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ANTITUMOR ACTIVITY OF *FLAX SEED & SESAME SEED* EXTRACT MIXTURE AGAINST BREAST CANCER

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Abstract:
Breast cancer is a long term survival results in increased mortality. Some plant lignans can be converted by intestinal microflora to the mammalian lignans, enterodiol and enterolactone, which may have protective effects against hormone-related diseases such as breast cancer. The rich source of lignans is flaxseed, sesame seeds. The extraction and purification of lignan from flax seed and sesamin from sesameseed was made by using high performance liquid chromatographic (HPLC). The MCF-7 cell lines were induced subcutaneously in mammary pad region of female rat and the animals are treated for 28days with sesamin (40 mg/kg body), lignin (20mg/kg) and lignin & sesamin mixture (1:1) orally. On 30th day blood was collected through retro orbital puncture and sacrificed the animals. Serum biochemical parameters such as lactate dehydrogenase (LDH), High-density lipoprotein (HDL) cholesterol, Triglyceride,alkaline phosphatase (ALP), alanine transaminase (ALT), and Carcino embryonic antigen (CEA). Histopathology examination at the end of the experiment (day 31), the breast tissue (breast cancer) were excised out and fixed .Statistical analysis was performed as the mean± standard deviation (SD).
Keywords: Antitumor, HPLC, MCF-7, Lignan, Sesamin, CEA

1. Aims and Objective:
In this study, we investigated for the first time whether mixture of sesamin & lignan isolated from sesame seed, and flaxseed is converted to the mammalian lignans in vivo in rats. In addition, we compared the mixture of sesamin & lignin effect in breast cancer model.

2. Introduction:
Cancer is one of the leading causes of mortality worldwide. Tumor cells circulating in the blood evidence the migration of tumor from the site of origin to another site leading to the formation of new metastatic lesion and establishment of metastatic tumors.
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 [1, 6], which may have protective effects against hormone-related diseases such as breast cancer.
Sesame seed (*Sesamum indicum)*, a traditional health food in Asian countries, contains large amounts of the plant lignans sesamin, sesamolin, and sesaminol glucosides [6, 7]. Sesamin, the major fat-soluble lignan in sesame seed, affects lipid metabolism [8–10] and has antihypertensive [11-14] , and anticancer activities [15,16] . However, only a limited number of studies have examined the metabolism of sesame seed lignans [17-21] .
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) Flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components: oil containing approximately 59% a-linolenic acid) and the presence.

3. Materials and Methods:

Flaxseeds were collected from the local market, identified as (Linumusitatissimum 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 grinding flaxseeds properly by a grinder machine eventually obtained on a homogenized powder that was ready for extraction.

This stage involved defatting of flax oils by using Soxhelt apparatus according to (AACC, 1984). Extraction of Crude Lignan by used the method which was described by Rickard et al,(1996), involves taking 25 g of defatted powder treats with a mixture of Dioxan and Ethanol alcohol (1:1),(v:v),respectively, with a ratio (1:8),(w:v),(powder: solvent),sample put on magnetic stir for 4 hrs., then filtrated and the solvent was evaporated by rotary evaporator at 40 0C to obtained crud lignan.

Separation of Lignan:
The process of separation Alkaline hydrolysis of SDG oligomers according to (Li et al. ,2008 and Yuan et al..,2008) by using an alkaline hydrolysis solution (a methanolic NaOH , 20 mM,pH=8) at 50 ºC for hydrolyzing SDG oligomers. The mixture was filtered by whatman filter paper no.1 then the supernatant was concentrated with a rotary evaporator within 45 ^{0}C. Eventually, a thick sticky texture material ,pH was corrected into 3.0 through adding drops of sulfuric acid 2 molar then the sample was stored in 4 ^{0}C

Seeds collection and preparation of crude extract Sesamum indicum seeds were collected and separated from undesirable materials or plant parts, then grinded into coarse powder by electrical grinder and kept in clean plastic cans until use. Ground sesame seeds (100 g) were defatted by mixing with n-hexane (500 ml) with magnetic stirrer at room temperature. The resulting slurry was filtered in Buckner funnel through whatman No.1 filter paper and then left to dry for 12 hour. The dried defatted residue (63 g) was mixed with 80% ethanol for 6 hours, the resulting ethanolic mixture were filtered and evaporated under vaccum by rotary evaporator at 40˚C, the final residue powder weighted 2.8 g and stored at 4˚C until use [14].

