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Investigation of in vitro cytocompatibility of Annona Muricata selenium nanoparticles (SeNps) and its impact on cell migration in human Gingival Fibroblast cells

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ABSTRACT

Background: Annona muricata has been used to treat a wide range of illnesses, making it a crucial species for ethnomedicine. Different parts of A. muricata are used in developing an alternative medicinal extract. Interestingly over the past few decades, metallic nanoparticles have captured the attention of scientists, and selenium is an essential component of amino acids. Hence in the present study A. muricata extracts coated with selenium particles (SeNps) were used to analyze the cytotoxicity of the extract in the gingival fibroblast cells.

Methods: Periodontal ligament tissues were taken from healthy adolescent patients' interdental papillae and the primary culture of gingival fibroblast was established. After two to three passages, the cells were treated with A. muricata SeNps. The cell proliferation, migration, and apoptosis assays were performed before and after treatment with A. muricata SeNps.

Results and Conclusion: The morphology of the isolated fibroblast were in good condition and the cytotoxicity assays revealed that the proliferation and migration increased on treatment with A. muricata SeNps while the apoptosis was less. Thus the findings from the current study proved that A. muricata SeNps had less cytotoxic effects and could be used as a potential therapeutic compound in human diseases.

Keywords: *Annona muricata, cytotoxicity, selenium nanoparticles, Cell proliferation, Apoptosis*

INTRODUCTION

Since the beginning of medicine, natural products, particularly phytochemicals, have been used to support human health. Even today, phytotherapy, also known as herbalism or herbal medicine, offers treatments for diseases like cancer [1-3]. Due to their demonstrated safety, low cost, and oral bioavailability, dietary phytochemicals have many inherent advantages over synthetic compounds. However, the molecular, cellular, and tissue levels of the action

of agents derived from plants have only recently started to be clarified by researchers. Numerous natural products have been thoroughly studied in recent years, and many of the compounds have shown anticancer and other positive effects in controlled studies [2,3]. Annona muricata or Graviola is one of the efficient phytochemicals studied for its potential medicinal value [4-7].

In addition, this lowland tropical Annona muricata is a member of the Annonaceae family. Preparations from A. muricata have been used to treat a wide range of illnesses, making it a crucial species for ethnomedicine. Different parts of A. muricata are used in developing tropical nations, including Africa, to treat diseases like diabetes, coughs, skin conditions, and cancers [5]. According to studies, annonaceous acetogenins (AGEs) are the primary bioactive substances that have been extracted from a variety of A. muricata parts. These are long-chain (C32 or C34) fatty acid derivatives with medicinal value that are produced via the polyketide pathway [6]. Though there are studies on A. muricata's effect on various diseases, still it is not validated due to the off-target effects of the effect [7]. Hence alternative approaches are being explored to prove their therapeutic efficacy.

Interestingly over the past few decades, metallic nanoparticles have captured the attention of scientists, and they are now extensively used in the fields of biology and engineering. Researchers have recently become very interested in the trace element selenium. In addition to organic and inorganic molecules, selenium is an essential component of amino acids [8,9]. Hence in the present study A. muricata extracts coated with selenium particles (SeNps) were used to analyze the cytotoxicity of the extract in the gingival fibroblast cells.

Several regulations for pharmaceutical development and drug manufacturing use the cytotoxicity assay. Simply put, a cytotoxic substance is one that destroys cells, frequently implying that these cells are being targeted for eradication [10]. Thus, in this study, we explore the cytotoxic effects of A. muricata SeNps by analyzing the proliferation, migration and apoptotic efficacy of gingival fibroblast cells before and after treatment with A. muricata SeNps.

METHODOLOGY

Ethical consent and approval

The Saveetha University Human Ethical Committee approved the periodontal ligament tissue collection methods. All the sample collection procedures were performed in accordance with ethical standards and in compliance with Helsinki's declaration.

Sample collection

Prior to tissue collection, all patients read and signed an approved consent form. During the extraction of their first or second premolars as part of their normal orthodontic therapy, periodontal ligament tissues were taken from healthy adolescent patients' interdental papillae.

Establishment of Gingival fibroblast primary cells

To establish a primary culture, the tissues were washed well with phosphate-buffered saline (PBS) and the tissue was minced into tiny fragments. The minced human gingival tissue was plated onto 25 cm^2 tissue culture flasks with Dulbecco's modified essential medium (DMEM) supplemented with 10% Fetal bovine serum (FBS) and necessary antibiotics, then left undisturbed for 48 hours at 37°C in a humidified incubator with 5% CO_2. The medium was changed after 48 hours. The cells were expanded until the number of cells was large enough to conduct experiments. The experiments were done after the completion of two to three passages to establish a stable cell line [11].

