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Antitumor Activity of Flaxseed Oil Cold Pressed Extract against Lung Cancer

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ABSTRACT

Cancer is one of the leading causes of mortality worldwide. Tumor cells circulating in the blood evidence the migmiceion of tumor from the site of origin to another site leading to the formation of new metastatic lesion and establishment of metastatic tumors. Thus, we designed the present study to investigate in detail the antitumor effects of *flaxseed oil*. The cytotoxic effect of flaxseed *oil* on A549 *in vitro* as determined by a Trypan blue assay and evaluated under an inverted microscope by hemocytometer. Furthermore, results of staining assays demonstmiceed that flaxseed oil has significant antitumor effects on A549 cells via apoptosis, in a concentmiceion-dependent manner. Finally, the antitumor effects of flaxseed *oil* were evaluated *in vivo* by using transplanted tumor nude mice, and the results confirmed that flaxseed *oil* has a notable antitumor effect on A549 cancer via mitochondria-mediated apoptosis. By assessing tumor volume, viable and nonviable tumor cell count, tumor weight, histopathology hematological parameters and biochemical estimations.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death in both men and women worldwide. [1, 2] Lung cancers are commonly classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), among which NSCLC constitutes approximately 75% of lung cancer cases nowadays .[3] Improvements have been made in diagnosis and treatment of lung cancers; however it remains aggressive cancer with a poor prognosis[4].

Surgical removal of tumors from the primary site could also benefit the patients providing long-term disease-free survival, but the presence of circulating tumor cells contributes to the development of metastasis in secondary sites[5]. Approximately, more than a million of cancer cells are shed into the bloodstream every day during the metastatic process of a primary tumor[6]. Although most of these cells are detected in the blood of end-stage cancer patients [7], they fail to form metastasis due to rapid destruction [8]. With this scientific knowledge, inhibition of metastatic processes could be a leading approach to control cancer metastasis [9]. Phenolic compounds containing antioxidants were used in the prevention or control of deleterious health effects and are therefore very useful for the reduction of risk of chronic diseases [10] including various cancers and inflammatory disorders [11].

Plant lignans occur in many foods, with flaxseed and sesame seed presently recognized as the richest source. Representative furfuran type lignans from edible plants, such as matairesinol, secoisolariciresinol, lariciresinol, and pinoresinol, are known to be converted by gut microflora to mammalian lignans, enterolactone or enterodiol [12, 17], which may have protective effects against hormone-related diseases such as lung cancer.

Flax is making its mark in the world's food supply as a functional food. It delivers a health boost beyond what might be expected from their traditional nutrient content. Flax fits this description perfectly, being rich in alpha-linolenic acid (ALA), the essential omega-3 fatty acid, and phytochemicals such as lignans (Morris 2003).

MATERIALS AND METHODS

Flaxseeds were collected from the local market, identified as (*Linum usitatissimum* L.) by the pharmacognosy Prof. Dr. Naglaa in the College of pharmacy/ Medical University.

Cleaning flax from derbies, which include other plants seeds, some parts of vegetarian of flaxseed and dust, Secondly flaxseeds properly extracted sepamiceely by a cold pressing grinder machine collected & filtered the oil and used for further studies of *in vivo* and *in vitro* studies.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was done to detect the presence of carbohydmicees, proteins, saponins, alkaloids, flavonoids, tannins, tri-terpenoids and phenolic compounds according to the procedure described in "Textbook of Practical Pharmacognosy" by *C.K. KOKATE*.

Detection of chemical compounds in cold pressed oil extract

Chemical detection was carried out using different reagents to determine the quality of active compounds exists in the extracted oils.

Detection of sesamin using (HPLC)

Quality and quantity analysis were done by HPLC technique analysis using C-18 column, 50 × 4.6 mm I.D column, the mobile phase used was 1% phosphate buffer(pH =4.5): acetonitrile: water (60:40), and the flow micee was 1ml/min at 264 nm. The volume of injected extract and standard lignin were 20µl.The peak area was calculated and compared with standard.

***In vitro* Cytotoxicity studies using A549 cell line**

A549 cell line was obtained from Sharjah University, UAE. The steps and procedure for cell culturing, Thawing, Revival, and Propagation of Cells were followed as described by D. F. Basri et al.