3.1 Detection of chemical compounds in ethanolic extract:

Chemical detection was carried out using different reagents as mentioned in [15] to determine the quality of active compounds exists in crude extract.

3.2 Detection of sesamin using (HPLC):

Quality and quantity analysis was 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 rate was 1ml/min at 264 nm. The volume of injected extract and standard sesamin were 20µl.The peak area was calculated and compared with standared.

3.3 Preliminary Phytochemical Screening:

Preliminary phytochemical screening was done for the presence of carbohydrates, proteins, saponins, alkaloids, flavonoids, tannins, tri-terpinoids and phenolic compounds according to the procedure described in “Textbook of Practical Pharmacognosy” by C.K. KOKATE.[22]

3.4Experimental Design:

Animals

Thirty female rodent rat for breast cancer weighing 25-30gm were obtained from Dubai pharmacy college, Dubai, UAE. All the animals were kept under constant environmental conditions with a 12/12 light-dark cycle and temperature of 23±2◦C, fed with standard granulated chow, and given drinking water *ad libitum*. The animal experiments were carried out in accordance with the Institutional Protocols of Animal Care. The experimental protocol (Reg no: DPC/AEC/2016-17/ 16) was approved by Dubai Pharmacy college Animals Ethic Committee.

3.5 Cell lines:
Cell lines for Breast cancer (MCF-7) were obtained from College of medicine, Sharjah Institute for Medical Research, University of Sharjah, Sharjah, and UAE. The cells were supplemented with a medium containing fetal bovine serum (FBS) and Calf serum with 10% glycerol and maintained at -4°c

3.6Tumor Transplantation & Treatment Schedule:
For the induction of breast cancer, female rat were transplanted with MCF-7 cell lines [(0.2ml) 2×10^6 cells/mouse] subcutaneously under mammary fat pads.
Animals are divided into five groups of four animals each. (N=6). Animals from groups 2, 3, 4 and 5 were transplanted with breast cancer cell lines. They are divided as follows:
Breast cancer
Group-I: Control group (1% tween 80-1ml/100g)
Group-II: MCF-7 induced ((0.2ml) 2×10^6 cells/mouse)
Group-III: MCF-7 induced + 5-fluorouracil (20mg/kg)
Group-IV: MCF-7 induced + flaxseed lignan *extract* (20mg/kg)
Group-V: MCF-7 induced + *sesame seed sesamin extract* (40mg/kg).
Group-V: MCF-7 induced + flaxseed lignan *extract* (20mg/kg) + *sesame seed sesamin extract (*40mg/kg).
Animals were divided into five groups as said above. The control group animals were given 1% tween80 1ml/100g for 30 days. Group 2 animals were transplanted with cancer cell line on the 1st day. Group 3 animals were transplanted with cancer cell line on the 1st day and the standard drug 5 fluorouracil 20mg/kg from the 3rd day intra peritoneally until 30th day. Group 4 & 5 animals were transplanted with cancer cell line on the 1st day and *test* extract 20mg/kg & 40mg/kg p.o., from the 3rd day until 30th day.

3.7 Serum parameters:
On 31st day the body weight and body circumference was noted and animals were sacrificed using light ether anesthesia. Blood was collected by carotid bleeding method. Blood was centrifuged using Remi cool centrifuge at 4000 rpm for 15 min. Serum was separated for the estimation of various biochemical parameters like lactate dehydrogenase (LDH), High-density lipoprotein (HDL) cholesterol, Triglyceride,alkaline phosphatase (ALP), alanine transaminase (ALT), and Carcino embryonic antigen (CEA).

3.8 Histopathological Estimation:
At the end of the experiment (day 31), all the animals were anesthetized by light ether and the breast tissue (breast cancer) were excised out and fixed in buffered formalin (10%). five micron think section were prepared by using microtome and these sections were stained with haematoxylin and eosin. For histological alterations these slides were observed under light microscope with 40x magnification.