Cell viability assay (MTT assay)

The Human gingival fibroblast cells were plated in 96 well plates with a concentration of 5×10^3cells/well in DMEM media with 1X Antibiotic Solution and 10 % fetal bovine serum (Gibco) in CO_2 incubator at 37˚C with 5% CO_2. The cells were washed with 100 µL of 1X PBS, then the cells were treated with Annona Muricata SeNps (10-100µg/ml for hGFs cells and 5-40µg) and 24 hours were used for incubation in an incubator with CO2 at 37°C with 5% CO2. At the conclusion of the treatment period, the media was aspirated from the cells. In a CO2 incubator, 0.5 mg/mL MTT produced in 1X PBS was added and incubated at 37 °C for 4 hours. Following the incubation period, the cells' medium containing

MTT was removed, and 100 L of PBS was used to wash the cells. The created crystals were properly mixed and dissolved in 100 L of DMSO. At 570 nm, the emergence of colour intensity was measured. Formazan dye changes to a purple-blue hue. With the use of a microplate reader, the absorbance was determined at 570 nm. The formula cell viability = [O.D. of treated cells/O.D. of control cells] 100 was used to calculate the percentage of viable cells.

Microscopic analysis

In a 6 well plate, 2x105 human gingival fibroblast cells were plated to study the effect of Annona Muricata SeNps on cell morphological changes. The cells were treated with and without Annona Muricata SeNps for 24h time point. After the treatment period, the cells were washed with PBS and observed in inverted phase contrast microscope [11].

Cell migration (wound healing or scratch assay)

On six-well culture plates, 2 105 human gingival fibroblast cells were plated. For 24 hours, Annona Muricata SeNps was subjected to treatment, and control cells were given a culture medium devoid of serum. With a 200-ul tip, a wound was made in the cell monolayer, which was then scratched, PBS-washed, and photographed with an inverted microscope. The injured area was captured using the same microscope after the healing time. Additionally, the studies were carried out three times, once for each treatment group.[11,12].

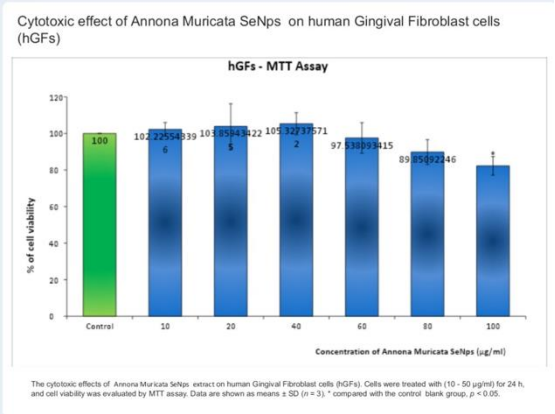
RESULTS

Primary culture of gingival fibroblast

The established primary culture of gingival fibroblast was microscopically observed for morphological confirmation. The morphology of the cells was in good condition and the cells were used for the further experiment after 2 passages.

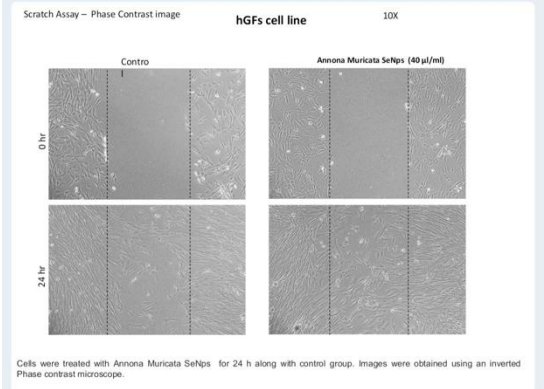
Cell proliferation

The cell proliferation assay defines the ability of the cells to multiply and is also associated with the viability of the cells. The cells after treatment with A. muricata SeNps showed increased proliferation when compared to the untreated cells. This shows the lower cytotoxicity of A. muricata SeNps. Figure 1 represents the proliferation of A. muricata SeNps in different concentrations.



Cell migration

Similar to cell proliferation, the ability of the cells to migrate was found to be relatively higher in A. muricata SeNps when compared to normal cells. Figure 2 represents the migration images taken at 0 hrs and 24 hrs in control and treated cells.



