RESEARCH ARTICLE

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Genotoxicity potentials of methanolic extracts of Mimosa pudica against oral cancer cells

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ABSTRACT

To study the genotoxic potentials of methanolic extracts of mimosa pud is against oral cancer cells. Oral cancer also called as mouth cancer is type of head and neck cancer and is cancerous tissue growth located in oral cavity It may arise as a primary lesion originating in any of the tissues in the mouth, by metastasis from a distant site of origin, or by extension from a neighboring anatomic structure, such as the nasal cavity. Alternatively, the oral cancers may originate in any of the tissues of the mouth, and may be of varied histologic types: teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, or melanoma from the pigment-producing cells of the oral mucosa. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37oC in a humidified atmosphere with 5% CO2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 104 cells /well and allowed to attach overnight at 37oC. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours. The cell suspension was then collected and subjected to DNA isolation procedures. The methanolic extracts of M. pudica show anticancer activity on cancer activity on oral cancer cells. Here DNA fragmentation is done where more fragmentation is seen with dosage increased. So this proves that extracts show anticancer activity. Our study shows that the extracts which we used show anti cancer activity against oral cancer cells

ARTICLE HISTORY

Received October 03, 2020
Accepted November 15, 2020
Published December 12, 2020

KEYWORDS

Genotoxic potential, Mimosa Pudica, DNA Fragmentation, DMEM medium, Gel electrophoresis

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INTRODUCTION

Oral cancer also called as mouth cancer is type of head and neck cancer and is cancerous tissue growth located in oral cavity(FERLAY and J, 2004) (Garrote et al., 2001) .It may arise as a primary lesion originating in any of the tissues in the mouth, by metastasis from a distant site of origin, or by extension from a neighboring anatomic structure, such as the nasal cavity. (Franceschi et al., 2000).(Gillison, 2000) Alternatively, the oral cancers may originate in any of the tissues of the mouth, and may be of varied histologic types: teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, (Franceschi et al., 2000; Castellsagué et al., 2004) or melanoma from the pigment-producing cells of the oral mucosa. There are several types of oral cancers, but around 90% are squamous cell carcinomas,originating in the tissues that line the mouth and lips (Sánchez et al., 2003)(Nascimento et al., 2000). Oral or mouth cancer most commonly involves the tongue. It may also occur on the floor of the mouth, cheek lining, gingiva (gums), lips, or palate (roof of the mouth).Most oral cancers look very similar under the microscope and are called squamous cell carcinoma, but less commonly other types of oral cancer occur, such as Kaposi's sarcoma.In 2013 oral cancer resulted in 135,000 deaths up from 84,000 deaths in 1990. Five-year survival rates in the United States are 63%.(Bosch et al., 2002)(Shah, 1998)

Mimosa pudica linn. (Family:mimosaceae) is used as an ornamental plant due to its thigmonastic and nyctinastic movements. M. pudica is also used to avoid or cure several disorders like cancer, diabetes ,obesity, and urinary infections. M. pudica is famous for its anticancer alkaloid, mimosine, along with several valuable secondary metabolites like tannins, steroids, flavonoids, triterpenes, and glycosyl flavones.a wide srrsy of pharmacological(Zampini, Vattuone and Isla, 2005) antioxidant,antibacterial,antifungal,anti-inflammatory,hepatoprotective,antinociceptive,anticonvulsant,antidepressant,antidiarrheal,hypolipidemic activities ,diuretic antiparasitic antimalarial and hypoglyciene have been attributes to M.pudica.The aim of the study is to evaluate the genotoxicity ability of M. pudica. Previously many studies have been done by our department like (Krishnan and Lakshmi, 2013; T et al., 2013; Lakshmi, Ramasamy and Thirumalaikumaran, 2015; Devaraj and Lakshmi, 2017; Ezhilarasan, Lakshmi, Nagaich, et al., 2017; Ezhilarasan, Lakshmi, Vijayaragavan, et al., 2017; Lakshmi et al., 2018; Ahamad et al., 2019; Trishala et al., 2019)

