

Research Article

Chemopreventive Role of Flaxseed Oil against Chemical Induced Skin Cancer in Mammals

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Abstract

Linum usitatissimum, commonly known as flax, is the established medicinal plant which has been used as antiviral, antibacterial, anti-inflammatory, anti-diabetic and cardio-protective agent. The present study was designed to evaluate the anti-tumorogenic potential of flaxseed oil using two stage skin carcinogenesis protocol in Swiss albino mice. A single topical application of 7,12-Dimethylbenz (a) anthracene (DMBA), followed 2 weeks later by the croton oil application thrice in a week till the end of experiment (i.e. 16 weeks), resulted in the 100% tumor incidence. The oral administration of flaxseed oil (100 μ l/animal/day) at post-initiation (i.e. starting from the day of croton oil application & continued for 16 weeks) and peri-post initiation stage (i.e. 7 days before DMBA application & continued till the end of experiment) resulted in the significant reduction in the tumor incidence, cumulative number of tumors, tumor burden and tumor yield. The average latent period of tumor appearance was prolonged to 11.92 and 12.22 in both these groups in comparison to carcinogen control group (i.e., 7.23). Thus, the results of this experiment demonstrate the potential anti-tumor activity in the flaxseed oil on DMBA/croton oil induced skin carcinogenesis in Swiss albino mice.

Keywords: Chemical carcinogenesis; Chemoprevention; Flaxseed oil; Tumor burden

Introduction

Cancer has emerged as a life threatening non-communicable disease and it is characterized by the abnormal proliferation, invasiveness and metastasis of cells. During the last few decades, there has been a sudden increase in the cancer global burden due to the change in life style, environment, genetic variation, virus infection etc. which directly or indirectly responsible for the cancer development [1]. Among all the cancers, skin carcinogenesis are the most prevalent

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as it represents almost one third of all the newly diagnosed cases. Melanoma and non-melanoma are the two major categories of skin cancer. The two most frequent type of non-melanoma skin cancer comprises Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC), which has annual incidences of about 80 and 16% respectively [2].

The two stage skin carcinogenesis model in mouse is a novel tool to study the stages and mechanism of cancer as it displays a pre-neoplastic condition in the form of papillomas that are visible and can be confirmed histopathologically. It involves classical tumor initiator 7, 12-Dimethylbenz (a) anthracene (DMBA) which causes the mutations leading to DNA damage. TPA, the phorbol ester present in Croton oil, acts as the promoter and alters gene expression resulted in hyper proliferation, tissue remodelling and inflammation [3].

Besides the traditional treatment methods like chemotherapy and radiotherapy, there should be an alternative preventive approach to reduce the cancer burden. The administration of herbal and natural products to reverse, inhibit and delay the carcinogenesis is a new area of research known as chemoprevention [4]. It postpones the cancer occurrence in high risk population and simultaneously reduces the side effects of various treatment therapies. Chemopreventive agents modulate different stages of cancer development either by blocking mutagenic carcinogens, scavenging of free radicals or reduction in apoptosis and abnormal cell proliferation [5]. As reported in scientific study cancer cases are directly associated with the nutritional value of diet consumed [6].

Linum usitatissimum, commonly known as flaxseed or linseed belonging to the family *linaceae*, is grown as important oil seed crop around the globe. Flaxseed oil has various health benefits including antiviral, antibacterial, anti-inflammatory, anti-diabetic and reduction in cardiovascular risks [7,8]. The high α -linoleic acid content, chlorophyll pigments, tocopherol, plastoquinone-8, phenolic acids and flavanoids may play significant role in the pharmacological quality of the oil [9]. The studies on *in vivo* and *in vitro* models suggested that flaxseed oil can reduce the effect of breast, prostate, colon and intestinal cancer [10-13].

Looking towards the health benefits of this plant, the present experiment was conducted to evaluate possible anti-cancer activity of flaxseed oil against chemical induced skin carcinogenesis in mammals.

