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Comparative Evaluation Of Amla, Neem Silver Nanoparticles Based On Anti Inflammatory And Cytotoxic Properties

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ABSTRACT

Background: With the exponentially growing number of applications across various sectors, silver nanoparticles (Ag-NPs) have become one of the most sought-after nanoparticles as nanotechnology has advanced. Ag-NPs enhanced products could become more harmful to the environment and living things as a result of greater use.

Materials and Methods: Extract preparation was prepared using neem leaves gathered from the university campus. To properly remove the dust and debris from the leaves' surface, they were extensively washed under running water. They were left in the hot air oven at 600C for 24 to 48 hours after being air dried for 10 days. Then, a fine powder was made from these leaves.And synthesis of nanoparticles and sample preparation for brine shrimp lethality assay was done.

Results: The cytotoxic effect of amla and neem leaves mediated silver nanoparticles was evaluated in five different concentrations (10µL, 20 µL,30 µL,40 µL and 50 µL).Plant extract of silver nanoparticles revealed a high level of cytotoxicity and antiinflammatory properties .This demonstrates that the cytotoxic effect of amla and neem leaf extract-mediated nanoparticles is better even at 80 L . The collected data was tabulated into Microsoft office Excel 2013 transferred to SPSS version 26.0 software (SPSS, Chicago, IL, USA) for statistical analysis.

Conclusion: According to our findings, the anti-inflammatory action of aloe vera and neem leaves, aided by silver nanoparticles, has nearly the same impact as traditional medicine and also shows minimal cytotoxicity. Within the limitations of this study we can conclude that this medication may be utilised as an alternative to diclofenac in the future.

Keywords: *silver, cytotoxicity, leaves, extract, inflammatory*

INTRODUCTION

The science and engineering involved in the design, synthesis, characterisation, and use of materials and devices whose smallest functional organisation, in at least one dimension, is on the nanoscale scale, or one billionth of a metre, is referred to as nanotechnology. With a high degree of functional specificity, these materials and devices may be developed to interact with cells and tissues at the molecular (i.e., subcellular) level for applications in medicine and physiology(1). Fluorescent biological labels, drug and gene delivery, pathogen detection, protein detection, DNA structure probing, tissue engineering, tumour detection, separation and purification of biological molecules and cells, MRI contrast enhancement, and pharmacokinetic studies are among the applications(2).

Neem (Azadirachta indica) is a member of the Meliaceae family, and its health-enhancing attributes are ascribed to its high antioxidant content. It has long been utilised in Chinese, Ayurvedic, and Unani medicine, particularly in the Indian Subcontinent, to cure and prevent a variety of ailments.

Quercetin and ß-sitosterol were the first polyphenolic flavonoids isolated from fresh neem leaves and demonstrated antifungal and antibacterial activity(3). Numerous biological and pharmacological activities, including antibacterial, antifungal, and anti-inflammatory properties, have been documented(4,5). Previous research has shown that they have anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, anti gastric ulcer, antifungal, antibacterial, and antitumor properties(6,7).

Aloe vera's botanical name is Aloe barbadensis miller. It is a perennial, xerophytic, succulent, shrubby or arborescent plant in the Asphodelaceae (Liliaceae) family. It grows mostly in arid areas of Africa, Asia, Europe, and America(8). This medicinal herb has traditionally been used to cure skin disorders (burns, wounds, and anti-inflammatory processes). Furthermore, Aloe vera has been proven to have anticancer, antioxidant, antidiabetic, and antihyperlipidemic effects. Vitamins (vitamin A, C, E, and B12), enzymes(i.e., amylase, catalase, and peroxidase), minerals (i.e., zinc, copper, selenium, and calcium), carbohydrates (monosaccharides such as mannose-6-phosphate and polysaccharides such as glucomannans), lignin, saponins, salicylic acids, and amino acids are among the 75 potentially active elements of aloe vera(9,10).

Though many researches have explored the beneficial properties of neem leaves and aloe vera individually, our study explores the cytotoxic activity and the anti-inflammatory properties of the combination of neem and aloe vera leaves formulation incorporated with silver nanoparticles.

