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Assessment of cytotoxic effects of lemon seed conjugated silver nanoparticles

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ABSTRACT

Cytotoxicity is defined as the toxicity caused due to action of chemotherapeutic agents on living cells , the cytotoxicity tests hold a significant value in nanoparticles as they help in determining the proposed bio medical use of nanoparticles.

Materials and Methods: The research project involved two basic steps as it's methodology; 1. Synthesis of lemon seed extract assisted silver nanoparticles and determination of cytotoxicity using brine shrimp lethality assay.

Result: The cytotoxicity assay using brine shrimp lethality assay showcased the death of very few nauplii, thus showcasing very little cytotoxicity.

Conclusion: The lemon seed extract assisted silver nanoparticles showcased very very little cytotoxicity.

Keywords: *Lemon, Nano-particles, Nanotechnology, Cytotoxicity assays, Brine shrimp lethality assay, etc*

INTRODUCTION

Lemon is an important medicinal plant of the family Rutaceae.It is farmed mostly for its alkaloids, which have anticancer properties, and it has been reported that crude extracts of Lemon's leaves, stem, root, and flower have antibacterial ability against clinically important bacterial strains(Yoshizawa et al. 2000).Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities.These bioactive substances may be relevant for antioxidant, anti-proliferative, and antiviral agents, as well as for the prevention of cardiovascular illnesses, according to recent in vitro studies that reveal they have health-promoting qualities.(de Oliveira 2022), (yuwanati et al 2022) (Jeevankumar et al 2022). Citrus fruits, as a major contributor to human diet, have received attention by researchers due to their multitude of bioactive compounds. (Roy and Saraf 2006)

The term "nanotechnology" refers to atomic, molecular, and macromolecular size research and technology development that enables the controlled manipulation and study of structures and devices with length scales between one and one hundred nanometers. The features and functions of objects at this scale, such "nanoparticles," are unique and very different from those of objects at a larger scale. The small size, surface tailorability, improved solubility, and multifunctionality of nanoparticles open many new research avenues for biologists(World Scientific Encyclopedia Of Nanomedicine And Bioengineering I, The: Nanotechnology For Translational Medicine: Tissue Engineering, Biological Sensing, Medical Imaging, And Therapeutics (A 4-Volume Set) 2016; McNeil 2005).

Silver Nanoparticles are one of most commonly used nanoparticles in dentistry. AgNPs) are widely utilised in a wide range of applications, from home disinfectants and medical devices to water purifiers, and are well renowned for their excellent antibacterial capacity and superior physical qualities. (Yu, Yin, and Liu 2013)Food packaging supplies, food storage containers, water purifiers, sock and underwear that resist odours, room sprays, laundry detergents, washing machines, lotions, and soaps are among the available goods. Additionally, AgNPs are frequently employed in medical applications such as implantable medical devices, surgical equipment, feminine hygiene products, and bone cements.(Maret and Wedd 2014)

Cytotoxicity is defined as the toxicity caused due to the action of chemotherapeutic agents on living cells. Cytotoxicity tests are very important in nanoparticles as they help in the determination of the proposed biomedical use.Drug screening often employs cell cytotoxicity and proliferation tests to determine whether the test compounds exhibit direct cytotoxic effects or have an impact on cell proliferation.There are a variety of assay methods based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. These methods could be basically classified into different categories: (I) dye exclusion methods such as trypan blue dye exclusion assay, (II) methods based on metabolic activity, (III) ATP assay, (IV) sulforhodamine B assay, (V) protease viability marker assay, (VI) clonogenic cell survival assay, (VII) DNA synthesis cell proliferation assays and (V) raman micro-spectroscopy. In order to choose the optimal viability assay, the cell type, applied culture conditions, and the specific questions being asked should be considered in detail.(Istifli and İla 2019).

This particular study aims to asses the cell viability via brine shrimp lethality assay.brine shrimp (Artemia salina, fairy shrimp or sea monkeys) lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals.It is a preliminary toxicity screening of plant extracts, (Bhat 2021)This assay was first proposed by Michael et al. in 1956. Subsequently, it was further developed by others. This lethality assay has been successively employed as a bioassay guide for active cytotoxic and anti- tumor agents in 1982 .The aim of this study was to evaluatethe cytotoxic effect of lemon seed extract and it’s assisted silver nano-particles using brine shrimp lethality assay.