Materials and Methods

Chemicals

The initiator, 7, 12-Dimethylbenz (a) anthracene (DMBA) and the promoter croton oil were procured from Sigma Chemicals Co., St. Louis, USA. DMBA was dissolved at a concentration of 100 μ g/100 μ l in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution. Flaxseed oil was procured from Prano flax India Private Ltd.

Animals

The protocol of the experiment was approved by Institutional Ethical Committee and the animal care (1678/GO/a/12/CPCSEA) and

handling was done according to guidelines set by the World Health Organization, Geneva (Switzerland) and the Indian National Science Academy, New Delhi (India). The present study was conducted on the female Swiss albino mice (7-8 weeks old & weighing 24 ± 2 g), selected from a random breed inbred colony. These animals were housed in polypropylene cages in the animal house under the controlled conditions of temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and light (14 light: 10 dark). The animals were fed a standard mouse feed (procured from Aashirwad Industries, Chandigarh, India) and water was given *ad libitum*.

Experimental design

Animals for this study were divided into following groups:

Group I: Vehicle treated control (Normal, n=10)

Animals of this group received topical application of acetone (100 μl /mouse) on the shaven dorsal skin and double distilled water (equivalent to FSO i.e., 100 μl /mouse/day) by oral gavage for 16 weeks.

Group II: FSO treated control (Drug control group, n=10)

Animals belonging to this group received flaxseed oil (100 μl /animal/day) by oral gavage throughout the experimental period i.e., 16 weeks and acted as drug treated control.

Group III: Carcinogen treated (Positive control group, n=10)

Mice of this group were treated with single topical application of 100 μl DMBA (100 μg /100 μl acetone) over the shaven area of skin. Two weeks later 100 μl croton oil (1% w/v in acetone) was applied topically three times in a week until the end of experimental period.

Group IV: FSO treated (Post-initiation group, n=10)

The treatment pattern of DMBA and croton oil was same as group II. This group received FSO (100 μl /animal/day) by oral gavage starting from the time of croton oil application until the end of experiment.

Group V: FSO treated (Peri-and post initiation group, n=10)

Animals of this group were administered FSO (100 μl /animal/day) orally starting from 7 days before DMBA application and continued throughout experimental duration (i.e., 16 weeks).

Induction of tumor

For the induction of skin tumors, dorsal hairs between the cervical and caudal portions of the animals of Group III - V were removed using a surgical clipper, 2 days prior to the initiation of the experiment, and 100 μl DMBA (100 μg /100 μl acetone) was applied. After 14 days, the tumor initiation by DMBA was promoted with the topical application of 100 μl croton seed oil (1% v/v in acetone), thrice a week for the next 14 weeks.

During the 16 weeks of experimentation, mice were observed daily and weighed weekly. Tumors appearing on the shaven area of the skin were examined and recorded at weekly intervals in all the above groups. Only those tumors which persisted for two weeks or more, with a diameter greater than 2 mm, have been taken into consideration for final evaluation of the data. Skin tumors, which regressed after one observation, were not accounted.

Optimum dose selection

Different doses (5,10,25,50,100 or 150 μl /animal/day) of flaxseed oil was orally administered to the mice for 15 days, and the alterations

in morphological parameters like body weight, food & water consumption, general behavior, gait, morbidity, mortality etc. were noted daily till 30 days. The biochemical parameters were evaluated in liver and skin on the 16th and 31st day in the form of Reduced Glutathione (GSH), Lipid Peroxidation (LPO) and total protein. The optimum dose of flaxseed oil was considered in which the highest level of GSH, total proteins and the lowest level of LPO were measured, and the same was used for the further experimentation. The following studies were performed:

Morphological analysis

Cumulative number of papillomas: The total number of papillomas appeared till the termination of the experiment.

Tumor incidence: The number of mice carrying at least one tumor expressed as a percentage incidence.

Tumor yield: The average number of tumors per mouse.

Tumor burden: The average number of tumors per tumor bearing mouse.

Diameter: The diameter of each tumor was measured.

Weight: The weight of each tumor was measured at the termination of experiment.

Body weight: The weight of each mouse was measured weekly.