Our team has extensive knowledge and research experience that has translate into high quality publications (11–20)

MATERIALS AND METHODS

It was an in vitro study conducted in the month of September 2022 – December 2022 in the city of Chennai, Tamil Nadu.

Ethical approval

The study was registered with the Institutional Review Board of the Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India. Ethical approval was obtained from the Institutional Review Board of the SIMATS.

Extract preparation

Crushed Indian Gooseberries were used to obtain the pure liquid. Neem leaves were gathered from the university campus. To properly remove the dust and debris from the leaves' surface, they were extensively washed under running water. They were left in the hot air oven at 600C for 24 to 48 hours after being air dried for 10 days. Then, a fine powder was made from these leaves.

Synthesis of nanoparticles

Separately, 200 ml of 1 mM silver nitrate was added to the plant extracts, centrifuged at 18,000 rpm for 25 minutes. Heating the supernatants ranged from 50 to 950C.

Sample preparation for brine shrimp lethality assay

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. Six well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10 µL,20 µL,40 µL,80 µL and control). Then the nanoparticles were added

according to the concentration level. The artificial sea water up to 10mL per test tube is the control. The test tubes were left uncovered under a lamp. The number of surviving shrimps were counted and recorded after 24hrs. The percentage of motility was calculated by dividing the total number of dead nauplii by the total number and then multiplied by 100%.This is to ensure that the death of nauplii is attributed to the compounds present in nanoparticles.

Sample preparation for Albumin Denaturation assay

The following protocol, as modified by Mizushima and Kobayashi, was used to test the anti-inflammatory activity. 0.45 mL of bovine serum albumin (1% aqueous solution) was combined with 0.05 mL of a solution of different fixation (10, 20, 30, 40, and 50 mL), and the pH of the resulting mixture was adjusted to 6.3 using a little amount of 1N hydrochloric acid. These samples were heated to 55 °C in a water bath for 30 minutes after being incubated at room temperature for 20 min. After cooling the samples, the absorbance at 660 nm was calculated spectrophotometrically. The standard was diclofenac sodium.

The percentage of protein denaturation was determined utilizing the following equation,% inhibition= Absorbance of control- Absorbance of sample×100 / Absorbance of control

Sample preparation for Egg Albumin Denaturation assay

A 5ml solution was created by combining 2.8ml of freshly manufactured, pH-6.3 phosphate buffered saline with 0.2ml of egg albumin that had been removed from hen eggs. Specific concentrations (10L, 20L, 30L, 40L, 50L) were made. The positive control used was sodium diclofenac. The mixes were subsequently heated for 15 minutes at 37°C in a water bath. The samples were then allowed to cool to ambient temperature, and absorbance at 660 nm was measured.

Statistical Analyses

The collected data was tabulated into Microsoft office Excel 2013 transferred to SPSS version 26.0 software (SPSS, Chicago, IL, USA) for statistical analysis. Descriptive data analysis was done to find the mean and standard deviation for the data. The confidence interval was set at 95%.Data are represented as the average values with standard error of at least three values of each independent experiment.

RESULTS

The cytotoxic effect of amla and neem leaves mediated silver nanoparticles was evaluated in five different concentrations of reaction mixture ranging from 10µL, 20 µL,30 µL,40 µL and 50 µL. For each concentration, the percentage of live nauplii was 100% on the first day and 100%, 100%, 100%, 70%, and 50% on the second day. Plant extract with an 80-L concentration of silver nanoparticles revealed a high level of cytotoxicity.This demonstrates that the cytotoxic effect of amla and neem leaf extract-mediated nanoparticles is better even at 80 L, which is greater than 50%. (Figure 1).

