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Genotoxicity Analysis of Mace (*Myristica fragrans*) on Oral Cancer Cell Line By DNA Fragmentation

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Abstract:

Aim :To analyse the genotoxic activity of mace(Myristica fragrans) on oral cancer cell lines

Objective : Since genotoxins are mutagens, they cause mutations.Mace shows genotoxic activity and therefore this research aims in analysing genotoxicity of mace(Myristica fragrant) on oral cancer cell lines

Background : Cancer cell lines are genetic representative of tumours, and therefore they are useful tools in understanding the molecular changes associated with oral cancer. Working on these cancer cell lines provides a basis for any drug formulation and the effect on cancer as a whole. Cancer is one growing concern in the Indian population and thus the use of something as common as mace would help in the same.One of the components of mace is a compound similar to menthol, which has natural pain-relieving characteristics.Thus it would potentially have an effect on oral cancer.

Reason:Exploring the genotoxicity potential of herbs will pave way for replacing the synthetic anti cancer drugs and its side effects.Result : This analysis was done to prove the genotoxicity potential of mace on cancer cell lines through DNA fragmentation.

Result : This analysis was done to prove the genotoxicity potential of mace on oral cancer cell lines through DNA fragmentation.

Keywords: Cancer, chemotherapy ,DNA fragment, genotoxicity ,Indigenous spice, oral cancer cell line

INTRODUCTION:

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxins are mutagens; they can cause mutations.A substance that has the property of genotoxicity is known as a genotoxin. Oral cancer is a major problem in the Indian subcontinent where it ranks among the top three types of cancer in the country [1]. Age-adjusted rates of oral cancer in India is high, that is, 20 per 100,000 population and accounts for over 30% of all cancers in the country [2]. The variation in incidence and pattern of the disease can be attributed to the combined effect of ageing of the population, as well as regional differences in the prevalence of disease-specific risk factors [3]. Squamous cell carcinoma is the most prevalent malignant neoplasm in the oral cavity. The risk factors are tobacco and heavy alcohol consumption[4].

Mace spice is a dried, outer aril, enveloping firmly around the nutmeg kernel. The spice contains fixed oil trimyristine, and many essential volatile oils, which gives a sweet aromatic flavour such as myristicin, elemicin, eugenol and safrole. Also it's trace elements could be directly or indirectly responsible for the antitumor activity of mace[5].This study was done to analyse the genotoxicity activity of mace by DNA fragmentation.

DNA fragmentation is the separation or breaking of DNA strands into pieces which can be done intentionally by laboratory personnel or by cells, or can occur spontaneously [6]. Spontaneous or accidental DNA fragmentation is fragmentation that gradually accumulates in a cell. Leukemic cells have been shown to generate several classes of DNA fragments after treatment with cytotoxic cancer chemotherapy agents[7].

Additionally, active principles in mace spice have many therapeutic applications in many traditional medicines as anti-fungal, anti-depressant, aphrodisiac, digestive, and carminative functions Their radical scavenging capacity was carried out on 2,2′-diphenyl-1-picrylhydracyl (DPPH) radicalm, and they showed strong scavenging activity in comparison with synthetic antioxidants. Their reducing power was also determined, which also proved strong antioxidant capacity of essential oil and extract[8]. Likewise, mace blades contain more riboflavin (vitamin B-2).Mace contains more calcium, copper, iron and magnesium than nutmeg. 100 g of mace powder has 13.90 mg of iron when compared to just 3.04 mg of nutmeg. Manganese and copper are utilised by the human body as co-factors for the antioxidant enzyme, Superoxide dismutase. Iron is essential for red blood cell production and as a co-factor for cytochrome oxidase enzyme.

MATERIALS AND METHODS:

Mace was procured in Nuts and spices store.Cell line was purchased from ATCC.Extract was obtained by adding Dichloromethane (DCM).

