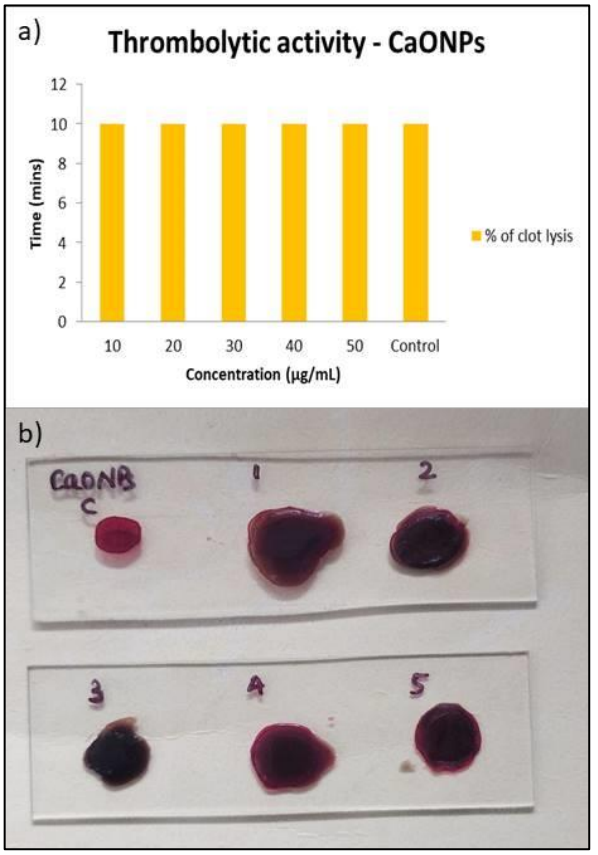
Figure 1: UV-visible spectrophotometer analyses of CaONPs



3.2 Thrombolytic activity

The thrombolytic activity of calcium oxide nanoparticles was assessed using clot lysis assay at concentrations of 10, 20, 30, 40 and 50 µg/ml with a clot lysis percentage of 10% for each concentration as displayed in figure 2. Therefore, the clot lysis remains the same for all the concentrations.

Figure 2: Thrombolytic activity of calcium oxide at varying concentrations with their clot lysis percentage a) Graphical representation b) Visual observation

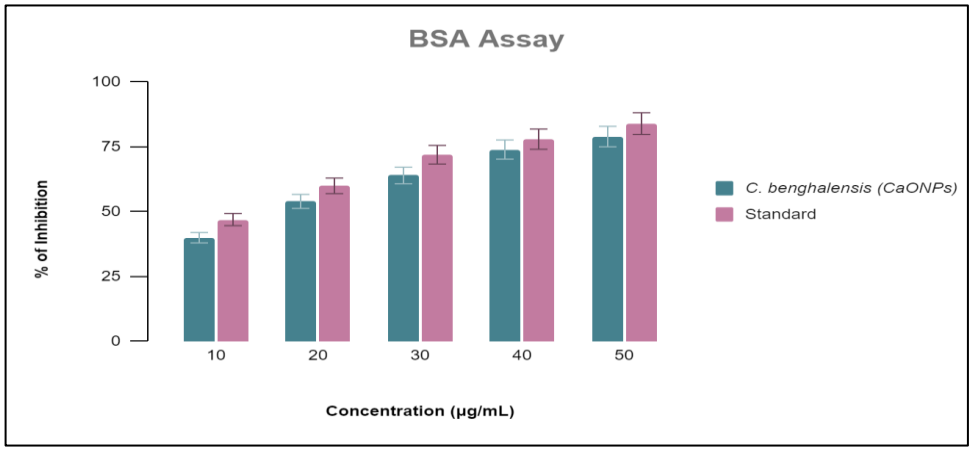


3.3 Anti-inflammatory activity

3.3.1 Bovine serum albumin denaturation assay

The bovine serum albumin denaturation of calcium oxide nanoparticles shows that the percentage of inhibition increases with increase in the concentration as that of the standard as shown in figure 3. In the lowest concentration of 10 µg/ml the CaONPs show 40% inhibition and standard shows 47% inhibition. In the highest concentration of 50µg/ml the CaONPsshow 79% inhibition and standard shows 84% inhibition. But when compared to the standard, the prepared CaONPs displayed a slightly low percentage of inhibition.

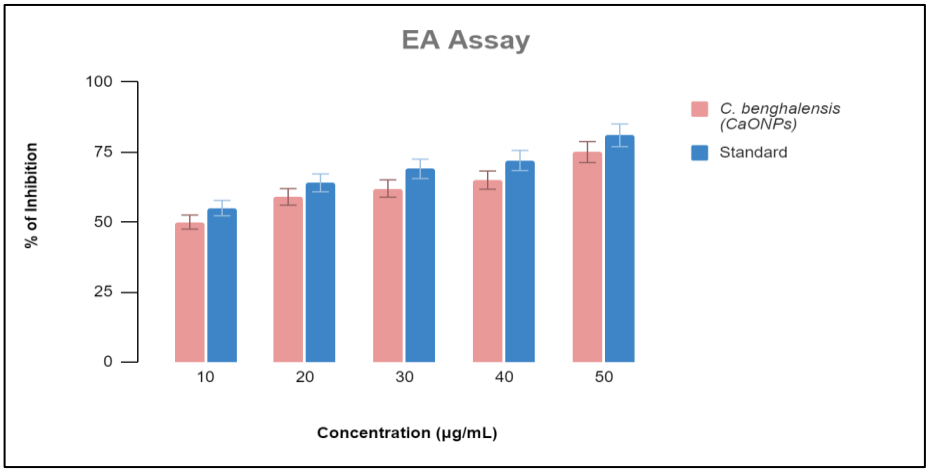
Figure 3: Graphical representation of the anti-inflammatory activity of Bovine serum albumin denturation assay



