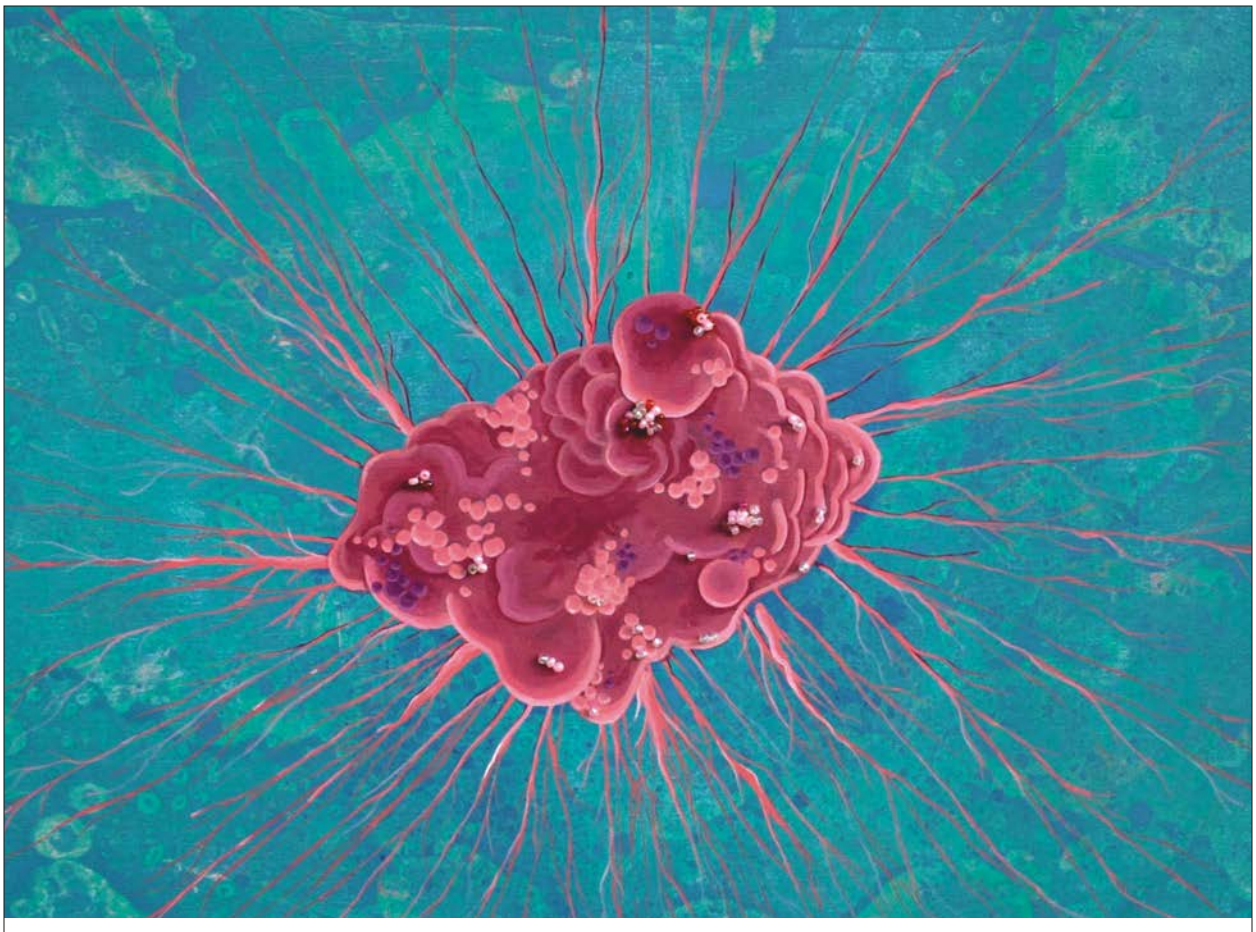

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Chemopreventive Effects of Flax Seed Oil and Flax Seed Meal on Azoxymethane-Induced Colon Tumors in Fisher 344 Male Rats