In Figure 2 it is seen that the albumin denaturation assay, a bar graph plotted against different concentrations (10µl, 20µl, 30µl, 40µl, 50µl) % of inhibition, it can be seen that at 10µL the anti-inflammatory activity was 50%, 20µL showed the anti-inflammatory activity was 62.5%, 30µL of nanoparticle showed 72% of anti-inflammatory activity, 40µL of nanoparticle showed 75% of inhibition, 50µL of nanoparticle showed 79% of anti-inflammatory activity and the standard which is diclofenac showed a maximum of 80%, the anti-inflammatory activity of neem and aloe vera leaves kept on increasing with the standard value as the concentration increases.

The egg albumin denaturation assay shows a bar graph plotted against different concentrations (10µl, 20µl, 30µl, 40µl, 50µl) % of inhibition, it can be seen that 10µL the anti-inflammatory activity was 60%, 20µL showed the anti-inflammatory activity was 65%, 30µL of nanoparticle showed 70% of anti-inflammatory activity, 40µL of nanoparticle showed 75% of inhibition, 50µL of nanoparticle showed 78% of anti-inflammatory activity and the standard which is diclofenac showed a maximum of 80% (figure 3).

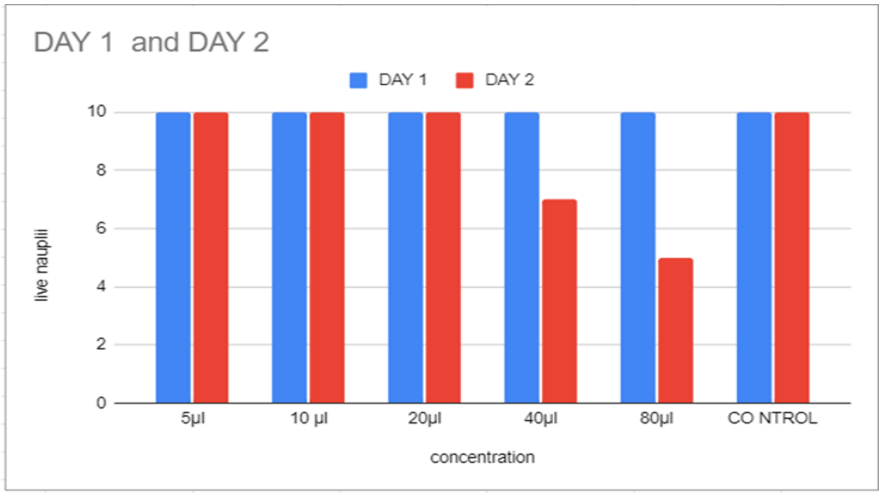

FIGURE 1: The live nauplii present at varying concentration over a period of 2 days