3.3.2 Egg albumin denaturation assay

The egg albumin denaturation of calcium oxide nanoparticles evaluates that the percentage of inhibition increases with increase in the concentration of C. benghalensismediated calcium oxide nanoparticles as that of the standard. As shown in figure 4, the lowest concentration of 10 µg/ml the calcium oxide nanoparticles show 54% inhibition and standard shows 50% inhibition. In the highest concentration of 50 µg/ml calcium oxide nanoparticles show 80% inhibition and standard shows 75% inhibition. But when compared to the standard, the prepared CaONPs displayed a slightly low percentage of inhibition.

Figure 4: Graphical representation of the anti-inflammatory activity by egg albumin denturation assay

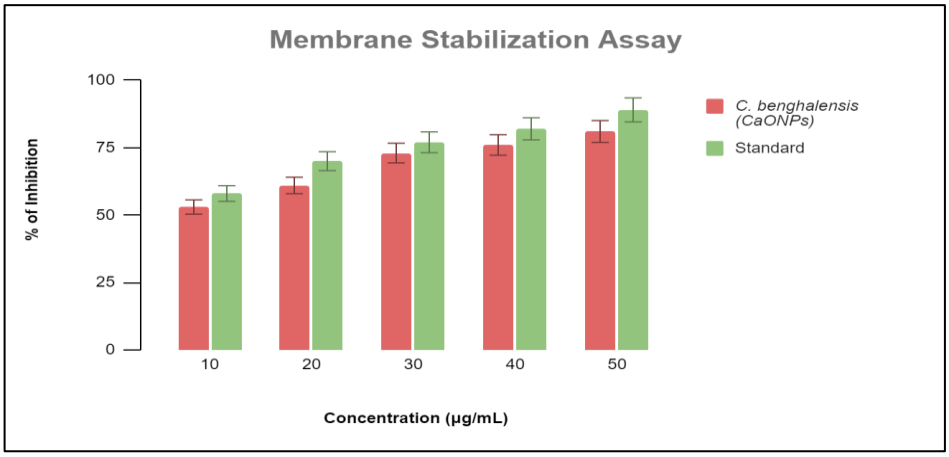


3.3.3 Membrane stabilization assay

The membrane stabilization assay of calcium oxide nanoparticles evaluates that the percentage of inhibition increases with increase in the concentration of C. benghalensismediated calcium

oxide nanoparticles as that of the standard. As displayed in figure 5, the lowest concentration of 10 µg/ml, the calcium oxide nanoparticle shows 54% inhibition and standard shows 50% inhibition. In the highest concentration of 50 µg/ml the calcium oxide nanoparticle shows 80% inhibition and standard shows 75% inhibition. But when compared to the standard, the prepared CaONPs displayed a slightly low percentage of inhibition.

Figure 5: Graphical representation of the anti-inflammatory activity by Membrane Stabilization assay



4. Discussion

Inflammation is most typically caused by the combination of these two kinds of stressors in place and time (Nathan, 2002). Acute inflammation brought on by a S. aureus skin infection (the common boil) is one type of inflammation; chronic inflammatory processes cause crippling joint deterioration in rheumatoid arthritis, bronchial wall inflammation in asthma and chronic bronchitis, and modification of the arterial wall in atherosclerosis (Punchard et al., 2004).

The calcium oxide nanoparticles absorption spectra show a characteristic absorption peak at 295 nm. The thrombolytic activity of herbal mixture was assessed using clot lysis assay at various concentrations with a clot lysis percentage of 10% for all. The BSA assay of C. benghalensismediated calcium oxide nanoparticles shows 79% inhibition and standard shows 84% inhibition in its higher concentration. TheEA assay of calcium oxide nanoparticles shows 50 µg/ml, the calcium oxide nanoparticle shows 80% inhibition and standard shows 75% inhibition in its higher concentration. The Membrane Stabilization assay of calcium oxide nanoparticles, the highest concentration of 50 µg/ml the calcium oxide nanoparticle shows 80% inhibition and standard shows 75% inhibition.

Overall, the study revealed that C. benghalensis mediated calcium oxide nanoparticles possess anti-inflammatory and thrombolytic activity. A study shows that the CaONPs synthesized from fruit extracts of C. colocynthis exhibit antioxidant, cytotoxic properties (Mazher et al., 2023). A study shows that CaONPs seed priming could strengthen carom(Trachyspermumammi L) plant's antioxidant defense, the results of the experiment were

CaONPs enhanced the antioxidant defense of carom plants and raised the storage of vitamin antioxidants (Mazher et al., 2023). A study proved that carob fruits (Ceratonia siliqua) mediated calcium hydroxide possess anti-inflammatory properties(Shahrajabian& Sun, 2024). Similarly, the calcium oxide nanoparticles prepared using C. benghalensisin this present study shows significant anti-inflammatory activity.

5. Conclusion

This study revealed that Commelinabenghalensismediated calcium oxide nanoparticles possess anti-inflammatory and thrombolytic activity which is used to treat inflammation. In future, research has to be done to ensure that the CaONPs from Commelinabenghalensis can be used for medical application.

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Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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