Procedure

A549 cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 10% Fetal Bovine Serum (FBS Invitrogen) and 1% penicillin/streptomycin. The cells were collected and when they reached 70-80% confluence. The viability was checked and centrifugation was performed. About 50,000 cells / well were seeded in a culture flask plate and incubated for 48 hrs at 37^{o}C, 5% CO_2 incubator and treated

with different concentmiceions of dose samples were tested since 0-50µl (2-fold variation) without FBS and incubated for 48 hours. After incubation with test samples, the medium was pipetted out without disturbing cells and the T flask washed with 1%PBS, then, trypsin EDTA 1ml/well DMEM 5ml/well was added and incubated for 5minutes. After incubation, the medium collected and centrifuged. The deposited pellets were mixed well with 1ml of fresh DMEM. 10 µl cells were diluted in 10 µl of trypan blue. The cells were placed and filled in a Hemocytometer, then the cell death and cell viability were calculated by the inverted fluorescent microscope

Calculating Inhibition

We used this equation to calculate the inhibitory activity of the oils:

% Inhibition = 100 – (OD of the sample/OD of Control) x 100.

***In vivo studies* of antitumor activity**

Animals

Thirty Albino mice, which have the weight range (18gm-30gm.) were obtained from Dubai. All animals were kept under constant environmental conditions with a 12/12 light-dark cycle and tempemiceure of 23 ± 2^0C, fed with standard granulated chow, and were given some drinking water ad libitum. The animal experiments were carried out in accordance with the Institutional Protocols of Animal Care.

Tumor Transplantation & Treatment Schedule

The albino mice were divided into five groups of four animals each. (N=6). The mice from the groups1, 2, 3, and 4 were transplanted with lung cancer cell lines A549 [(0.2ml) 2×10^6 cells/mouse] subcutaneously under the lungs. The animals were divided and administered daily through to, group 1 control and group 2 received 5 fluorouracil 20mg/kg, group 3& 4 administered flaxseed oil (2ml/kg&5ml/kg)after 3rd third until the twenty-eighth day.

Serum parameters

On a twenty-ninth day, the body weight and body circumference were noted and mice were sacrificed using light ether anesthesia. Blood was collected by the carotid bleeding method

and centrifuged using Remi cool centrifuge at 4000 RPM for 20 min. the Serum was sepamiceed and various biochemical parameters were estimated; LDL, HDL, triglyceride, and total cholesterol and carcinogenic embryonic antigen (CEA)

Histopathological Estimation

At the end of this experiment (day 29), all animals were anesthetized with light ether and the Lung tissue (lung cancer) were excised out and fixed in buffered formalin (10%). five microns think sections were prepared using microtome, these sections were stained with hematoxylin and eosin for histological altemiceions and observed under the light microscope with 40x magnification.

Statistical Analysis

Statistical analysis was performed as the mean± standard deviation (SD). The results were analyzed for statistical significance by unpaired t-test followed by Dunnett's post hoc test of significance. A P value less than 0.05 were considered as statistically significant.

RESULTS

***In vitro* studies**

The cytotoxic activity of the *flaxseed oil* was against A549 cell lines. The percentage of cell line viability were examined after 48 hrs dumiceion under the inverted microscope using hemocytometer its shown that the percentage viability was decreased in *flaxseed oil* group flask compared to the standard drug 5-fluorouracil and the control cell percentage viability is increased shown figure: 1. Apoptosis is considered the most potent defense against cancer cell development**.**

***In vivo* studied animals**

Effect of flaxseed *oil* on Serum Parameters

Treatment with *flaxseed oil* 5ml/kg had a significant decrease in tumor marker and lipid profile comparable to those of 5-fluorouracil treated animals. There was a significant ($p<0.001$) decrease in CEA, Triglyceride, Cholesterol, LDL, ALT, ALP level and Tumor size in cell line induced group 4 *flaxseed oil(5ml/kg)* when compared to the normal control animal similar to the levels in standard 5-Fluorouracil treated group in the lung cancer induced

group *'s* animals, when compared to the normal control animals. (As shown in figure2& Table 1).Treatment flaxseed oil group 4 showed the significant increase in the levels of HDL ($p<0.01$) (As shown in figure2& Table1)

Estimation of Tumor size

There was a significant ($p<0.01$) decrease in the tumor size on group 4 *flaxseed oil* administered animals and group 2 standard 5- fluorouracil drug compare to the control animal tumor mass volume shown in figure 2.