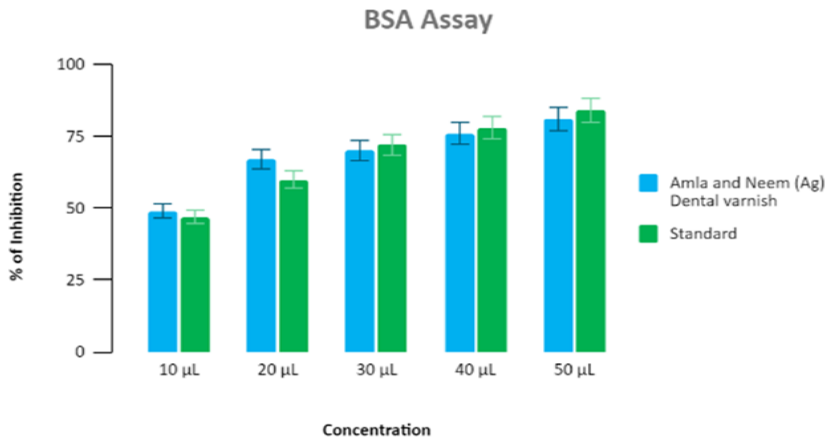

FIGURE 2: Percentage of inhibition using Albumin Denaturation assay

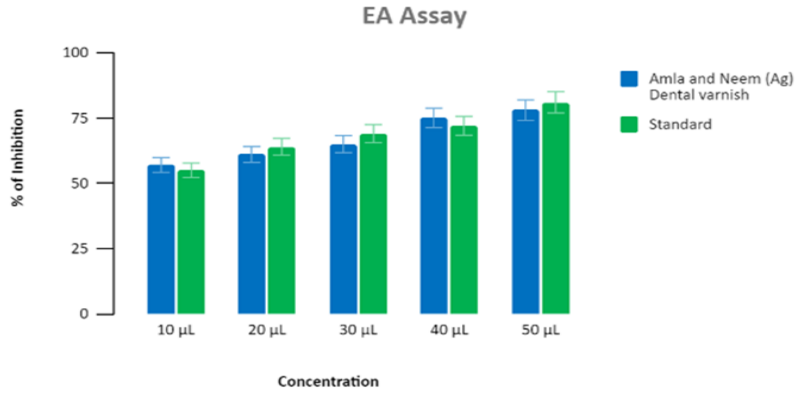

FIGURE 3: Percentage of inhibition using Egg Albumin assay

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DISCUSSION

Due to their larger surface area-to-volume ratios, which are attributed to nanosilver's marked reactivity, and their extremely small size, which enables AgNPs to enter cells, interact with organelles, and yield distinct biological effects, silver nanoparticles (AgNPs) have unlocked numerous novel disciplines in nanobiotechnology protocols. AgNPs have the ability to bypass immune cells, stay in the system for longer periods of time and with a greater distribution, reach target tissues at higher concentrations, avoid diffusion to adjacent tissues, release therapeutic agents or drugs for specific stimuli to achieve a longer duration at a specific rate, and yield desired effects(21).

According to Arivazahagan and colleagues, neem leaf extract may exercise its chemopreventive benefits by inhibiting lipid peroxidation while boosting the levels of glutathione (GSH) and GSH-dependent enzymes(22). AgNPs were able to trigger selective apoptosis in cancer cells at concentrations as low as 0.78 g/mL for HT-1080 and 1.56 g/mL for A431. Biogenic AgNPs synthesised from Fagonia indica (73.37 nm, 12.35 g/mL) were able to trigger caspase-3 in human breast cancer cells(23). Because of the aberrant metabolism, high growth rate, and greater absorption of AgNPs by cells, it has been proposed that biogenic AgNPs have selective cytotoxicity towards malignant cells(24).

The free silver ion can then attach to enzyme thiol groups(25). Both gram positive and gram negative bacteria were shown to be harmful to AgNPs produced at 100 ℃ for 6 hours. This might be owing to the smaller size of the AgNPs produced under these circumstances, resulting in a larger surface area. The release of silver ions is a size-dependent process(26). The antibacterial action of the synthesis AgNPs might be attributed to the release of silver ions and the subsequent result in genotoxic activity of aloe vera(27).

Using rat liver microsomal and mitochondrial enzymes, antioxidant components of Aloe vera were tested for lipid peroxidation. Isorabaichromone, one of the aloesin compounds studied, has significant antioxidative action. Isorabaichromone, along with feruloyl aloe skin and p-coumaroylaloesin, was one of the most effective components, exhibiting significant DPPH radical and superoxide anion scavenging properties(28).

In a study by Thue et al, Aloe vera extract exhibited no significant toxicity against brine shrimp with the LC50 value of above 500 ppm. This signified that Amla and neem are not toxic to humans(29).

From this study we can observe that it is the first study where neem and amla and neem leaf extracts have been taken in combination to assess for their anti inflammatory and cytotoxicity and we have observed that this combination along with silver nanoparticles has proved to be an effective anti inflammatory at specific concentration and also less cytotoxic. This result is in accordance with previous research.

CONCLUSION

Nanomedicine has emerged as the medical field's future. The benefits of utilising silver nanoparticles to treat inflammation include minimal medication dosage effectiveness. According to our findings, the anti-inflammatory action of amla and neem leaves, aided by silver nanoparticles, has nearly the same impact as traditional medicine and also shows minimal cytotoxicity. Within the limitations of this study we can conclude that this medication may be utilised as an alternative to diclofenac in the future.

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Nil.

CONFLICT OF INTEREST

There are no conflicts of interest.

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