MATERIALS AND METHODOLOGY

Plant Material Mimosa pudica is obtained from green chem herbal extracts and formulations, Bengaluru, India. SCC25 cells were procured from NCCS Pune.Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37oC in humidified atmosphere with 5% CO2.The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 104 cells /well and allowed to attach overnight at 37oC. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours. The cell suspension was then collected and subjected to DNA isolation procedures. DNA fragmentation, reagents required ,cell lysis buffer, 40ml 1M tris 40ml of 0.5M EDTA, 20ml of 10% SDS, Final volume was made upto 200ml, 3.5 M ammonium acetate, Tris saturated phenol ,Chloroform: isoamylalcohol (24:1), Ice cold isopropanol ,70% ethanol

Procedure

$1*10^6$ cells were incubated with 100µl of cell lysis buffer at room temperature for one hour.This was centrifuged for 15 min at 3000rpm at 4°C to sediment the cell debris.To the supernatant equal volume of phenol: chloroform: isoamyl alcohol mixture was added to the supernatant and mixed well. This was centrifuged at5000 rpm for 15min.The supernatant was transferred to new tube.The 3rd step was repeated once.To the final aqueous phase 40µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA.This was incubated at -20°C for 1hour, followed by the centrifugation at 10000 rpm for 15min.The pellet was retained and washed with 70% ethanol and stored in 20-50µl of TE buffer.The samples were analyzed in 2% agarose gel stained with Ethidium bromide.(L, Harsha and Thangavelu, 2017)

Agarose gel electrophoresis

Preparation of agarose gel with 1X TAE buffer and stained with 2µlof ethidium bromide.The % of agarose depends upon the molecule to be separated. Samples loaded with loading dye (2µl of loading dye is used).Electrophoresis of DNA fragments at 50volts.Visualization of DNA fragments in the UV trans-illuminator.

RESULTS AND DISCUSSION

The(fig 1) below shows the DNA fragmentation in 6 lanes. Here more the fragmentation means more activity against cancer cells. So as the dosage of extracts. Increases the fragmentation also increases so does the anti cancer activity.

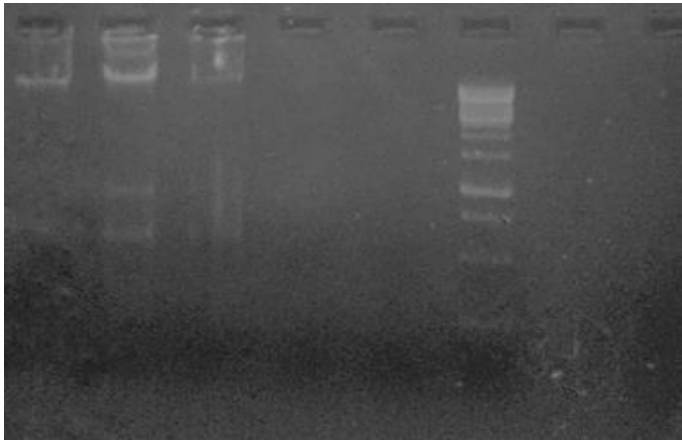

Figure 1: DNA fragmentation assay

Lane 1 – DNA from untreated cells
Lane 2 – DNA from cells treated with 125 µg sample
Lane 3 – DNA from cells treated with 75 µg sample
Lane 6 – 1 KB ladder