Average latent period: The time lag between the application of the promoting agent and the appearance of 50% of tumors was determined. The average latent period was calculated by multiplying the number of tumors appearing each week by the time in weeks after the application of the promoting agent and dividing the sum by total number of tumors.

$$\text{Average latent period} = \frac{\sum FX}{N}$$

Where F is the number of tumors appearing each week, X is the numbers of weeks, and n is the total number of tumors.

Inhibition of tumor multiplicity:

$$\frac{\text{Total no. of papillomas in carcinogen control} - \text{Total no. of papillomas in treated X 100}}{\text{Total no. of papillomas in carcinogen control}}$$

Statistical analysis

Data from different experimental groups were analyzed and expressed as mean +SE. The significant levels of difference between carcinogen treated control and FSO treated experimental groups were statistically computed using χ^2 -test at 5% probability level.

Results

The oral administration of flaxseed oil did not alter the body weight and it was measured near to normal in all groups (Table 1). The animals of Group I and II did not show any tumor development throughout the experimental period. As depicted in (Figure 1), FSO treatment resulted in the significant ($P \leq 0.05$) reduction in the cumulative number of tumors i.e., 28 and 22 in the Group IV and V respectively when compared with the carcinogen treated Group III (i.e., 53). The average weight of tumors was reduced from 1.24gm (Group III) to 0.74gm (Group IV) and 0.52gm (Group V) in animals (Table 1). Morphological appearance of tumors in carcinogen treated control group was scaly, larger and darker while in FSO administered groups the tumors were soft, smaller and lighter in color (Figure 2).

Group	Treatment	Body Weight (gm)		Tumor Diameter (mm)			Tumor Weight (gm)	Tumor Incidence	
		Initial	Final	2-5mm	6-9mm	Total			
Vehicle treated control	I	Acetone	25.56 ± 1.73	32.67 ± 0.45	--	--	--	--	
Flaxseed oil treated Control	II	100µl/animal/day	27.48 ± 1.87	33.53 ± 1.32	--	--	--	--	
Carcinogen treated control	III	DMBA+Croton oil	25.63 ± 1.95	31.67 ± 1.23	38	15	53	1.24	100
FSO Experimental I	IV	(DMBA+Croton oil)+FSO	25.66 ± 1.75	34.71 ± 1.12	15	13	28*	0.74*	70
FSO Experimental II	V	FSO+(DMBA+Croton oil)+FSO	25.84 ± 2.06	35.77 ± 1.37	12	10	22*	0.52*	60

Table 1: Variations in tumor size, weight, incidence and body weight during chemical induced skin carcinogenesis in mice.

Each Value represents mean ± S.E.

Statistical comparison: Control v/s Experimental *p ≤ 0.05.

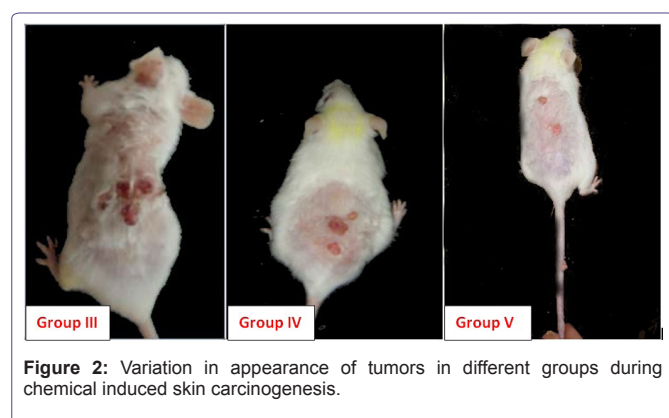


Figure 2: Variation in appearance of tumors in different groups during chemical induced skin carcinogenesis.