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Abstract: This study was designed to evaluate the anticarcinogenic effect of Flax Seed Meal (FSM) (10 and 20%) and Flax Seed Oil (FSO) (7 and 14%) on Azoxymethane (AOM)-induced colon tumors in Fisher 344 male rats during initiation (I), promotion (P) and Initiation + Promotion (I+P) stages of carcinogenesis. After an acclimatization period of 1 week, 14 groups of Fisher 344 male weanling rats, 3-4 week old (15 per group) were assigned to 2 control groups fed AIN 93G diet and AIN 93G + 14% soybean oil (SBO) (high fat control). The remaining 12 groups were assigned to 10 and 20% FSM (I, P and I+P) and 7 and 14% FSO (I, P and I+P). All rats received 16 mg kg^{-1} body weight of AOM at 7 and 8 week of age. At 20 weeks of age all animals were switched to AIN-93 M diets and at 45 week of age all rats were killed by CO_2 asphyxiation. Tumor incidence (%) in colon of rats fed C (7 and 14%) was both 100. Tumor incidences for rats fed FSO (7 and 14%) at I, P and I+P were: 100, 100, 61, 60, 58 and 61, respectively and 80, 80, 66.6, 66.6, 66.6 and 31, respectively for rats fed FSM (10 and 20%) at I, P and I+P. Tumors per tumor-bearing ratios for groups fed C; 10 and 20% FSM (I, P and I + P) were 3.86, 1.28, 1.70, 1.75 and 1.0, 0.94, 0.64, respectively. In rats fed C (7 and 14%) and 7 and 14% FSO (I, P and I + P) T/TBR ratios were 3.86, 5.96; 1.4, 0.6, 0.6; 1.90, 0.8, 0.8, respectively. Glutathione-S-Transferase activity (a phase II detoxification enzyme) was significantly ($p<0.05$) higher in rats fed 10 and 20% FSM and 7 and 14% FSO compared to controls. The results of this study indicate that bioactive phytochemicals such as dietary fiber and lignans such as secoisolariciresinol diglycoside (SDG) found in flax seed meal and essential fatty acids such as α-linolenic acid (ALA) found in flax seed oil suppress colon tumors, particularly at the promotion stage and flax seed products may therefore be effective chemopreventive agents.

Key words: Flax seed meal, flax seed oil, azoxymethane, colon tumors

INTRODUCTION

Cancers of the colon and rectum combined are the third most common type of cancer and the second most common cause of cancer deaths in the US, with about 145,290 new cases and 56,290 deaths expected in 2005 (American Cancer Society, 2005). Colorectal carcinogenesis is a multi stage, multi step process that occurs over a period of decades. During this time, cancer-associated genetic mutations accumulate successively and a benign (but initiated) enterocyte progresses to an

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invasive cancer (Markowitz *et al*., 1994). Colon cancer etiology is complex and involves both genetic and environmental factors. A genetic predisposition, such as Familial Adenomatous Polyposis (FAP), is the major risk factor for colorectal cancer development. Among the environmental factors, dietary factors play a critical role. Low intakes of fibers, fruit and vegetables and high intake of fats have been linked with increased colon cancer risk (WHO, 2002). A pioneering epidemiologic study (Burkitt, 1971) showed that a fiber-rich, low fat diet consumed by an African indigenous population was associated with a very low incidence of colon cancer compared with a high incidence associated with a high fat, low fiber diets consumed in Western Countries. The preferred diet to reduce colon cancer risk is considered to be a high fiber, low fat diet (Dixon *et al*., 2004; Reddy, 1995; Giovannucci and Goldin, 1997). However, the sources as well as the quantity of fat and fiber consumed must be considered when examining their effects on colon carcinogenesis (Burkitt, 1971).