MATERIALS AND METHODS

Research project involved two steps:

1. Synthesis of lemon seed extract assisted silver nanoparticles.
2. Cytotoxicity assay us using brine shrimp lethality assay.

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Synthesis of lemon seed extract assisted silver nanoparticles

Lemon seeds were obtained from a local market in Chennai. The seeds were exposed to sunlight for a period of three days, also the moisture content was removed using a dehumidifier. The seeds were grounded and then the powder obtained was weighted to obtain one gram accurately. The mixture was then mixed in 100ml of distilled water, which was then boiled for 10-15 mins. This was then left for cooling and then filtered using filter paper. 30ml of filtrate was then mixed with 70ml of silver nitrate (AGNO3). This mixture was then covered with aluminium paper and was left in static condition for a period of over 3 days, the colour change was observed which has been demonstrated in the images. The mixture was then transferred to six fifteen milliliter conical tubes that were centrifuged at 3000 rpm for ten minutes. The nanoparticles accumulated at the conical tips which were collectively stored in a single tube, the super tenant was then discarded.

Cytotoxicity assay us using brine shrimp lethality assay

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.

6 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 plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula, number of dead nauplii/number of dead nauplii+number of live nauplii×100

Lemon seed extract in distilled water

Lemon seed extract and silver

After few days

Post-centrifugation

RESULTS

The number of live nauplii were counted after incubation with various concentrations for 24hrs. The results have been shown in the below table.

Measurements	moving larva count
control	10
5ul	10
10ul	10
20ul	9
40ul	8
80ul	8

DISCUSSION

This study aimed to evaluate the cytotoxicity of lemon seed extract silver nanoparticles using brine shrimp lethality assay , the results showed that the cytotoxicity increased with the increase in the dosage of lemon seed extract. Very little to no cytotoxicity was observed in control, 5ul, 10ul, doses resulting in zero nauplii death.One nauplii death was observed in 20 ul dose and 2 nauplii death in 40 and 80ul doses. Thus , we can say that our extract has very little cytotoxicity.

Cytotoxicity research has been carried out by various other researchers in the past.Plant-related elements like leaves, stems, roots, shoots, flowers, barks, seeds, and their metabolites have been successfully used for efficient biosynthesis.Beg etal have reported green synthesis of Ag NPs from seed extract of Pongamia pinnata(Husen and Iqbal 2019) (Gopinath et al 2022).

The formation of nanoparticles was confirmed by an absorption max at 439 nm. The well dispersed nanoparticles with an average size of 16.4 nm had zeta potential equal to − 23.7 mV which supports dispersion and stability. Interaction of Ag NPs with human serum albumin was investigated and showed negligible change in α helics.Karatoprak et al. have described the green synthesis of Ag NPs from the medicinal plant extract Pelargonium endlicherianum in a fairly recent article. Gallic acid, apocyanin, and quercetin from the plant function as reducing agents to create silver nanoparticles.

Nanomaterials' cytotoxicity is influenced by their size, shape, coating or capping, and the pathogens used to test their toxicity. Green technique nanoparticles are typically more hazardous than those made using a non-green process. Due to the presence of both the released

Ag ions and Ag NPs, some diseases are more susceptible to nanomaterials, especially Ag NPs, than others.Our sample shows very little to no cytotoxic activity and this can turn to be very helpful as NPs are used as an antimicrobial agent, electrochemical sensors, biosensors, in medicine, health care, agriculture and biotechnology.Although, the long-term effect of nanoparticles on human health and crops is not clear (Ramya et al 2021). (Thampan et al 2021. A large number of nanoparticles are being explored in many areas of industry technology, biotechnology and agriculture. It is known that various forms of silver from laundry, paints, clothes etc. and biosolids reach the sewage and sludge.Further studies on lemon seed silver nanoparticles can turn out to be fruitful.

CONCLUSION

The lemon seed extract assisted silver nanoparticles showcased very very little cytotoxicity and thus can be used to produce anti -cancer drugs upon further detailed studies.

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