Histopathological studies

Severe submucosal inflammation, severe alveolar and nuclear cell wall damage noticed in control and group2 and 4 flaxseed oil reduced inflammation and alveolar damages noticed as shown in figure3

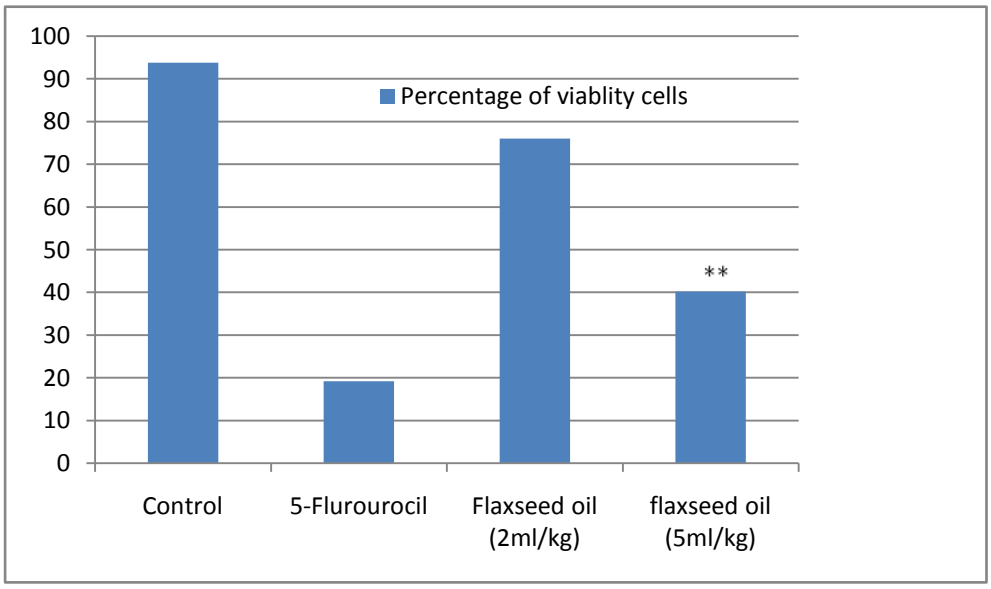

Figure 1: Investigations of cell viability on *in vitro* studied

Figure 1: There was a significant ($p<0.001$) decrease the percentage of cell viability in the flaxseed oil gorup4 compared to the standard drug 5-fluorouracil