Both epidemiological and experimental studies support a protective role of omega-3 fatty acids against colon cancer. Omega-3 fatty acids are now known to be essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, diabetes, arthritis, hypertension, other inflammatory and autoimmune disorders and cancer (Simopoulos, 1999). Therefore, consumption of omega-3 fatty acids offers a way to enhance cancer therapy, support chemotherapy and may increase life span (Caygill *et al*., 1996). Mounting evidence shows that dietary omega-3 polyunsaturated fatty acids (PUFAs) inhibit the promotion and progression stages of carcinogenesis. Mechanisms accounting for these anti-tumor effects of fatty acids are reduced levels of PGE_2 and inducible NO synthase as well as an increased lipid peroxidation, or translation inhibition with subsequent cell cycle arrest. Further, omega-3 eicosapentaenoic acid is capable of down-regulating the production and effect of a number of mediators of cachexia, such as IL-1, IL-6, TNF-alpha and proteolysis-inducing factor (Stehr and Heller, 2006). Other mechanisms of action whereby omega-3 fatty acids may inhibit tumor progression may include the suppression of Arachidonic Acid (AA)-derived eicosanoid biosynthesis; influence transcription factor activity, gene expression and signal transduction pathways; modulate estrogen metabolism; increase or decrease the production of free radicals and reactive oxygen species; and influence insulin sensitivity and membrane fluidity (Larsson *et al*., 2004).

Flax seed contains a mixture of fatty acids. Flax seed is a rich plant source of alpha-linolenic acid (ALA), an essential fatty acid in the human diet and the parent fatty acid of the omega-3 family. Flax (*Linum usitatisimum*) is grown as either an oil crop or a fiber crop, with fiber (linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen and Richards, 2003; Vaisey-Genser and Morris, 2003).

Flaxseed has about 41% fats and is low in saturated fat (9% of total fatty acids), moderate in monounsaturated fat (18%) and rich in polyunsaturated fat (73%) (Cunnane *et al*., 1993). ALA, which is found in green leafy vegetables, flaxseed, rapeseed and walnuts; desaturates and elongates in the human body to the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and by itself may have beneficial effects in health and in the control of chronic diseases (Simopoulos, 1999). Flaxseed contains both soluble and non-soluble fiber, which accounts for about 28% of the weight of full-fat flax seeds. The major insoluble fiber fraction in flaxseed consists of cellulose and lignin and the major soluble fiber fractions are the mucilage gums (Vaisey-Genser and Morris, 2003). The chemopreventive effects of fish oil (containing omega-3 fatty acids) have been attributed to the inhibition of oxidative metabolism of arachidonic acid through the cyclooxygenase (COX) pathway (Takemura *et al*., 2002). Flax seed oil contains a higher percentage of α-linolenic acid (an omega-3 PUFA) than fish oils.

The hepatic detoxification system exhibits a critical role in carcinogenesis. This system is composed of phase I and phase II enzymes. Cytochrome P_{450}, a phase I enzyme, is involved in the bioactivation of chemical carcinogens, the biotransformation of some endogenous compounds and

xenobiotic detoxification (Guengerich and Shimada, 1991). Phase II enzymes such as GST catalyze the conjugation of small water-soluble molecules to xenobiotics and initiate their excretion (Kensler, 1997). GST and cytochrome P_{450} are highly inducible in animals and humans and their expression is affected by nonnutritional and nutritional factors. In addition to the source of dietary lipid, the amount of dietary lipid is also critical in the modulation of hepatic bioactivation (Chen *et al*., 2001). Glutathione-S-Transferase (GST) levels in the liver of rats were analyzed to study the possibility that flax seed meal and flax seed oil may increase levels of detoxifying enzymes. Therefore, this study was designed to evaluate the anticarcinogenic effect of Flax Seed Meal (FSM) (10 and 20%) and Flax Seed Oil (FSO) (7 and 14%) on azoxymethane (AOM)-induced colon tumors in Fisher 344 male rats during initiation (I), promotion (P) and initiation + promotion (I+P) stages of carcinogenesis.

