CYTOTOXIC EFFECT OF RED SANDAL MEDIATED SILVER NANOPARTICLES MOUTHWASH USING BRINE SHRIMP LETHALITY ASSAY - AN IN VITRO STUDY

ABSTRACT:

Background:

Silver Nanoparticles (AgNP) plays a crucial role in nanoscience, nanotechnology and nanomedicine. AgNP has antibacterial, antifungal, antiviral, anti-inflammatory, anti-antigenic, anticancer and cytotoxic properties. Cytotoxic effects of mouthwashes on the gingival tissue have always been a priority. Pterocarpus santalinus, with the common name of red sandalwood. The aim of the study is to evaluate the cytotoxic effect of red sandal mediated silver nanoparticles mouthwash using brine shrimp lethality assay.

Materials and Methods:

The mouthwash was prepared using red sandal mediated silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, sodium dodecyl phosphate with 0.1% in concentration. The methodology includes green synthesis of Red sanders mediated Silver Nanoparticles synthesis followed by tests for Cytotoxic effect using mouthwash.

Results:

Red sanders mediated silver nanoparticles have high potent cytotoxic potential, it has been proved with the help of brine shrimps. LD50 concentration was obtained to be 80µL, with half the population of nauplii in the respective well surviving post incubation.

Conclusion: From the observed result, it has been concluded that Pterocarpus santalinus/red sandal mediated silver nanoparticles mouthwash has a considerably moderate cytotoxic effect. It is a rich source of various potential phytochemicals and is a simple, rapid, stable and cost effective method for nano based medical applications.

Keywords: Green synthesis, Cytotoxic effect, Mouthwash, Pterocarpus santalinus, Silver nanoparticles

INTRODUCTION:

Various medicinal plants have been used for hundreds of years and are found to be effective in the treatment of many diseases(1). Natural products of plants possess several biological activities including cytotoxic effects. The cytotoxic level of medicinal plants must even be evaluated against host cells. The safety of plants as potential therapeutically agents must be ascertained and the therefore side effects should be acceptable to the host(2). Bioactive compounds with no or less toxic effect to the host are good for formulation of medicine. Many researches are done to understand the cytotoxic effect constituents of medicinal plants for the treatment of the disease(3).

Nanosized inorganic particles, of either simple or composite nature, display physical and chemical properties and represent an increasingly important material development of novel nanodevices which may be utilised in numerous physical, biological, biomedical, and pharmaceutical applications(4). Silver Nanoparticles (AgNP) plays a crucial role in nanoscience, nanotechnology and nanomedicine. AgNP has antibacterial, antifungal, antiviral, anti-inflammatory, anti-antigenic, anticancer and cytotoxic properties(5). AgNP has engrossing properties and its low cost and simply available in natural sources. AgNP has an impractical potential in comparison to gold nanoparticles(6). AgNPs are increasingly utilized in various fields, including medical, food, health care, cosmetics, and industrial purposes, due to their unique physical and chemical properties(7). Silver nanoparticles may be a popular additive in many health products as listed above due to its unique ability to fight infectious diseases, slow the growth of bacteria, mold and germs(8). Biologically prepared AgNPs show high yield, solubility, and high stability(9). Several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches which will produce well-defined size and morphology under optimized conditions for translational research(10).

Cytotoxic effects of mouthwashes on the gingival tissue have always been a priority. Chlorhexidine gluconate mouthwash had no cytotoxic effects on human gingival fibroblasts. Mouthwashes are composed of strontium chloride, potassium nitrate, sodium citrate and

sodium fluoride as desensitizing agents(11). Pterocarpus santalinus, with the common name of red sandalwood. The wood is traditionally considered not aromatic. A wide array of biological activities and potential health benefits of P. santalinus have been reported including antioxidative, antidiabetic, antimicrobial, anticancer, anti-inflammatory cytotoxic properties as well as protective effects on the liver, gastric mucosa and nervous systems(12). All these protective effects were attributed to bioactive compounds present in P. santalinus. The effect of this herb against most of the oral pathogens is essentially unexplored(13). Our team has extensive knowledge and research experience that has translated into high quality publications (14–26),(27–31),(32),(33). The aim of the study is to evaluate the cytotoxic effect of red sandal mediated silver nanoparticles mouthwash using brine shrimp lethality assay.