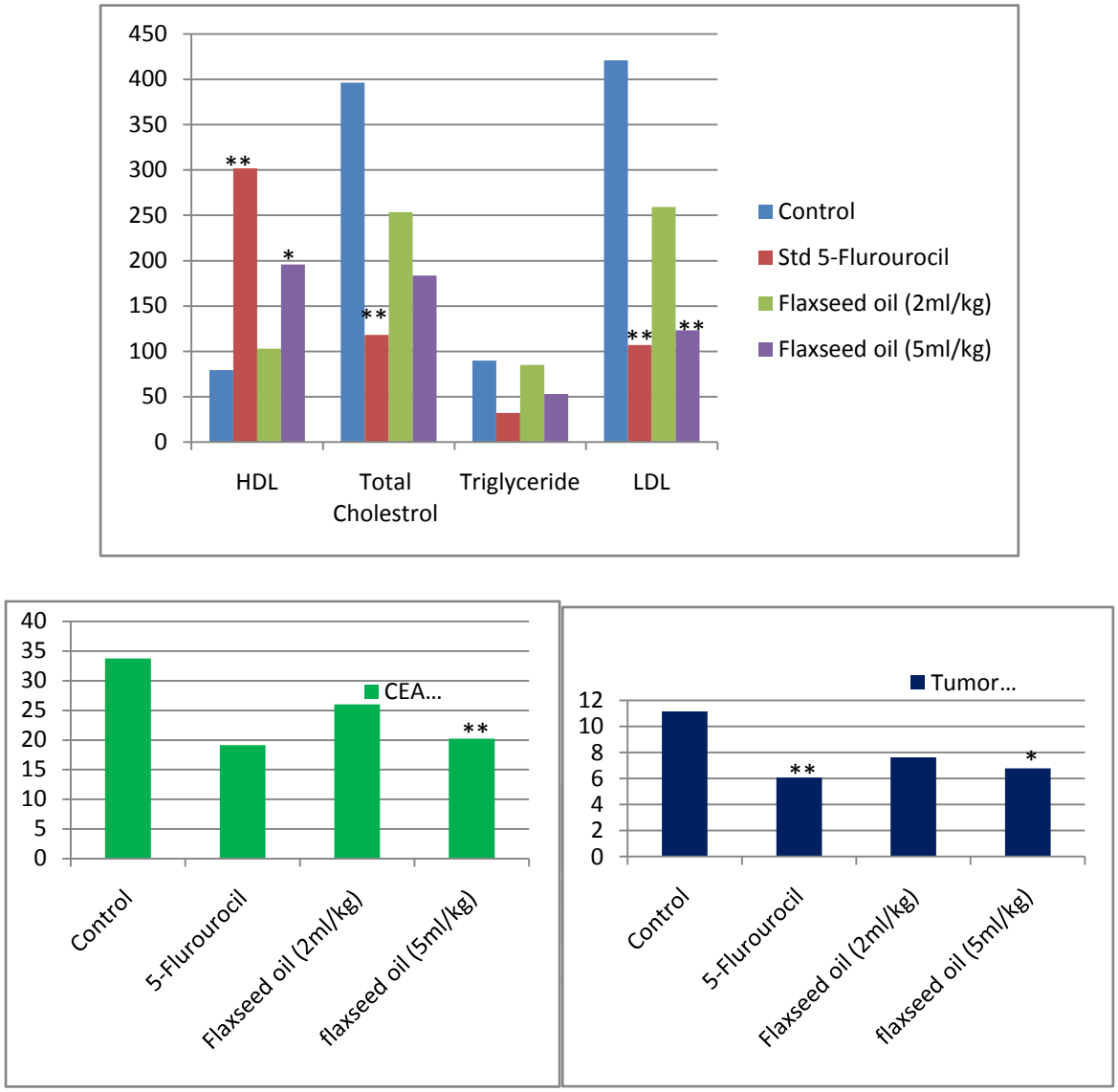

Figure 2: Biochemical Investigations of *In vivo* studied animals

Figure 2: There was a significant($p<0.001$) decrease in CEA, Triglyceride, Cholesterol, LDL level and Tumor size in cell line induced group4 flaxseed oil compared to the normal control animal similar to the levels in standard 5-Fluorouracil treated group in the lung cancer induced groups.

Table 1: Biochemical parameter of treated animals Serum analysis

Animal Group	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	CEA (ng/ml)
Control	396.3±1.53	89.66±0.95	79.33±6.05	421.13±2.24	41.08±1.46
Standard	118±0.99**	32±1.63***	301.6±3.36*	107±0.96	18.03±1.82***
Flaxseed oil 2ml/kg	253.66±1.08**	85.01±6.75	103±2.06*	259.33±0.96	35.24±1.65
Flaxseed oil 5ml/kg	183.5±0.46**	53.03±1.82	195.75±3.31	123.25±0.87**	39.89±0.99**

*Values are represented as mean ±SD, where n=6, ***P<0.001 as compared to normal control, **p<0.01 as compared to normal control*

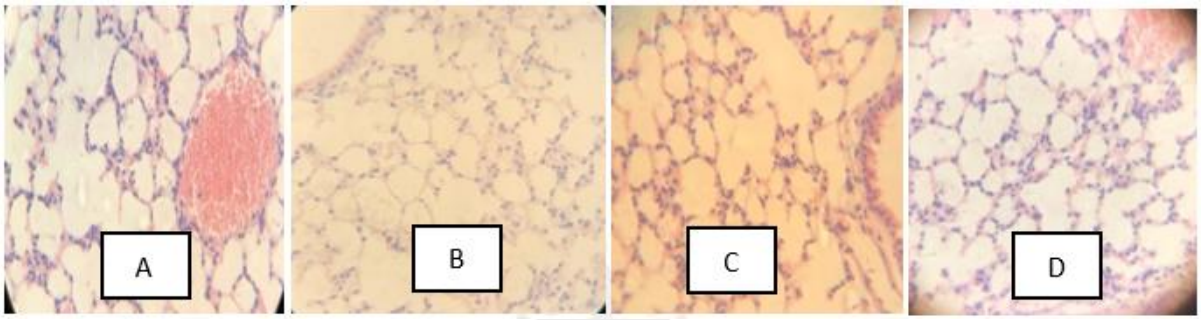

Figure 3: Histopathology of breast tissues

Figure 3: A: Control B: standard C: flaxseed OIL 2ml/kg D: flaxseed oil 5ml/kg. A549 Cell line induced: Severe submucosal inflammation and alveolar nuclear cell wall damage noticed in group A&C.