MATERIALS AND METHODS

Animals, Housing and Diet

Male Fisher 344 weanling rats were obtained from Harlan, IN and housed in stainless steel wire cages at Alabama Agricultural and Mechanical University. The temperature and relative humidity were maintained at 21°C and 50%, respectively. Light and dark cycles were 12 h each. Feed and water were provided *ad libitum*. There was a one-week period of acclimatization. After this period, the animals were randomly divided into groups and fed control and experimental diets until 46 weeks of age. Each of the control and flax product diets were fed during initiation (I), promotion (P) and initiation plus promotion (I+P) stages of carcinogenesis. In the initiation group, rats were fed flax products in the diet for 5 weeks (3 weeks prior to the first azoxymethane (AOM) injection and 1 week after the second AOM injection); rats were then switched to the control diet. In the promotion group, the rats received the control diet up to 9 weeks of age (1 week after the second AOM injection) followed by flax product diets for the duration of the experiment. In the initiation plus promotion group, rats received a flax seed product diet throughout the experiment. (Fig. 1). Rats were fed AIN-93G control (American Institute of Nutrition 93 Growth diet until 20 weeks and later switched to AIN-93M maintenance diet according to standard recommendations by the American Institute of Nutrition (Reeves *et al*., 1993). At 46 weeks of age, the rats were killed by CO_2 asphyxiation. All diets were prepared weekly/biweekly and stored at refrigeration temperature (4°C). During this time, biweekly body weights and daily feed intakes were recorded. The diets were refreshed daily (Table 1).

Chemicals and Dietary Ingredients

All biochemicals, except azoxymethane (Midwestern Research Institute, NCI Repository, Kansas, MO) were obtained from Sigma Chemical, St. Louis, MO. Flax seed meal was obtained from Hylden Farms of North Dakota and Flax seed oil was obtained from Nature‘s Distributors, Arizona.

Carcinogen Injection

The animals were randomly assigned and started on treatment diets at 3 weeks of age. For induction of colon tumors, all animals received 2 subcutaneous injections of azoxymethane (AOM) in saline at the rate of 16 mg kg^{-1} body weight at 7 and 8 weeks of age.

Collection of Samples

At 46 weeks of age, all rats were killed using CO_2 asphyxiation. Livers were excised and flash frozen using liquid nitrogen and stored at -80°C until Glutathione-S-Transferase (GST) analysis. The colons from rats of each group were removed and flushed with PBS (0.1 M, pH 7.2) and prepared for counting the tumors. The colons were split open longitudinally and the colonic mucosa scraped using a glass slide. The colons were excised and the cecum was removed and weighed. The rectum was not

Table 1: Composition of diets used in the experiment

Ingredients (g)	C+7% SBO	C+14% SBO	C+10% FSM	C+20% FSM	C+7% FSO	C+14% FSO
Cornstarch	397.5	397.5	347.5	247.5	397.5	397.5
Soybean oil	70.0	140.0	70.0	70.0	0.0	0.0
Flax seed meal	0.0	0.0	100.0	200.0	0.0	0.0
Fiber	50.0	50.0	0.0	0.0	50.0	50.0
Flax seed oil	0.0	0.0	0.0	0.0	70.0	140.0
Common ingredients[1]	482.5	412.5	482.5	482.5	482.5	412.5

[1]: Common ingredients (g), Casein (>85% protein), 200; Dextrose, 132; Sucrose, 100; Alphacel (fiber); Mineral Mix (AIN 93G-MX), 35; Vitamin Mix (AIN 93-VX), 10; L-Cystein, 3; Choline Bitartate, 2.5, Formulation of diets based on AIN93G (J. Nutr., 123: 1939-51 (1993)

