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Research Article

GENOTOXICITY OF WALNUT OIL (*JUGLANS REGIA*) ON ORAL CANCER CELL LINE BY DNA FRAGMENTATION

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ABSTRACT

Aim: To assay genotoxic activity of walnut oil on oral cancer cell line.
Objective: This study is to analyse the genotoxicity of walnut oil on oral cancer cell line by DNA fragmentation. The genotoxicity of walnut oil on oral cancer cell line is to be studied.
Background: Genotoxicityis a word in genetics defined as a destructive effect of a compound on a cell's genetic material (DNA, RNA) affecting its integrity. Genotoxins are mutagens; they can cause mutations. Genotoxins include both radiation and chemical genotoxins. Walnut oil (Juglans regina) is the most important of the commercially available products of walnuts for organic farming and medicines. Walnut is unique in that, unlike most other vegetable oils, it closely resembles sebum, a waxy substance produced by our skin glands, so it can act as a natural skin conditioner. It has nearly replaced animal fats in the manufacture of skin lotions and creams. The genotoxic substance invades the nucleus and causes damage to the nucleic acid. This changes caused can be viewed by DNA fragmentation. This study is to analyse the genotoxicity of walnut oil on oral cancer cell line by DNA fragmentation. The genotoxicity of walnut oil on oral cancer cell line was studied.
Reasons: Oral cancer has become very prevalent nowadays. This research was done to see if walnut oil can be effective against oral cancer cell line.
Result: From the above experiment and research it was proved that walnut oil has the potential to be an anti carcinogenic drug.

INTRODUCTION

Genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic. Genotoxic substances are known to be potentially mutagenic or carcinogenic when inhaled, ingested or penetrate the skin. The drug used in chemotherapy to kill cancer cells depends on its ability to halt cell division. Usually, cancer drugs work by damaging the RNA or DNA that tells the cell how to copy itself in division. If the cancer cells are unable to divide, they die. Chemotherapeutic techniques have a range of side- effects that depend on the type of medications used. The most common medications affect mainly the fast-dividing cells of the body, such as blood cells and the cells lining the mouth, stomach, andintestine. Chemotherapy- related toxicities can occur acutely after administration, within hours or days, or chronically, from weeks to years. Thus the property of genotoxicity is explored in herbs as it is safe. Herbs possessing genotoxicity property will act only on the cancerous cells leaving the normal cells safe. Herbs with genotoxicity can be tested for its anti cancer property. To assay for genotoxic molecules, researchers assay for DNA damage in cells exposed to the toxic substrates. 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. The compromised integrity of the genetic material has been known to cause cancer. As a consequence, many sophisticated techniques including Ames Assay, *in vitro* and *in vivo* Toxicology Tests, and Comet Assay have been developed to assess the chemicals' potential to cause DNA damage that may lead to cancer.

DNA fragmentation is the separation or breaking of DNA strands into pieces. It can be done intentionally by laboratory personnel or by cells, or can occur spontaneously. Spontaneous or accidental DNA fragmentation is fragmentation that gradually accumulates in a cell. DNA fragmentation is often necessary prior to library construction or sub cloning for DNA sequences. A variety of methods involving the mechanical breakage of DNA have been employed where DNA is

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fragmented by laboratory personnel. Such methods include sonication, needle shear, nebulisation, point-sink shearing and passage through a pressure cell.DNA Fragmentation plays an important part in forensics, especially that of DNA profiling.

Walnut oil is the oil extracted from walnuts (*Juglans regina*). Each 100 g of oil contains 63.3 g polyunsaturated fatty acids22.8 g monounsaturated fatty acids, and 9.1 g saturated fats. It contains no cholesterol. Unlike most nuts that are high in monounsaturated fatty acids walnut oil is composed largely of polyunsaturated fatty acids (72% of total fats), particularly alpha-linolenic acids (14%) and linoleic acids (58%), although it does contain oleic acid as 13% of total fats. There are two types of walnut oil commercially available: cold pressed and refined. Cold pressed walnut oil is typically more expensive due to the loss of a higher percentage of the oil. Refined walnut oil is expeller pressed and saturated with solvent to extract the highest percentage of oil available in the nut meat. The solvents are subsequently eliminated by heating the mixture to around 400 °F (200 °C). Both methods produce food grade culinary oils. Walnut oil, like all nut, seed and vegetable oils will undergo rancidificationaccelerated by heat, light, and oxygen.