This study shows us that methanolic extracts of mimosa pudica shows genotoxic potentials on oral cancer cells. Similar study on Cytotoxic and genotoxic effects of Lavandula stoechas aqueous extracts was conducted The aim of this study was to investigate cytotoxic and genotoxic effects of aqueous extracts (40, 80 and 120 g/L) from L. stoechas flowers on Allium cepa root tip meristem cells. For this purpose, A. cepa onion bulbs were treated with the above-mentioned L. stoechas flower extracts for 72 h. Spring water (pH 7.3) was used as a control. The result of this study showed that aqueous extracts reduced mitotic index, but induced chromosome aberrations and mitotic aberrations in comparison with control, significantly ($p < 0.05$). Aqueous extracts induced breaks, stickiness, pole deviations and micronuclei. Furthermore, these effects were related to extract concentrations. These results showed that L. stoechas aqueous extracts have cytotoxic and genotoxic effects.(Talamini et al., 2000)

Similarly for Inula viscosa was done I. viscosa has been used for years in folk medicine for its anti-inflammatory, antipyretic, antiseptic, and paper antiphlogistic activities. In this study, cytotoxic and genotoxic effects of I. viscosa leaf extracts on the root meristem cells of Allium cepa have been examined. Onion bulbs were exposed to 2.5 mg/ml, 5 mg/ml, and 10 mg/ml concentrations of the extracts for macroscopic and microscopic analysis. Tap water has been used as a negative control and Ethyl methanesulfonate (EMS) ($2 \cdot 10{-}2$ M) has been used as a positive control. The test concentrations have been determined according to doses which are recommended for use in alternative medicine. There has been statistically significant ($P < .05$) inhibition of root growth depending on concentration by the extracts when compared with the control groups.(Zink and Chaffin, 1998) (Basaran et al., 1996) All the tested extracts have been observed to have cytotoxic effects on cell division in A. cepa. I. viscosa leaf extract induces (Plewa and Wagner, 1993)(Barboza et al., 2009) the total number of chromosomal aberrations and micronuclei (MNC) formations in A. cepa root tip cells significantly when compared with control groups. Also, this paper shows for the first time the induction of cell death, ghost cells, cells with membrane damage, and binucleated cells by extract treatment. These results suggest the cytotoxic and genotoxic effects of the I. viscosa leaf extracts on A. cepa.

Acacia aroma, native plant from San Luis, Argentina, is commonly used as antiseptic and for healing wounds. The present study was conducted to investigate the in vitro cytotoxicity and genotoxicity of hot aqueous extract (HAE) and ethanolic extract (EE) of A. aroma (Shama, Hridhya and Kulandhaivel, 2018) (del V. Carrizo, Palacio and Roic, 2002) The cytotoxic activity was assayed by neutral red uptake assay on Vero cell. Cell treatment with a range from 100 to 5000 µg/mL of HAE and EE showed that 500 µg/mL and 100 µg/mL were the maximum noncytotoxic concentrations, respectively. The CC_{50} was 658 µg/mL for EE and 1020 µg/mL for HAE. The genotoxicity was tested by the single-cell gel electrophoresis comet assay.

The results obtained in the evaluation of DNA cellular damage exposed to varied concentrations of the HAE showed no significant genotoxic effect at a range of 1–20 mg/mL. The EE at 20 mg/mL showed moderate genotoxic effect related to the increase of the DNA percentage contained in tail of the comet; DNA was classified in category 2.(Nascimento et al., 2000) (Basnakian and Jill James, 1994) At concentrations below 5 mg/mL, the results of cytotoxicity and genotoxicity of aqueous and ethanolic extracts of Acacia aroma guarantee the safety at cell and genomic level.(Brugés and Reza, 2007)However further

studies are needed for longer periods including animal models to confirm the findings. So all these studies above show that genotoxic potentials are very useful in various medicinal fields and in our study we used them against the cancer cells which showed its genotoxic potential and was able to act against cancer cells.

Authors Contribution

Both authors have equal contribution in collecting and preparing the manuscript for bringing out this research work .

CONFLICT OF INTEREST

Nil

CONCLUSION

So our study conducted shows us that the genotoxic potentials of methanolic extracts of mimosa pudica shows anti cancer activity on oral cancer cells. Here the fragmentation of DNA is seen increasing with increase in the dosage of the extract which attributes its anti cancer activity.

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