Maintenance of cell line- The oral cancer cell line was purchased from ATCC.The oral cancer cells were seeded in 24 well plate and kept in an incubator . Incubation of cell lines with multiple concentrations of Mace extract. Cells were treated with the mace extract in 2 different concentrations (125 µl, 70 µl) for 24 hrs. Treated cells were

subjected to DNA fragmentation assay according to Alexei.G[9].

Isolation of genomic DNA

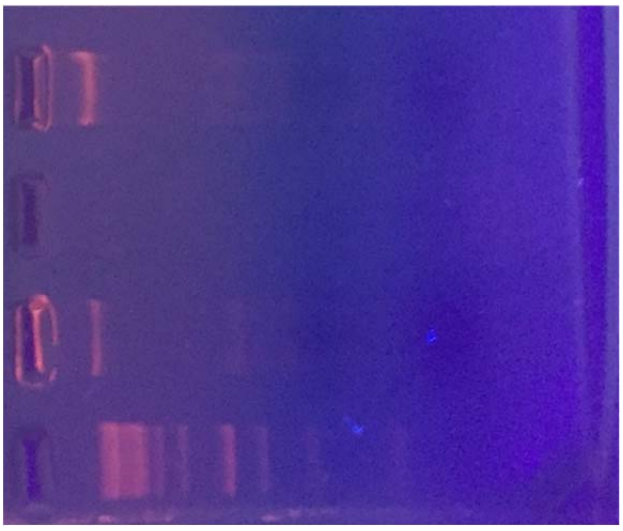
$1*10^6$ cells were incubated with 100µl of cell lysis buffer at room temperature for one hour. This was centrifuged for 15 min at 3000rpm at 4°C to sediment the cell debris. To the supernatant equal volume of phenol: chloroform: isoamylalcohol mixture was added to the supernatant and mixed well. This was centrifuged at 5000 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 analysed in 2% agarose gel stained with Ethidium bromide.

Analysis of DNA fragmentation by Agarose gel electrophoresis method

Preparation of agarose gel with 1X TAE buffer and stained with 2µl of 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.Visualisation of DNA fragments in the UV trans-illuminator.

RESULTS:



Lane 1 – 1 kb Ladder
Lane 2 – DNA from cells treated with 125 µg sample
Lane 3 – DNA from cells treated with 75 µg sample
Lane 4 – DNA from untreated cells

DNA fragmentation was observed with the two concentrations of mace extract as seen in Lane 2 and Lane 3 (as seen in figure 1) on oral cancer cell lines by agarose gel electrophoresis method. This proves that myristica fragrans(mace) shows genotoxicity on the oral cancer cells by degrading its DNA. Hence Mace has the potential to be an anticancerous drug[10].

DISCUSSION:

Genotoxicity refers to the property of a chemical agent which can alter the genetic information of an organism that can cause mutations which may lead to cancer.All mutagens are genotoxic[11]. Chemotherapy is the most effective and widely used treatment in most types of malignancies [12].Firstly it was thought that chemotherapy drugs specifically kill the cancer cells only but now it is well known that it also damages the normal cells resulting in the chemotherapy dose dependent side effects such as fatigue, nausea, hair loss, vomiting, etc and even death may also occur in severe cases. The main strategy of chemotherapy drugs is based on the phenomenon that these drugs selectively target the tumour cells, largely by the means of genotoxicity partially caused by the production of reactive oxygen species [13],which does not specifically damage the cancer cells but also the normal cells [14]. Total 132 cancer chemotherapy drugs are approved by the US Food and Drug Administration, of which 56 drugs have been reported to cause oxidative stress [15].Therefore, herbs and spices are considered a safer alternative.They spare the normal cells unlike the chemotherapeutic drugs.

CONCLUSION:

Mace shows genotoxicity property against oral cancer cell lines(KB).Its widely available and indigenous to few countries.They are natural remedies which are readily available and they pose no threat to a human's health. The use of synthetic drugs and radiation not only destroy cancer cells but they also cause damage to other cells thereby causing delayed wound healing[16] .Further research must be carried out and more of these herbs or spices must be incorporated into medicines given for oral cancer.

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