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INVITRO CYTOTOXICITY ANALYSIS OF SOLANUM XANTHOCARPUM INCORPORATED SILVER NANOPARTICLES ON PERIODONTAL LIGAMENT FIBROBLAST CELLS

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Abstract

Introduction

Due to the low toxicity of herbs, less side effects, therapeutic efficacy, and improved patient tolerance, herbal alternatives to chemicals are being extensively explored. Because of its ecofriendliness and simplicity, plant-mediated biological production of silver nanoparticles is important. Solanum xanthocarpum has medicinal benefits in all parts, including the stem, flowers, fruits, and roots. As a result, the current investigation was carried out to assess the cytotoxicity of medicated silver Nanoparticles containing solanum xanthocarpum on pdl fibroblasts cells.

Methodology

Silver aqueous extracts of Solanum xanthocarpum Nanoparticles have been created. The periodontal ligament cells' viability was determined using the MTT test. The ANOVA test was used to undertake statistical analysis of the results.

Results

In comparison to NaOCl, Ag-SXENP had no deleterious effect on fibroblasts even at 50 percent concentration, indicating a benign effect even at 200 ug/ml dosage. Add another line

Conclusion

Solanum xanthocarpum induced with silver Nanoparticles shows low cytotoxicity on the periodontal ligament fibroblast cells.

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Keywords: Cytotoxicity, silver Nanoparticles ,periodontal ligament cells, solanum xanthocarpum

Introduction

The purpose of endodontic treatment is to eliminate germs from an infected canal. The process of removing microorganisms from the canal necessitates irrigation.(1)However, an intracanal medicament is administered between sessions to eliminate any remaining germs that survive the cleaning and shape of canals.(2) Medicinal plants are being investigated as a possible replacement to chemical chemicals.(3,4)

Nanoparticles have attracted a lot of attention because they meet the requirements where antibiotics fail to prevent the formation of MDR mutants. Many researches have demonstrated the benefits of introducing a material with improved qualities, such as the fabrication of antibacterial agents using nanotechnology.

Because of its Eco friendliness and simplicity, plant-mediated biological production of silver nanoparticles is important. Silver nanoparticles are biosynthesized from plants such as Euporbia hirtaki,(Elumalai et al., 2010) Svensonia hyderabadrensis,(M. L. Rao & Savithramma, 2011) Trianthena decandre,(Geethalakshmi & Sarada, 2010) Shorea tumbuggaia (Venkateswarlu, Ankanna, Prasad TNVKV, Nagajyothi, & Savithramma, 2010) have been reported previously.

The prickly, perennial, diffuse, patch-forming herb Solanum Xanthocarpum of the Solanaceae family is most typically found in Southeast Asia, Malaysia, and all districts of Tamil Nadu, India, flowering and fruiting throughout the year.(Mathew, 1983)(Pandey, 2004)The stem, flowers, fruits, and roots, as well as other elements of the plant, have therapeutic benefits. The entire dried plant is used in India to treat ailments such as leprosy, dropsy, and cough.(Prempeh & Mensah-Attipoe, 2008)

Antibacterial, antifungal, antinociceptive, antioxidant, hypoglycemic, and larvicidal qualities are among the plant's pharmacological activities.(Samiei et al., 2013)Seed vapours of this plant were effective in treating dental discomfort and pain associated with gingival swellings, as well as fever, rheumatism, pneumonia, and other respiratory problems. (Sheeba, 1970)

Previously our team had a rich experience in working on various research projects across multiple disciplines; (Azeem & Sureshbabu, 2018; Felicita, 2017; Felicita, Chandrasekar, & Shanthasundari, 2012; A. R. Jain, 2017; Krishnan & Lakshmi, 2013; Kumar, Vamsi, Sripriya, & Sehgal, 2006; Mp, 2017; Patturaja, 2016; T. D. Rao & Kumar, 2018; Sekar, Lakshmanan, Mani, & Biruntha, 2019; Sivamurthy & Sundari, 2016)

In this research study cytotoxicity of solanum xanthocarpum mediated silver Nanoparticles in pdl fibroblasts was evaluated.