MATERIALS AND METHOD:

Preparation of Silver Nanoparticles:

The dried powdered plant material 0.5gm of red sandal was diluted with 100ml of distilled water, and heated for 9 mins at 60-80°C under vacuum. Then it was filtered using Whatman's filter paper and plant extract was prepared. 80ml of distilled water was taken in a conical flask and 20ml of plant extract was added to it. 1mM of Silver particles were added and then kept in a shaker. Preliminary reading was taken every 2 hours for about 72hrs. After 72hrs fill the AgNP plant extract into the 6 centrifuge tube, 12ml each and centrifuged for about 10 mins.

Preparation of mouthwash:

The mouthwash was prepared using red sandal mediated silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, sodium dodecyl phosphate with 0.1% in concentration. Silver nanoparticles are the main constituent, ethanol acts as a solvent to solubilise the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavouring agent.

Cytotoxic effect:

Brine shrimp eggs were obtained from a new aqua laboratory. The seawater was put in a small plastic container(hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. After 2 days, when the shrimp were ready, 5 different nanoparticles solution was added to each test tube, 10 shrimps were introduced in

each tube. After 24 hrs, the number of surviving shrimps were counted and recorded. LC50 of less than 100 ppm was considered as potent.

RESULTS

The test for cytotoxic properties was assessed using brine shrimps. Descriptive statistics were used in this study. Ten nauplii were placed in each of six wells with one standard and the remaining with nanoparticle concentrations 5 µL, 10 µL, 20 µL, 40 µL and 80 µL. LD50 concentration was obtained to be 80µL, with half the population of nauplii in the respective well surviving post incubation. 10 numbers of nauplii are alive on day1 in different concentrations. On day 2, 9 nauplii are alive on 5 µL concentration, 8 nauplii are alive on 10 µL concentration, 9 nauplii are alive on 20 µL concentration, 8 nauplii are alive on 40 µL concentration and 7 nauplii are alive on 80 µL concentration. All nauplii are alive in control value(Table 1, Figure 1)

Table 1 depicts the cytotoxic activity of red sandal silver nanoparticles mouthwash

Concentration(µL)	Day 1	Day 2
5µL	10	9
10µL	10	9
20µL	10	9
40µL	10	8
80µL	10	8
Control	10	10