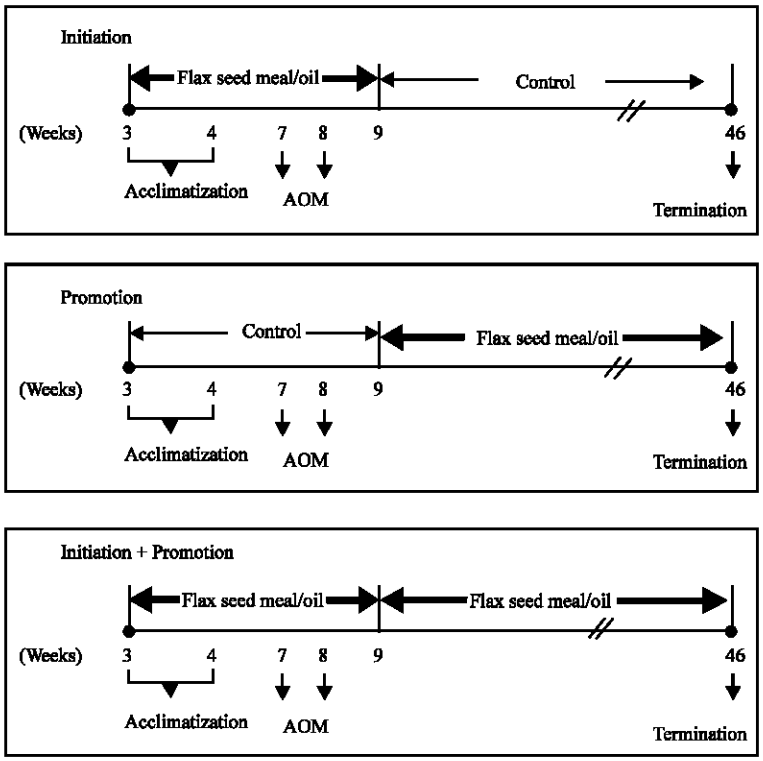

Fig. 1: Feeding dietary flax seed meal and flax seed oil in the initiation, promotion and initiation + promotion stages of carcinogenesis[1], AOM = Azoxymethane, [1]Scale is not proportional

considered as part of the colon. The colon was identified starting right at the point of excision of the cecum. The total length of the colon was divided in half and the one-half region closer to the cecum was considered as the proximal and the remaining one-half was considered as the distal region. The colonic mucosal scrapings were stored in vials at -80°C until GST analysis. The tumors were removed/excised and tumors were characterized as described by Shackelford *et al*. (1983). The tumors were visually counted and the size was determined using ruler/caliper.

Cecal Weight and Cecal pH

The cecum from each rat was excised, weighed and split open and the pH of the cecal contents was recorded.

Glutathione-S-Transferase (GST) Assay

Approximately 1 g of liver sample was homogenized in 10 mL of potassium phosphate buffer (pH 7.0, 0.1 M) in a Potter-Elvejem homogenizer (10 strokes) at 4°C. The homogenate was then centrifuged at 100,000 x g for 30 min. The clear supernatant was mixed with 1, chloro 2, 4-dinitrobenzene (1 mM), potassium phosphate buffer (0.1 M) and glutathione (1 mM). Assay sample (50-100 μL) was analyzed using Cary1/3 UV/VIS dual beam spectrophotometer at 340 nm (Habig *et al*., 1974).

Statistical Analysis

Data are expressed as means±SEM and were analyzed using the SAS statistical program by analysis of variance (ANOVA) and means were separated using the Tukey's studentized range test. Differences were determined for statistical significance using two-way ANOVA. Differences were considered significant at $p<0.05$.

RESULTS

Weight Gain, Daily Feed Intake, Cecal Weight and pH in Rats Fed FSM and FSO

The effect of oral administration of FSM and FSO on feed intake, weight gain, cecal weight and cecal pH is shown in Table 2 and 3, respectively. In rats fed FSM, there were no significant differences in weight gain, feed intake, cecal weight and cecal pH as compared to the control. Highest weight gain was (338.19 g) seen in the group fed 20% FSM (I+P) and lowest (312.47 g) in rats fed the control diet (Table 2). There were significant ($p<0.05$) differences in average weight gain (g) between rats fed 7% SBO (low fat diet) and 14% SBO (high fat diet) as shown in Table 3. Although there were no significant differences in feed intake among rats fed 7% FSO and SBO and 14% FSO and SBO at I, P and I+P stages, the weight gains for rats fed 7% SBO and 7% FSO were significantly ($p<0.05$)