Methodology

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For preparation of the aqueous extract fruits of the plant were collected and washed .10 g was weighed and mixed with 100 ml of distilled water in a beaker. The mixture was heated to 60°C for 20 minutes before cooling to room temperature. The extract was then filtered with Whatman filter paper

To make a 10 mM AgNO3 stock solution, AgNO3 powder was dissolved in distilled water. In a flask, the AgNO3 solutions were combined with the aqueous extract at a 1: 1 ratio in a volume of 50 mL. The flask was wrapped in aluminium foil and heated for 5 hours in a water bath at 60°C.

Chemicals Used

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 10% FBS, 100 units/ml penicillin, DMSO, human fibroblast cell lines, Eagle's minimum essential medium (EMEM), kanamycin, and phosphate-buffered saline

PDL fibroblast cell lines were donated by NCCS Pune for this investigation. The PDL Cells were grown at 37°C in a humidified CO2 (5%) chamber with 95 percent air in DMEM media with 10% foetal bovine serum, L–glutamine, 1% penicillin (100 U/ml), and streptomycin (100 g/ml). The cells were separated using 0.25 percent EDTA Trypsin. The cells were mechanically detached with a pipette after the Trypsin was neutralised with DMEM containing 10% FBS and PSGF. The 96-well culture plates were filled with 200 l of medium in each well. To allow the cells to adhere to the plates, the plates were incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO2 and 95% air.

As a negative control, the microplates were filled with 100 l of PDL cells at a density of 1105 for the cell viability assay. The cells were allowed to attach for 24 hours before being rinsed twice with MEM without FBS to eliminate dead cells and excess FBS from the growing medium using a micropipette. In each well, 1 ml of media (without FBS) containing different dilutions of nanoparticles generated from SXE (25, 50, 100, 200 g/ml) and 2.5 percent NaOCl were added; 20 l of MTT (5 mg/l in PBS) was added to each well, and the cells incubated for another 6-7 hours in a 5 percent CO2 incubator. Then 1ml of DMSO was added. Propanol was added in the amount of 50l. MTT enters the cells and goes to the mitochondria, where it is transformed into an insoluble coloured (dark purple) formazan product. After 15 minutes of shaking, the plates were read at 570 nm using an enzyme-linked immunosorbent assay (ELISA) (MINDRAY90) reader. The IC50 of the test samples was calculated as the proportion of cells that survived after three repetitions of each experiment.

SPSS software was used to determine statistical significance using one-way analysis of variance (ANOVA) and the post hoc least-significant difference test (version 22.0).

Results

Table 1:MTT assay absorbance value of PDL cells after the treatment with nanoparticles

S.No	Treatment	Conc (µg/ml)	Mean ± SEM

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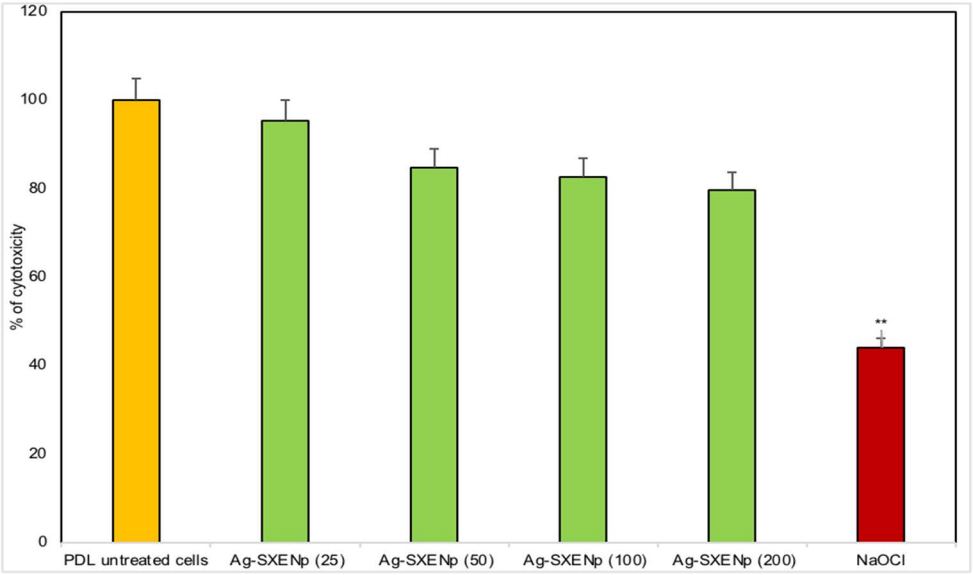
1	PDL untreated cells	-	0.513± 0.04
2	Ag-SXENp	25.0	0.489 ± 0.40a
3	Ag-SXENp	50.0	0.436 ± 0.35a
4	Ag-SXENp	100.0	0.424 ± 0.22a
5	Ag-SXENp	200.0	0.409 ± 0.33*a
6	Naocl	2.5%	0.226 ± 0.24*