3.9Statistical Analysis:
Statistical analysis was performed as the mean± standard error of mean (SEM). The results were analysed for statistical significance by unpaired t-test followed by Dunnet"s posthoc test of significance. P value less than 0.05 were considered as statistically significant.

4. Results:
4.1 Biochemical Investigations of *In vivo* studied animals'
Effect of lingnan and sesamin mixture on Serum Parameters
Treatment with mixture of sesamin & lignan had significant influence on liver enzymes. The effect of mixture of sesamin & lignan was 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 5 mixture of flaxseed and sesame animals, when compared to the normal control animal similar to the levels in standard
5-Fluorouracil treated group in the breast cancer induced groups animals, when compared to the normal control animals. (As shown in figure1& Table) Treatment with mixture of sesamin & lignin group 5 showed significant increase in the levels of HDL ($p<0.01$) (As shown in figure1& Table)

4.2 Histophathological studies

Severe sub mucosal inflammation and fibrosis noticed. Infiltration of inflammatory (lymphocytes, neutrophile) and fibrosis noticed in control and group3 and the mixture *lignin and sesamin* group 5 reduced inflammation and fibrosis noticed as shown in figure2

5. Discussion:

Natural products have been regarded as important sources that could produce potential chemoprevention. Cancer chemoprevention can be achieved by the use of natural, synthetic or biologic compounds that reverse, suppress or prevent the development of epithelial malignancies. Many chemopreventive agents are able to block or delay the promotion and/or progression of premalignant or malignant cells by modulating cell proliferation and/or differentiation. Apoptosis is conceivably the most potent defence against cancer development. Activation of apoptosis in pre-cancerous cells is one of the most important mechanisms of cancer chemoprevention.

The mixture of *flaxseed and sesame extracted lignans* and standard groups of animals shows the blood serum levels of ALT,ALP,LDH and CEA were decreased in conditions compare to breast cancer induced control animals . But the HDL level is increased in the mixture of *flaxseed and sesame extracted lignans* and standard groups compare to breast cancer induced control animals .The tumor size of mixture of *flaxseed and sesame extracted lignans* is significantly decreased compare to the standard group animals.

Increases in LDH levels are usually found in cellular death and/or cell membrane damage. This cell membrane damage or cell death is due to increase in ROS (reactive oxygen species). In cell line induced groups of breast cancer the increase in LDH was observed and the *lignin and sesamin mixture* 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 mice in both breast cancer and colon cancer when compared to normal control rat. The animals treated with *lignin and sesame* extract showed significant decrease in levels of CEA in the serum when compared to cell line induced groups.
Based on our data we can assume that mixture of flaxseed and sesame showed the antioxidant and antitumor properties by inducing apoptosis and thereby indicating the chemopreventive nature of natural products.