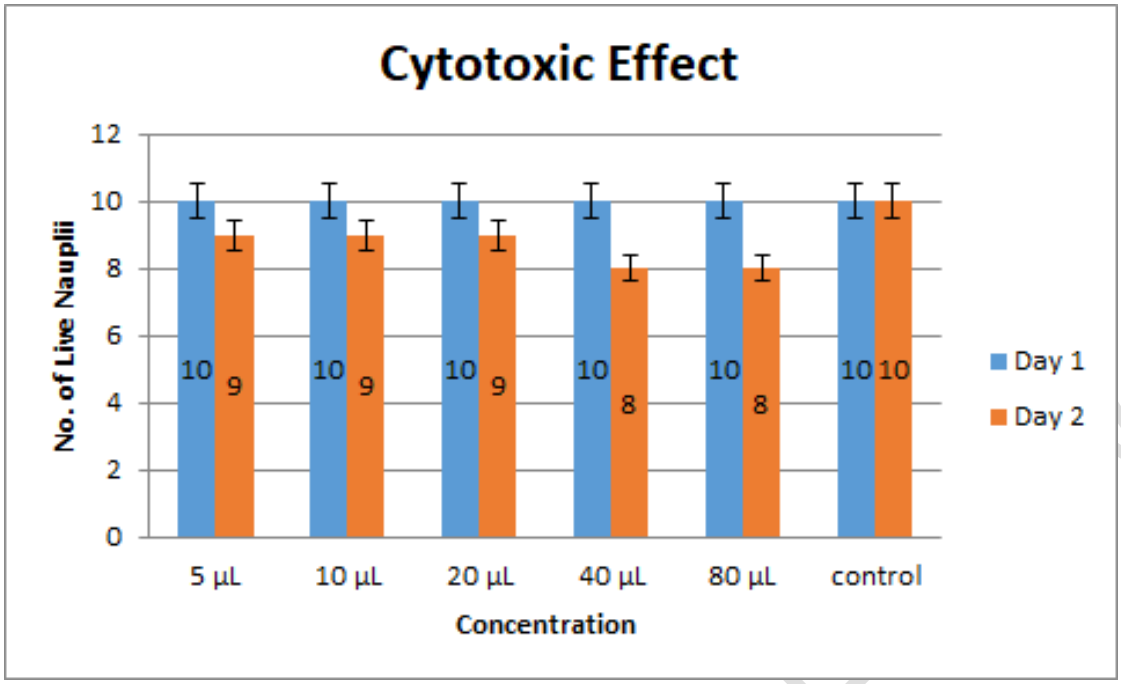

Figure 1: Graph showing cytotoxic effect of silver nanoparticles based mouthwash of red sandal. Blue represents day 1 and orange represents day 2. X axis denotes number of nauplii alive and Y axis denotes concentration. All nauplii are alive on day 1. On day 2, 9 nauplii are alive in 5µL, 8 nauplii are alive in 10µL, 9 nauplii are alive in 20µL, 8 nauplii are alive in 40µL and 7 nauplii are alive in 80µL. In comparison between day 1 and day 2, as concentration increases, the number nauplii decreases.

DISCUSSION:

Medicinal plants have unlimited capacity to synthesize bioactive compounds that are effective and have fewer side effects compared to synthetic drugs. Bioactive compounds from plants have shown over the years to have various biological activities(34). Scientists have developed a greater interest in using these compounds in formulation of new and novel drugs, because of their biological activities and reliability(35). P.santalinus could be used to treat various medical complications such as hemorrhage, dysentery, eye diseases, and mental aberrations, and to act as an aphrodisiac and diaphoretic.

P.santalinus is highly valued for its heavy, dark claret-red sanders, which yields 16% of the red coloring matter santalin, used as a coloring agent in pharmaceutical preparations and

foodstuff (36). The presence of various types of plant secondary metabolites such as anthocyanins, flavonoids, glycerides, isoflavone glucoside, phenols, pterocarpol, pterocarptriol, pterocarpodiolones with β-eudeslol, saponins, steroids tannins and triterpenoids in the P. santalinus L. has been reported ((37).

Cytotoxicity of dental materials and oral hygiene products has always been a concern for dental clinicians. These products should be biocompatible; otherwise, they would cause inflammatory reactions. Nanotechnology favorably changed the properties of many dental materials. Mouthwashes are composed of strontium chloride, potassium nitrate, sodium citrate and sodium fluoride as desensitizing agents. Clinical and human intervention studies are very limited; therefore the biological and physiological effects of the isolated compounds of the heartwood are also worth investigation. The key advances in the tissue culture-based biotechnology of economically important red sandals (38). The development of treatment methods has evoked great expectations in the future. This plant has been exploited to treat a wide variety of ailments, with reported antimicrobial and antioxidant properties, as well as cytotoxic effects against some human cancer cell lines. Invitro cytotoxicity effects of leaf, stem and bark of Pterocarpus santalinus Linn.F. was evaluated in a study where they found that P.santalinus showed a cytotoxic effect against cancer cell lines(39). The limitation of this study is that we did not do clinical trials in patients.

CONCLUSION:

From the observed result, it has been concluded that Pterocarpus santalinus/red sandal mediated silver nanoparticles mouthwash has a considerably moderate cytotoxic effect (40-53). It is a rich source of various potential phytochemicals and is a simple, rapid, stable and cost effective method for nano based medical applications.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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