Figure 1: MTT assay absorbance value of PDL cells after the treatment with nanoparticles



Values are expressed as Mean ± SEM (n=3); ***P<0.001 statistically significant as compared with Negative control. Silver Nanoparticles incorporated with solanum xanthocarpum fruit extract in comparison to NaOCl, did not harm fibroblasts even at 50% concentration.(Figure1,Table 1)

Discussion

The advantage of primary cultures is that they have the same features as the original tissue. Cytotoxicity tests should be carried out in vitro on the same cells that will be exposed to the substance under investigation. Another benefit of primary cultures is that, when comparing

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samples from various patients, cellular response heterogeneity in each organism to the presence of a harmful agent, substance, or medicine is possible due to the biological variety of the individuals.(14,15)

Antibacterial, antifungal, antinociceptive, antioxidant, hypoglycemic, and larvicidal qualities are among the pharmacological actions of the plant Solanum xanthocarpum. The plant's medicinal benefits can be found in every part of it.

Silver nanoparticles (NNPs) are widely used in medical research for a variety of antibacterial purposes.(16)The use of plant material as a reducing agent for the manufacture of silver nanoparticles has a number of advantages. These benefits include its accessibility, ease of handling, cost-effectiveness, low maintenance costs, and environmental friendliness.(17) It contains a number of metabolites that speed up the reduction reaction, resulting in the quick production of nanoparticles. It can also act as a capping agent, resulting in very stable nanoparticles for future applications.(18)Silver compounds are considered to be among the most powerful and fast-acting antimicrobials.(19,20)Green AgNPs have a number of challenges before moving forward with human trials, including confirming their cytotoxicity in a variety of cells, being compatible, degradable, and having few or no side effects. According to previous studies, the toxicity of AgNP is dependent on its size, concentration, form, and the reducing chemicals utilized. The size and surface area of nanoparticles determine their cytotoxicity; smaller nanoparticles have a higher surface area.(21)Our institution is passionate about high quality evidence based research and has excelled in various fields.(R. K. Jain, Kumar, & Manjula, 2014; Johnson et al., 2019; Keerthana & Thenmozhi, 2016; Lakshmi, Krishnan, Rajendran, & Madhusudhanan, 2015; Neelakantan, Subbarao, Subbarao, De-Deus, & Zehnder, 2011)
The cytotoxicity of Solanum xanthocarpum generated by silver Nanoparticles on periodontal ligament fibroblast cells was investigated in this research. In comparison to NaOCl, Ag-SXENP had no harmful effects on fibroblasts even at 50% concentration, demonstrating a benign effect even at 200 ug/ml dosage.

Conclusion

From this current study Solanum xanthocarpum produced with silver nanoparticles has low cytotoxicity on periodontal ligament fibroblast cells. Because of its high antibacterial characteristics and biocompatibility, it could also be used as an intracanal medicament. Although the efficiency and cytotoxicity results of solanum xanthocarpum extracts in vitro are promising, more investigation into its interaction with silver nanoparticles is needed before definitely recommending it as an intracanal medicament.

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Conflicts of Interest

There are no conflicts of interest.

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