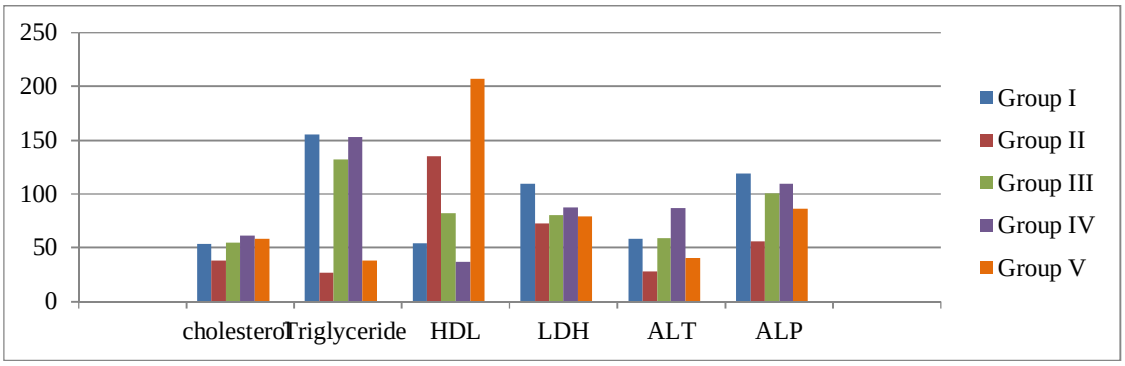

Figure 1: Biochemical Investigations of *In vivo* studied animals

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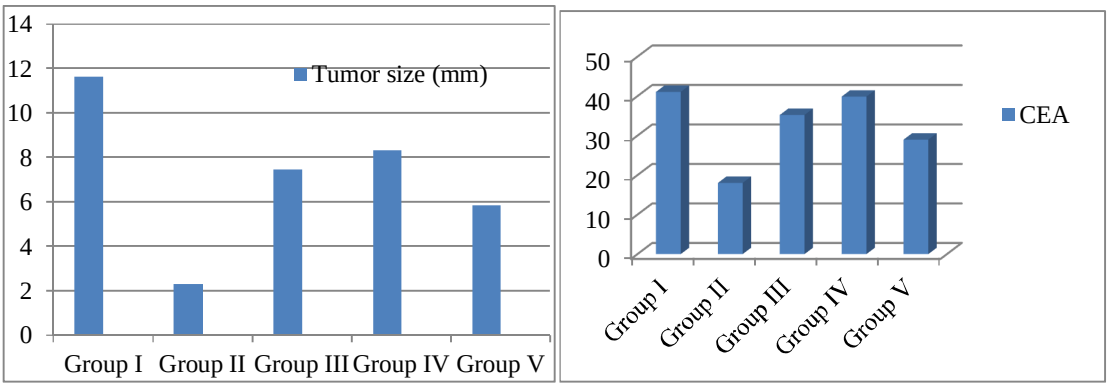

Figure 1: There was a significant(p<0.001) decrease in CEA, Triglyceride, Cholesterol, LDL, ALT, ALP level and Tumor size in cell line induced group 5 mixture of flaxseed and sesame animals, when compared to the normal control animal similar to the levels in standard 5-Fluorouracil treated group in the breast cancer induced groups.

Animal Group	Cholesterol mg/dl	Triglyceride mg/dl	HDL (IU/L)	LDL (IU/L)	ALT (IU/L)	ALP (IU/L)	CEA (ng/ml)
Group I	53±1.53	155±0.95	54±6.05	109±2.24	58±2.29	119±0.88	41.08±1.46
Group II	38±0.99**	27±1.63***	135±3.36*	72.19±0.96	28±1.60**	56±1.87**	18.03±1.82***
Group III	55±1.08**	132±6.75	82.3±2.06*	80.09±0.96	58±1.45	100±1.82	35.24±1.65
Group IV	61.3±0.46	153±1.82	87.11±3.31	87.11±0.87	87±1.48	109±6.22	39.89±0.99
Group V	58.3±0.92*	38.3±1.15**	78±1.73***	79±2.27**	40±0.76**	86±0.43**	28.99±0.98**

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

Table 1: Biochemical parameter of treated animals Serum analysis

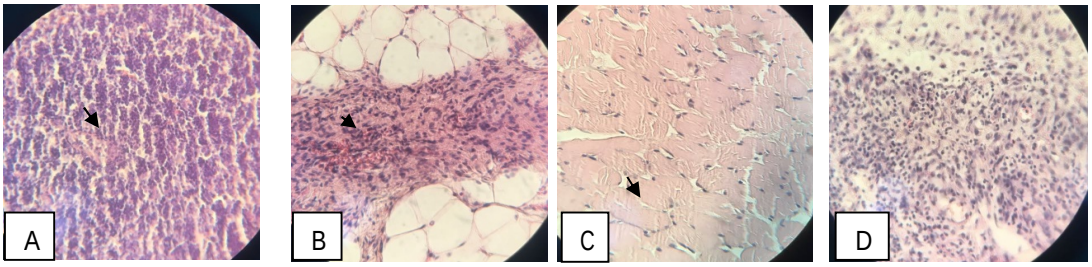

Figure 2: Histopathology of breast tissues

Figure2: A: Control B: standard C: flaxseed lignan D: sesamin E: mixture lignin and sesamin MCF-7Cell line induced: Severe sub mucosal inflammation and fibrosis noticed. Infiltration of inflammatory (lymphocytes, neutrophile) and fibrosis noticed

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