INTRODUCTION

Cytotoxicity is the quality of being toxic to cells. Types of venom are some examples of toxic agents or immune cell. Chemotherapy as a treatment of cancer relies on the ability of toxic agents to damage cells or to kill cells which are reproducing; this preferentially targets rapidly dividing cancer cells(8). Antibody dependent cell mediated cytotoxicity describes the cell killing ability of certain lymphocytes, which requires which required the target cells being marked by the antibody. Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Cytotoxicity can also be measured by sulforhodamine B assay, WST assay, etc(9).

Walnut oil is an oil extracted from walnut (juglans regina). Each 100g of oil contains 63.3g of polyunsaturated fatty acid, 22.8g monounsaturated fatty acid and 9.1g saturated fatty acid. It contains no cholesterol. Unlike most nuts that are high in monounsaturated fatty acid. Walnut oil has large amount of polyunsaturated fatty acid. Particularly, alpha-linolenic acids, linolenic acids (1).

There are two types of walnut oil they are cold pressed and refined. Cold pressed walnut oil is actually more expensive due to the loss of higher percentage of oil. Refined walnut oil expeller pressed and saturated with the solvent to extract the highest percentage of the oil available in the nut meal. Walnut oil is very much rich in vitamins and minerals.(2)

MATERIALS AND METHODS

Procurement of oil

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

Maintainence of kb cell line: The vial containing KB cell lines where 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)

The cells were treated with walnut oil in three different concentrations (100μl, 200 μl, 300μl) and left along 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 (5). 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)(6). It was then incubated in a CO2 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
RESULTS AND DISCUSSION

Cytotoxicity Of Walnut Oil On Oral Cancer Cell Lines

Cytotoxicity analysis using various concentrations of walnut oil (100, 200, 300 micrograms) was performed. The viability of the KB cell lines shows a gradual decrease as the concentration of the walnut oil is increased. This exhibits the Cytotoxicity of walnut oil extract with increasing concentration.

MTT Assay

The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X10^4 cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) X 100

Cytotoxicity % = 100 - Viability %

RESULTS AND DISCUSSION

Cytotoxicity analysis using various concentrations of walnut oil extract with increasing concentration.

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 chemopreventive 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.

References