Research Article

Gentotoxicity Analysis of Neemoil on Oral Cancer Cell Line by DNA Fragmentation

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ABSTRACT

The aim of this study was to determine the genotoxicity of neem oil on oral cancer cell line by DNA fragmentation. Genotoxicity is defined as a destructive effect of a compound on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxins are mutagens and they can cause mutations. Neem oil (*Azadirachta indica*) is the most important commercially available products of neem for organic farming and medicines. Nimbin is a triterpenoid which has been credited with some of neem oil's properties. Neem oil also contains several sterols, including (campesterol, beta-sitosterol, stigmasterol). The genotoxic substance invades the nucleus and causes damage to the nucleic acid thereby causing cell death. Here the oral cancer cell lines were treated with three different concentration of neem oil and were maintained. DNA was isolated and on agarose gel electrophoresis it was evident that DNA was fragmented. This concluded that neem oil has the ability to degrade the DNA of oral cancer cells. Hence further research on neem oil can help in the invention of new anticancer drugs.

Keywords: Genotoxicity, neem, anti-cancer drug, oral cancer.

INTRODUCTION

Genotoxicity is a property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. The chemicals used has destructive effect on cell's genetic material i.e. DNA and RNA, which affects the integrity of the cell. All mutagens are genotoxic, whereas not all genotoxic substances are mutagenic. The alteration can have direct or indirect effects on the DNA: the induction of mutations, mistimed event activation, and direct DNA damage leading to mutations. The permanent, heritable changes can affect either somatic cells of the organism or germ cells to be passed on to future generations.[1] This DNA damage can be in the form of single- and double-strand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations, and structural and numerical chromosomal aberrations.[2] The compromised integrity of the genetic material has been known to cause cancer. As a consequence, many sophisticated techniques have been developed to assess the chemicals' potential to cause DNA damage that may lead to cancer.

The neem tree (*Azadirachta indica*), a member of the Meliaceae family widely distributed in Asia, Africa and other tropical parts of the world, elaborates a vast array of over 300 bioactive phytochemicals.[3] Although aqueous extracts of neem are anti-fungal, anti-bacterial and anti-viral,[4,5,6] several studies have provided evidence for the chemopreventive potential of neem extracts, it has become increasingly important to identify the phytochemicals responsible for chemoprevention.[7–11] Neem, or isolated compounds, have shown impressive efficacy against a wide variety of human cancer cell lines, and animal models for human cancers including oral carcinoma.

DNA fragmentation is the breaking or separation of DNA strands into pieces. It can be done intentionally by laboratory personnel or by cells, or can occur spontaneously. The genotoxic substance invades the nucleus and causes damage to the nucleic acid and the changes can be viewed by DNA fragmentation. In this article we will see about the genotoxic activity of neem oil on oral cancer cell lines by DNA fragmentation method.

MATERIALS AND METHODS

Maintenance of cell lines

The Oral cancer cell lines i.e KB (ATCC CCL-17) were acquired from ATCC. Oral cancer cells were seeded in 24 well plate and kept in CO2 incubator. Cells were treated with the neem oil in three different concentrations (100µl, 200 µl, 300µl) for 24 h. Treated cells were subjected to DNA fragmentation assay. (Alexei G.et.al., 1994)[12]

Isolation of DNA

Reagents

The cell lysis buffer comprising of 40ml 1M tris, 40ml of 0.5M EDTA, 20ml of 10% SDS and the final volume was made upto 200 ml. 3.5 M ammonium acetate, Tris saturated phenol, Chloroform: isoamylalcohol (24:1), Ice cold isopropanol, 70% ethanol was also used.

Procedure

1*106 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 at5000 rpm for 15min and the supernatant was transferred to new tube and the above mentioned step was repeated once again. Then to the final aqueous phase 40µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA. It 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.[12]

Analysis of DNA fragmentation by Agarose gel electrophoresis method

Reagents

TAE buffer (stock solution 50X) comprising Tris base – 242g,Acetic acid glacial – 57.1ml, EDTA 0.5M. The Working concentration being 1X. The gel loading buffer with Bromophenol blue, Xylene cynol and Sucrose was used including Agarose, Ethidium bromide 20mg/ml and Gel loading buffer.

Procedure

The agarose gel was prepared with 1X TAE buffer and stained with 2µl of ethidium bromide. The % of agarose depended upon the molecule to be separated. The samples were loaded with loading dye (2µl of loading dye was used). Electrophoresis of DNA fragments was carried out at 50volts. DNA fragments were visualised under the UV trans-illuminator.[12]

RESULTS AND DISCUSSION

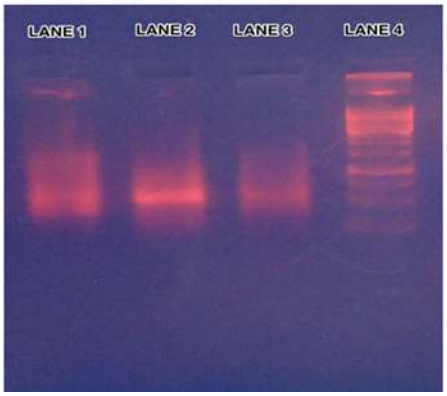

Figure 1: Gel showing the DNA fragmentation in lanes 1,2 and 3. Lane 4 shows the DNA ladder.

DNA fragmentation was seen in all the three lanes (1, 2, 3)[fig1]

Lane 1 – DNA from KB cells treated with 100µl sample

Lane 2 – DNA from KB cells treated with 200 µl sample

Lane 3 – DNA from KB cells treated with 300 µl sample

Lane 4 – DNA ladder.

DNA fragmentation was observed with all the three concentrations of neem oil on oral cancer cell lines by agarose gel electrophoresis method. Apoptosis has been characterised biochemically by the activation of a nuclear endonuclease that cleaves the DNA into multimers of 180-200 base pairs and can be visualised as an 'oligosomal ladder' by standard agarose gel electrophoresis.[12] This proves that neem oil shows genotoxicity on the oral cancer cells by degrading its DNA. Hence neem oil has the potential to be an anti-cancerous drug.

Genotoxicity is the ability of a substance to invade a cell and to destruct the DNA of the cell which leads to cancer on mutation. The genotoxic substances induce damage to the genetic material in the cells through interactions with the DNA sequence and structure. The purpose of genotoxicity testing is to determine whether a substrate, product, or environmental factor induces genetic damage. This entails cytogenetic assays using different mammalian cells.[13]

Oral cancer is one of the major worldwide threats to public health and it is associated with severe morbidity and long-term survival of less than 50% despite advances in the treatment (surgery, radiation, and chemotherapy). The survival of the patients remains very low, mainly due to their high risk of developing a second primary cancer. Therefore, the early detection and prevention of oral cancer and pre malignancy are quite important.[14–18] 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. Hence new approaches can be initiated by using neem tempered with other natural compounds may be of great promise in finding a sure cure for cancer patients and can be used to create further scope in the discovery of chemopreventive drugs.[19]

CONCLUSION

It is evident that neem oil has induced genotoxicity in the oral cancer cell lines. Further advanced research with neem can be highly beneficial for patients suffering from oral cancer. Neem as an anticancer drug is undoubtedly needed to reduce the devastating worldwide consequences of malignancy.

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