Walnut oil is high in both vitamins and minerals. It works wonders on the skin and is highly recommended for people who want a flawless and ageless skin. Other benefits of walnut oil include:Fighting wrinkles, Remedy for infection, Great antioxidant, Helps fight hair loss, Reduces risk of cardiovascular diseases, Cuts belly fat, Boosts blood vessel functioning.

This study was done to analyse the genotoxicity of walnut oil on oral cancer cell line by DNA fragmentation. The genotoxicity of walnut oil on oral cancer cell line was studied.

MATERIALS AND METHODS

Procurement of oil

The walnut oil was procured from Cyrus India ltd. And further analysis was done using this oil.

Maintaining KB cell lines

The vial containing the KB cell lines were procured from National Centre for Cell Sciences (NCCS), Pune.The oral cancer cells were seeded in 24 Welles plate and kept in CO2 incubator.

Treatment of KB cell lines with drug (walnut oil)

Cells were treated with walnut oil in three different concentrations (100 µl, 200 µl, 300µl) and left alone for 24 hours.

Isolation of genomic DNA

The Cells were placed in a 37°C water bath. It was continuously until the medium thawed. Then it was centrifuged at 1000rpm for 5 minutes at room temperature. The supernatant was discarded and cells were washed with fresh medium to remove residual DMSO(Dimethyl Sulphoxide) which is an important polar aprotic solvent that dissolves both polar and non-polar compounds and is miscible in a wide range of organic solvents as well as water.The cell pellet was re-suspended in 3ml of of DMEM(Dulbecco's Modified Eagle's Medium: a composition that helps in maintaining mammalian cell culture) with 10% FBS(Fetal Bovine Serum which helps in easier coagulation of cells). It was then incubated in a CO_2 incubator at a humidified 37°C. The medium was changed every 2-3 days or when ph indicator (e.g. Phenol red) in medium changed colour. The culture was kept in a medium with 10% FBS until cell line were re-established.

The treated cells were then subjected to DNA fragmentation.

Analysis of DNA Fragmentation by Agarose Gel Electrophoresis Method

The extracted DNA is loaded to Agarose gel with the loading dye, DNA fragments was visualised under UV transilluminator.

RESULTS AND DISCUSSION

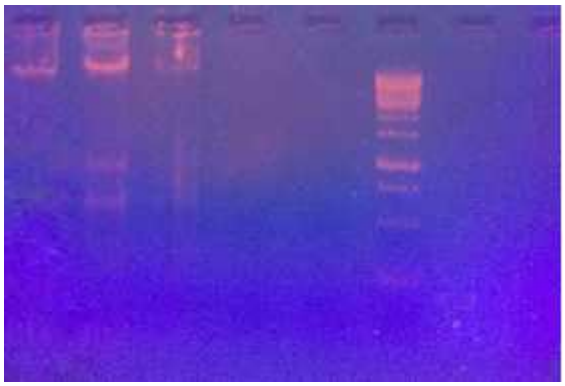

Figure 1

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

DNA fragmentation was observed with all the three concentrations of walnut 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 bevisualisedas an 'oligosomal ladder' by standard agarose gel electrophoresis .This proves that walnut oil shows genotoxicity on the oral cancer cells by degrading its DNA. Hence walnut oil has the potential to be an anti-cancerous drug.

Oral cancer is one of the major worldwide threats to public health. It is associated with severe morbidity and long-term survival is 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. 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. Therefore in near future new approaches can be initiated by using walnut oil 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 chemo preventive drugs.

CONCLUSION

From the above experiment and research it is proven that walnut oil has the potential to treat oral cancer. Walnut oil is

the most commonly available product of *Juglans regia*and is easily available in the market. Though research is still proceeding in various parts of the world to make use of this plant extract to treat cancer, oral-cancer in specific, there is less awareness among the masses. In near future the phytochemical properties of walnut oil may be used to design anti-cancer drugs. Also the medicinal property of the various natural herbs should be explored because than the other chemotherapeutic drugs, they don't affect the normal and healthy cells and they don't cause any side effects.

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