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Cytotoxicity potential of flaxseed oil on oral cancer cell lines

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ABSTRACT

Flax seed, one of the oldest crops is a potential dietary supplement for healthy lifestyle. It is a very nutritious food that is rich in omega 3 fatty acids than any other plant source. It contains lignans which is a potential component in reducing tumorous growth effectively. To prove the cytotoxicity of flax seed oil on oral cancer cell lines, this study was conducted on KB cells, oral cancer cell lines. Flax seed oil is the main material for study and certain salts and chemicals are used for the cell viability assays. The cell viability assays used was the MTT assay and neutral red incorporation assay. The cell viability assays are done and the results showed the cytotoxicity of flax seed oil on the oral cancer cell lines. Hence, it can be used as a biomedicine against cancer which can reduce the adverse effects of cancer treatment and prove to be a life saving drug for oral cancer suffering people which can help many in the future.



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INTRODUCTION

Flaxseed is one of the most seasoned products that was developed from the beginning of human advancement. The Latin name of the flaxseed is *Linum usitatissimum*, which signifies "extremely valuable". Flax was first presented in United States by settlers, essentially to deliver fiber for apparel (Laux, 2011). All aspects of the flaxseed plant is used economically, either specifically or in the wake of preparing. The stem yields great quality filaments having high quality and solidness. Flax has been utilized until 1990s basically for the creation of materials (cloth) and papers, while flaxseed oil and its sub-items are utilized cows sustain (Singh *et al.*, 2011). In the modern viewpoint, there is a distinction in utilizing terms flaxseed and linseed. Flaxseed is utilized as sustenance while the

linseed is utilized as a part of extraction of oil and utilized as cows bolster (Morris, 2008). In the course of the most recent two decades, flax seed has been the territory of enthusiasm for the field of ailment cure and eating routine as it has numerous potential medical advantages and organically dynamic segments. Flaxseeds have numerous nourishing attributes and they are a rich wellspring of following ω -3 unsaturated fats: α -linolenic corrosive (ALA), short chain polyunsaturated unsaturated fats (PUFA), dissolvable and insoluble strands, phytoestrogenic lignans likewise called as secoisolariciresinol diglycoside-SDG, proteins and a cluster (Alhassane and Xu, 2010, Ivanova *et al.*, 2011, Oomah, 2001, Bhatta, 1995). Its developing ubiquity is because of its properties in the treatment of cardiovascular sicknesses, treatment of mammary and prostate disease, mitigating action, purgative impact, and lightening of menopausal side effects and osteoporosis.

Flaxseeds are accessible in two essential assortments in particular darker and yellow assortments. Both have comparative nutritious attributes and equivalent quantities of short-chain ω -3 unsaturated fats. The main flaxseed special case is a kind of yellow flax called solin (can also be called as Linola), which has a totally unique oil profile and is low in ω -3 unsaturated fats (Dribnenki *et al.*, 2007). Darker flax is also called a fixing in paints, varnish, fiber and dairy cattle nourish (Drouillard

et al., 2000, Kozłowska *et al.*, 2011, Faintuch *et al.*, 2011). Different palatable types of flax are accessible in the sustenance advertise entire flaxseeds, flax powder, simmered flax and flax oil. As indicated by its physical and concoction piece, flaxseed is an exceptionally valuable compound with dynamic parts of numerous plant substances, for example, oil, protein, dietary fiber, solvent polysaccharides, lignans, phenolic mixes, vitamins (A, C, F and E) and mineral (P, Mg, K, Na, Fe, Cu, Mn and Zn) (Faintuch *et al.*, 2011). Many cancer patients seek treatments with complementary and/or alternative medicine (CAM), especially when they have developed drug resistance towards chemotherapies. Flaxseeds as well as its oil components have been observed to exhibit various activities and reactions against different types of cancer making flaxseed a CAM candidate for human cancer treatment. Flaxseed oil could even increase the therapeutic efficacy against HER2-overexpressing breast cancer. The high mortality was noted in the society due to oral cancer so, in the current study we tested the cytotoxicity of flaxseed oil on KB oral epidermal cancer cell lines.