CONCLUSION

The *flaxseed oil 5ml/kg* and standard groups of animals shows the blood serum levels of ALT, ALP, LDH and CEA were decreased in conditions compare to lung cancer induced control animals. But the HDL level is increased in the *flaxseed oil 5ml/kg groups* compare to lung cancer induced control animals .The tumor size of *flaxseed oil 5ml/kg group* is significantly decreased as compare to the standard group animals.

Increases in LDH levels are usually found in cellular death and/or cell membrane damage and the *flaxseed oil* extract treated groups showed significant decrease in LDH levels.

An increased level of CEA was observed in colorectal cancer, breast cancer, gastric cancer, lung cancer. CEA measurement is mainly used as a tumor marker to monitor cancer treatment. In the present study increased levels of CEA were observed in cell line induced lung cancer in mice when compared to normal control mice. The animals treated with *flaxseed oil 5ml/kg group* showed significant decrease in levels of CEA in the serum when compared to cell line induced control groups.

Based on our data we can assume that flaxseed oil showed the antioxidant and antitumor properties by inducing apoptosis and thereby indicating the chemopreventive nature of natural products

REFERENCES

1. Jemal, A.; Siegel, R.; Ward, E. Cancer statistics. CA Cancer J. Clin. 2007, 57, 43–66. Molecules 2014, 19 8750
2. Wen, J.; Fu, J.H.; Zhang, W.; Guo, M. Genetic and epigenetic changes in lung carcinoma and their clinical implications. Modern Pathol. 2011, 24, 932–943.
3. Liu, Y.; Sun, W.Y.; Zhang, K.T.; Zheng, H.W.; Ma, Y.; Lin, D.M. Identification of genes differentially expressed in human primary lung squamous cell carcinoma. Lung Cancer 2007, 56, 307–317.
4. Smith, E.L.; Denissenko, M.F.; Bennett, W.P. Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. J. Natl. Cancer Inst. **2000**, 92, 803–811.
5. Gul N, Bogels M, Grewal S et al. Surgery-induced reactive oxygen species enhance colon carcinoma cell binding by disrupting the liver endothelial cell lining. Gut 2011;60:1076-1086
6. Liotta LA, Kleinerman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res 1974;34:997-1004
7. Peck K, Sher YP, Shih JY et al. Detection and quantitation of circulating cancer cells in the peripheral blood of lung cancer patients. Cancer Res 1998;58:2761- 2765
8. Liotta LA, DeLisi C. Method for quantitating tumor cell removal and tumor cell-invasive capacity in experimental metastases. Cancer Res 1977;37:4003- 4008
9. Herd ZL. Suppression of B16 melanoma lung colonization by syngeneic monoclonal antibodies. Cancer Res 1987;47:2696-2703.
10. Nicloson GL. Organ-specific tumor metastasis: the role of preferential adhesion, invasion, and growth of malignant cell at specific sites. Cancer Metastasis Rev 1988;7:143-188
11. Chandrasekara A, Shahidi F. Antiprolifemiceive potential and DNA scission inhibitory activity of phenolics from whole millet grains. J Funct Foods 2011;3:159- 170
12. Lignans in man and in animal species. Nature. 1980; 287:740–2.
13. Wang LQ, Meselhy MR, Li Y, Qin GW, Hattori M. Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol, and enterolactone. Chem Pharm Bull (Tokyo). 2000;48: 1606–10.
14. Wang LQ, Meselhy MR, Li Y, Nakamura N, Min BS, Qin GW, Hattori M. The heterocyclic ring fission and dehydroxylation of catechins and related compounds by Eubacterium sp. strain SDG-2, a human intestinal bacterium. Chem Pharm Bull (Tokyo). 2001;49:1640–3.
15. Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignin production from various foods. Nutr Cancer. 1991;16:43–52.
16. Mazur W, Fotsis T, Wa¨ha¨ la¨ K, Ojala S, Salakka A, Adlercreutz H. Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. Anal Biochem. 1996; 233:169–80.

17. Fukuda Y, Osawa T, Namiki M, Saki T. Studies on antioxidative substances in sesame. Agric Biol Chem. 1985;49:301–6.