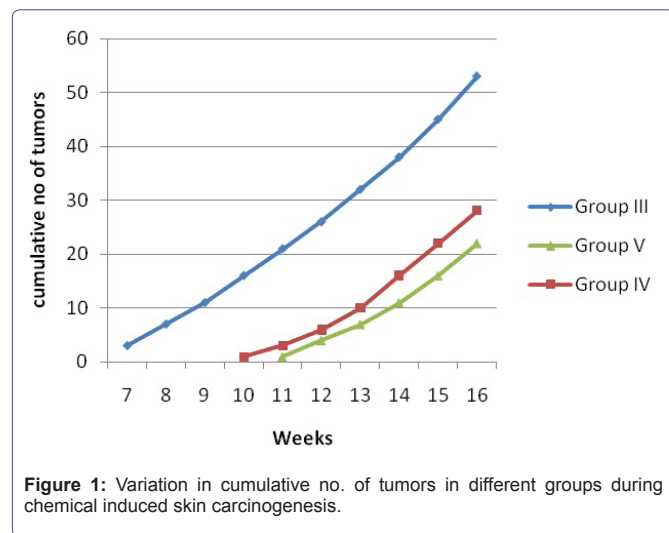


Figure 1: Variation in cumulative no. of tumors in different groups during chemical induced skin carcinogenesis.

Similarly, the tumor yield and tumor burden were also observed to be decreased to 2.8 and 4.0 in post treated group and to 2.2 and 3.5 in peri-post treated group while the same were recorded as much higher i.e., 5.3 and 5.3 in carcinogen control group (Figure 3-4).

A cent percent tumor incidence was observed in the animals after the exposure to carcinogen, however, the tumor appearance with FSO administration was reduced to 70% (7 mice out of 10) and 60% (6 mice out of 10) in Group IV and V respectively.

In carcinogen treated control group, the average latent period was recorded as 7.23 weeks which was found to be considerably prolonged to 11.92 and 12.22 weeks in the FSO administered experimental Group IV and V respectively (Figure 5). The maximum inhibition of

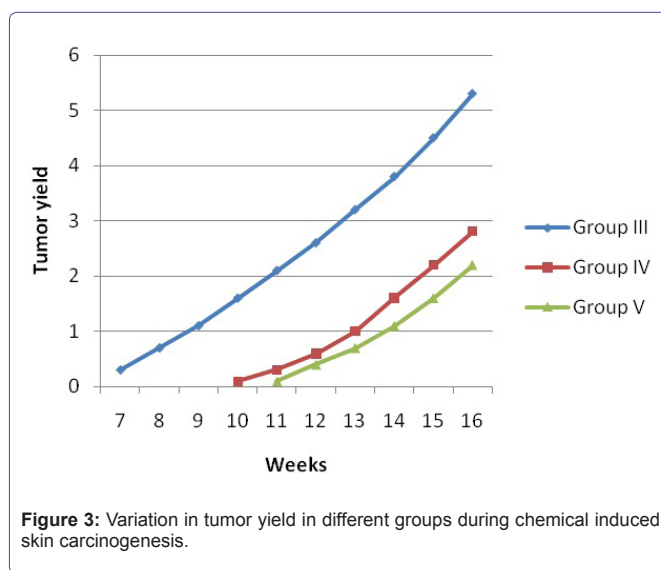


Figure 3: Variation in tumor yield in different groups during chemical induced skin carcinogenesis.

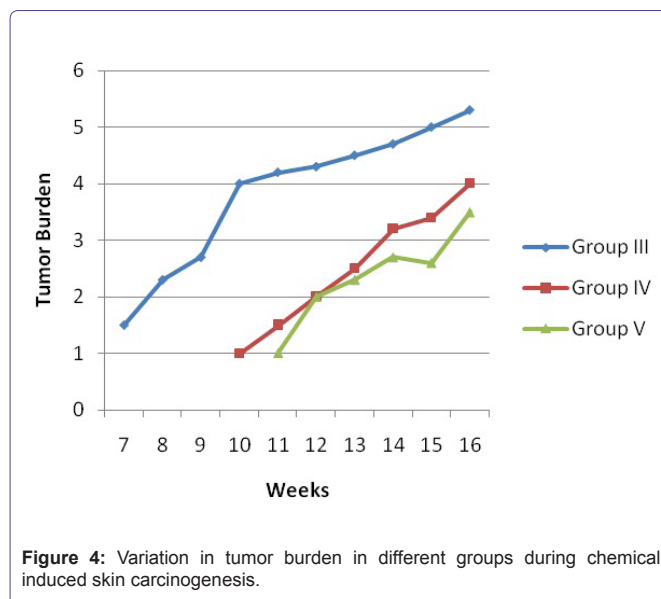


Figure 4: Variation in tumor burden in different groups during chemical induced skin carcinogenesis.

tumor multiplicity was recorded as 58.49% in Group V where animals received FSO treatment from one week prior to DMBA application and continued till the end of experiment while the same was noted as 47.17% in the Group IV in which animals received FSO treatment two weeks later DMBA application till the end of experiment.