MATERIALS AND METHODS

Cell lines

KB cell lines were obtained from National Center for Cell Sciences, Pune. The cells were kept up in Minimal Essential Medium improved with 10% FBS, streptomycin (100 µg/ml) and penicillin (100 U/ml), in a humidified climate of 50 µg/ml CO₂ at 37°C.

Maintenance of cell lines

The vial containing the KB cell lines gained from ATCC (CCL - 17) was expelled from fluid nitrogen cooler and promptly put in a 37°C water shower. It was fomented constantly until the point that the medium defrosted. At that point it was centrifuged for 10 min at 150 to 200 × g, room temperature. Supernatant was disposed of and cells were washed with new medium to expel leftover DMSO.

Cell viability assay

MTT Assay is a colorimetric assay that measures the diminishment of yellow 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The KB cells were routinely developed and subcultured as monolayers in DMEM supplemented with 10% infant calf serum. At this stage, the cells were plated roughly 30,000 cells/well in 96 well-microtitre plates and left to rest for 24 h at 37°C of a humidified environment of 5% CO₂. The cells were then presented to with/without different centralizations of the flaxseed oil or the medium alone (as would be expected). Groupings of the flaxseed oil

extending 25 - 100 µg/ml and standard medication cyclophosphamide (100 µg) were utilized. Toward the finish of the period, cytotoxicity was surveyed by assessing the reasonability of the KB cells by the MTT decrease measure. After 1 h hatching, the test arrangement from each well was expelled by yearning and supplanted with 50 µl of MTT arranged in MEM without phenol red (MEM-PR). The plates were delicately shaken and hatched for 3 h at 37°C of humidified 5% CO₂ air. The supernatant was expelled and 50 µl of propanol was included and the plates were tenderly shaken to solubilize the framed formazan. The MTT enters the cells and goes into the mitochondria where it is decreased to an insoluble, shaded (dim purple) formazan item. The cells are then solubilised with a natural dissolvable (eg. isopropanol) and the discharged, solubilised formazan reagent. Since lessening of MTT can just happen in metabolically dynamic cells the level of movement is a measure of the feasibility of the cells.

The cell culture suspension was washed with 1x PBS and afterward added with 200µl MTT answer for the way of life (MTT 5mg/volume broke down in PBS). Evacuate all MTT wash with 1x PBS and add 300ml DMSO to each one culture. The absorbance was measured using a microplate reader at 540 nm.

Cytotoxicity assay by neutral red incorporation

The neutral red (NR) fuse strategy, depicted by Borenfreund and Puerner (1985), was used to survey cytotoxicity through lysosome possibility. The flaxseed oil in a range from 0-2.5mg/mL was incubated for 24 hours, the test was performed by Trintinaglia *et al.* (2015). Cell monolayers created in 48-well culture plates were brought forth for 24h at 37°C with different doses of oil, in triplicate. By then, medium was emptied and 500 µL of NR (30 µg/mL in MM) was added to each well. The plates were brought forth once more for 3 h at 37°C to propel the take-up of the shading by cells. In this way, the supernatant was cleared. The monolayers were washed with PBS, and 500 µL of extraction course of action (H₂O: acidic destructive: ethanol) (49: 1: 50) was merged in each well. After carefully shaking the plates, the absorbance was read on spectrophotometer at 540 nm.

Statistics

The comparisons were performed utilizing one-way (ANOVA) trailed by the LSD test for post hoc examination. Measurable noteworthiness was acknowledged at a level of p<0.001. Information were analysed utilizing SPSS (edition 11).

RESULTS AND DISCUSSION

The Figure 1 showed the results of MTT assay of flaxseed oil and positive control. Positive control is a parameter in which the response is a known response and the unknown response of the treatment is compared with it. So, these results depict that the apoptosis of the KB cells increases as the concentration of the flax seed oil increases (Chen *et al.*, 2009). The cytotoxicity of flax seed oil was seen at the peak when the concentration of flax seed oil is at its highest. The observation seen in this assay is the varying color difference in the test tube solutions after incubation. As the color intensity of the solutions increases, the viability of the cell also increases (Adlercreutz,2007).