DISCUSSION

The remarkable anti-inflammatory and anti-cancer properties of Annona muricata extracts (AME) make it a targeted plant extract to be researched for its immunomodulatory properties. Traditional healers have used different elements of AME to treat a range of illnesses, such as cancer, diabetes, and inflammation. In 2019, Sabapathi and his colleagues established a study to identify the characteristics and cytotoxicity of AMEs. Using the MTT and Apoptosis assays, the in vitro cytotoxicity efficacy of AMEs loaded with solid lipid nanoparticles (SLNs) was assessed and compared to a free extract. When compared to free extract, the SLNs were more effective at killing MCF7 cancer cells and had a

noticeable apoptotic effect. Therefore, extract-loaded SLNs may be a different dosage form that controls therapeutic action while minimizing side effects [13].

Later in 2020, Alshaeri and his colleagues evaluated the antiproliferative effects of AMEs and one of their active fractions on BT-20 TNBC cells as well as the mechanisms underlying those effects. The MTS assay revealed that the AME significantly reduced the expression of the NF-B p65 protein and the viability of BT-20 cells. Eight fractions were obtained after preparative thin layer chromatography (TLC) was performed on the AME. Only fraction 4 (F4) out of the eight fractions significantly reduced cell viability in the MTS assay. According to an immunoblotting analysis, AME and F4 had an antiproliferative effect. A downregulation of cyclin D1 assembly, which resulted in cell-cycle arrest at the G1/S phase, complemented these effects. In addition, NF-B was assessed due to its role in the development of cancers. The study concluded that inhibition of the EGFR-mediated signalling pathways, which include AKT, MAPK, NF-B, and cyclin D1, results in the antiproliferative effect [14].

In a study by Akpan et al (2021), crude AME extracts, poly(lactic-co-glycolic acid)/gelatin (PLGA/Ge), and pluronic F127 were used to create electrospun hybrid scaffolds. The drug loading capacity was found to be increased and the sustained AME release from the fibers was found to be prolonged by the addition of Pluronic F127. Due to the induction of apoptosis, the released AME significantly reduced the in vitro growth of breast cancer cells more than non-tumorigenic cells. This finding supports the use of pluronic F127-containing electrospun fibers for sustained and targeted AME delivery to breast cancer cells [15]. In another study by Wahab et al (2022), dose-dependent inhibition of neutrophil migration, ROS production, phagocytic activity, and expression of CD11b/CD18 integrin was identified on treatment with AME which showed its ability of significant immunosuppressive effects on innate immune parameters. The study provided proof that AME has the potential to be a significant source of efficient immunosuppressive agents due to its strong inhibitory effects on the various humoral and cellular response parameters [16].

In addition, Mahmood with his colleagues in 2022 studied the effect of AMEs in copper oxide nanoparticles for cytotoxicity in breast cancer cell lines. The findings implied that the prepared copper oxide nanoparticles (CuONPs) inhibited the expansion of particular cell lines associated with breast cancer. After 24 hours of continuous CuONPs exposure, it was found that cancer cells had minimal colony formation using the concentration for AMJ-13 than MCF-7 cells. It shows that CuONP uptake by cancer cells results in apoptosis. [17,18]. Additionally, treatment with CuONPs increased the production of lactate dehydrogenase (LDH), which was likely brought on by cell membrane damage that led to leaks containing cellular components like lactate dehydrogenase. Thus, according to the research findings, the synthesized CuONPs precipitated anti-proliferative effects by inducing apoptosis, which results in cell death [19,20].Similarly, several studies have tested the cytotoxicity and efficacy of A. muricata extracts in various diseases. This is the first study to explore the cytotoxicity of A. muricata SeNp extracts in gingival fibroblasts [21,22].Our study has shown the proliferation, migration and apoptosis effects of A. muricata SeNp to be a potential therapeutic value. Thus further studies could validate its effect in the treatment of various diseases.

CONCLUSION

A. Muricata is one among the phytochemicals being studied for their potential role in the treatment of various human diseases. An in-depth analysis of the chemical makeup of A. muricata's leaves and seeds revealed acetogenin compounds, which explain the plant's medicinal properties. The findings from the present study imply that A. muricata SeNps is a promising candidate for disease treatment and merits additional study as a potential replacement for traditional medications. In terms of its potential therapeutic impact on various human diseases, it is a crucial compound with less cytotoxicity. Thus, future studies on A. muricata SeNps might pave way for the clinical breakthrough of the compound.

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