Discussion

Human body has the constant exposure of chemical carcinogens in the form of packed food products, beauty products, industrial waste and environmental pollutants. Chemical carcinogens can be categorized into exogenous, which include all physical, chemical and biological agents present outside the body that cause cancer after penetration into the organism, and endogenous carcinogens include metabolic intermediates in the body [14].

In the present study, the carcinogenesis is initiated by the DMBA, the potent indirect chemical carcinogen which is metabolically activated by Cyto. P450 into the 3,4-diol-1,2-epoxide that is capable of binding covalently to DNA and causing gene mutation which ultimately leads to skin carcinogenesis in Swiss albino mice. The main mutation associated with carcinogenesis is in Ras gene that confers selective advance to the epithelial cells. 12-O-Tetradecanoyl Phorbol-13-Acetate (TPA), main constituent of croton oil, is reported to cause activation of protein kinase C which ultimately leads to epidermal proliferation, apoptosis and activation of inflammatory mediators [15].

A wide variety of natural and synthetic compounds has been identified for having chemopreventive potential which can prolong the cancer initiation and progression in various experimental studies [16-20]. Similarly, the observations in the present study indicate that the oral administration of flaxseed oil during post initiation and peri-post initiation stages cause the decrease of 30-40% in the tumor incidences and 47-58% in cumulative number of papilloma of mice.

Chemopreventive agents target the different stages of carcinogenesis as some plant products are noted to be more effective in initiation and others are efficient during promotion stage [21-23]. In the present experiments, the flaxseed oil treatment increased the average latent period in mice when given during promotion stage. The outcome is even better when the treatment is started before the application of initiator. Thus, it is evident that like other plant extracts, the flaxseed oil also exhibits the tumor inhibition during initiation as well as promotion stage.

The medicinal properties of flaxseed oil is assigned due to the presence of high α -linoleic acid content, chlorophyll pigments, γ -tocopherol, plastoquinone-8, phenolic acids and flavonoids [9]. The antioxidative potential of these active constituents reduce the free radicals in the body generated during promotion phase. The results of previous studied clearly indicate that flaxseed oil decrease the oxidative stress by decreasing the lipid per oxidation in biological tissues [24]. The linolenic acid present in flaxseed oil conserves the fatty acid content in membranes and checks the formation of peroxides [25].

As documented earlier in the study carried out by Demark-Wahnefried et al., that the prostate cancer and its associated markers are inhibited by the flaxseed supplemented fat restricted diet [11]. Further, the flaxseed oil is effective against intestinal tumorigenesis and colon cancer by decreasing the expression of COX-1 & COX-2, which are responsible for metabolism of arachidonic acid into prostaglandins, finally leading to inflammation and an increase the apoptosis of tumor cells. Hence it might be possible mechanism of tumor inhibition in the present study after FSO administration [13].

Dietary flaxseed lignans and oil were reported to reduce the growth and metastasis of estrogen receptor negative human breast cancer and they also suppress the metastasis after the surgical excision of primary tumors [26]. Experimental evidences suggested that the oil and mucilage obtained from flaxseed have the preventive potential during the kidney and hepatic injury [27,28]. Similarly, administration of FSO also showed reduction in the length and number of gastric ulcer [29].

The results obtained from the present study are preliminary, and biochemical and histopathological study are under progress to know the chemopreventive potential of flaxseed oil in mice. However, it can be concluded that the synergistic effect of various phytochemicals and omega-3-fatty acid content in the flaxseed oil has the anti-cancer effect to reduce the tumorigenesis in mammals.

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