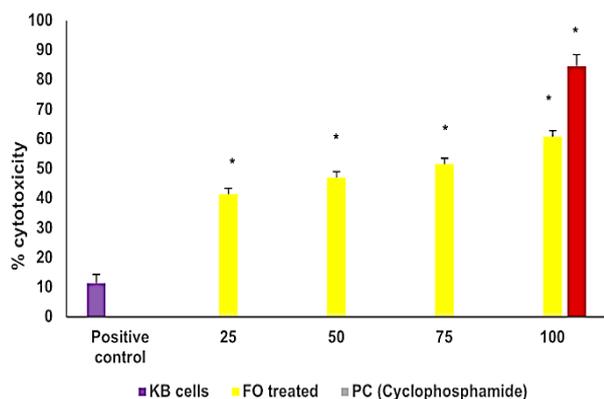


Figure 1: Impact of flaxseed oil and positive control cyclophosphamide cytotoxicity activity on KB cells, 24h after presentation

The information are mean ± SD of three free tests. *p<0.001 fundamentally as contrasted and positive control.

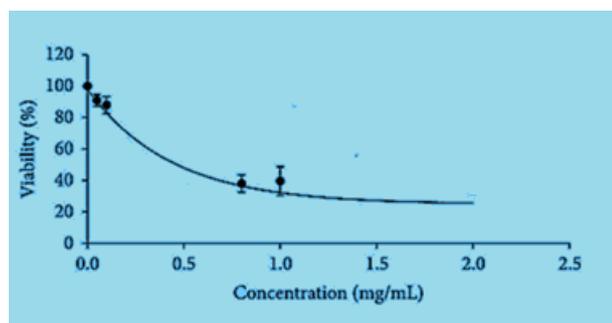


Figure 2: Level of reasonability of refined KB cells, brooded for 24 h within the sight of flaxseed oil at various fixations controlled by impartial red take-up (NRU).

Each point speaks to the mean ± SD of three autonomous trials.

The Figure 2 speaks to the cell reasonability by unbiased red joining measure. The impartial red measure is utilized to check the cytotoxicity of the part utilizing lysosome feasibility. The viability percentage of the KB cells decreases as the concentration of the flax seed oil increases. The oral can-

cer cell lines are effectively killed when the concentration of the flax seed oil is increased (Austria *et al.*, 2008). The Figure 3 is before the intake and Figure 4 after the uptake and accumulation of neutral red dye into the cells.

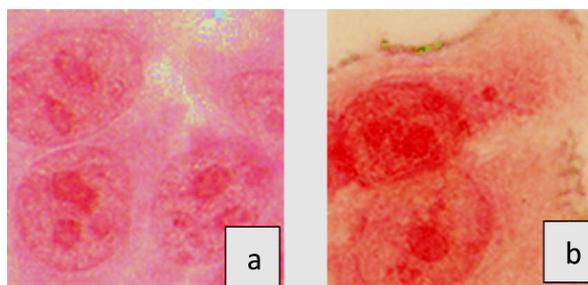


Figure 3: Uptake and accumulation of neutral dye into cells. (a) Cells incubated with flaxseed oil for 24 h (b) Control cells visualized by bright field microscopy

The flax seed oil was proven to be a very good alternative for treating different types of oral cancer. This has proven to be a good cytotoxic agent in this study as well as a very good nutritious food to be added in our diet (Austria *et al.*, 2008, Cunnane *et al.*, 1995). The fatty acids found in the flax seed oil was seen to prevent different types of cancers like breast cancer and the oral cancers (Faintuch *et al.*, 2011). From the correlation from various investigations of flax seed oil, it is dissected that the plant item is sheltered to use in treatment of oral disease (Ashwini, 2017). This examination can help in sparing numerous lives later on as very nearly 7% of the total populace is passing on of various sorts of malignancy.

CONCLUSION

Flaxseed oil could be considered as potential well-springs of anticancer mixes yet additionally contemplates are fundamental for detachment and distinguishing proof of organically dynamic substances. This product can be used as an effective adjuvant for the treatment of cancer in the near future.

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