Table 2: Weight gain, feed intake, cecal weight and cecal pH of rats fed 10 and 20 g/100 g flax seed meal at initiation, promotion and initiation + promotion stages

Groups	n	Weight gain (g/41 week)	Feed intake (g day^{-1})	Cecal weight (g)	Cecal pH
C (C + 7% SBO)	15	312.47±8.42^{b}	15.55±2.24^{a}	1.02±1.03^{b}	7.16±2.24^{a}
I (C + 10% FSM)	15	318.36±8.01^{b}	15.96±3.16^{a}	1.06±0.09^{b}	7.04±2.46^{a}
P (C + 10% FSM)	15	320.24±9.46^{ab}	15.98±3.12^{a}	1.86±1.10^{ab}	6.82±2.14^{b}
I+P (C + 10% FSM)	15	332.10±9.89^{a}	15.02±3.22^{a}	2.64±1.16^{a}	6.60±2.01^{b}
I (C + 20% FSM)	15	326.27±8.22^{a}	15.06±2.48^{a}	1.11±0.08^{b}	6.95±1.96^{ab}
P (C + 20% FSM)	15	328.90±8.14^{a}	15.88±3.14^{a}	2.10±1.16^{a}	6.42±1.84^{b}
I+P (C + 20% FSM)	15	338.19±9.42^{a}	15.37±3.11^{a}	2.90±1.86^{a}	6.05±1.75^{b}

Values are means±SEM. Means in a column without a common letter(s) differ, $p<0.05$, C = Control; I = Initiation; P = Promotion, FSM = Flax Seed Meal, SBO = Soybean oil

Table 3: Weight gain, feed intake, cecal weight and cecal pH of rats fed 7 and 14 g/100 g flax oil at initiation, promotion and initiation + promotion stages

Groups	n	Weight gain (g/41 week)	Feed intake (g day^{-1})	Cecal weight (g)	Cecal pH
C+7% SBO	15	312.47±8.42^{c}	15.55±2.24^{a}	1.02±1.03^{a}	7.16±2.24^{a}
C+14% SBO	15	346.82±8.96^{a}	15.92±1.80^{a}	1.00±1.01^{a}	7.58±2.46^{a}
I (C+7% FSO)	15	323.28±8.14^{b}	15.84±1.94^{a}	1.60±2.28^{a}	7.85±2.22^{a}
P (C+7% FSO)	15	322.33±8.10^{b}	15.94±1.98^{a}	1.10±2.20^{a}	7.44±2.48^{a}
I+P (C+7% FSO)	15	321.53±8.04^{b}	15.99±1.74^{a}	1.00±2.19^{a}	7.62±2.46^{a}
I (C+14% FSO)	15	334.68±8.68^{a}	15.06±2.38^{a}	1.42±0.09^{a}	7.68±2.54^{a}
P (C+14% FSO)	15	340.16±8.92^{a}	15.02±1.95^{a}	1.36±1.06^{a}	7.54±2.06^{a}
I+P(C+14% FSO)	15	344.28±8.93^{a}	15.09±1.84^{a}	1.49±1.10^{a}	7.26±1.98^{a}

Values are means±SEM. Means in a column without a common letter(s) differ, $p<0.05$, C = Control; I = Initiation; P = Promotion, FSO = Flax seed oil, SBO = Soybean oil

Table 4: Effect of feeding 10 and 20% flax seed meal on tumor incidence in azoxymethane-induced colon tumors in Fisher 344 male rats

Groups	N^1/N^2	Tumor incidence	Proximal (%)	Distal	Proximal	Distal (n)	Total
C	15/15	100.0	16.0	84.0	10.0	47.0	57.0
I-10%	12/15	80.0	10.0	90.0	2.0	17.0	19.0
I-20%	12/15	80.0	10.0	90.0	2.0	18.0	20.0
P-10%	10/15	66.6	10.0	90.0	1.0	10.0	11.0
P-20%	10/15	66.6	0.0	100.0	0.0	11.0	11.0
I+P-10%	11/15	73.3	0.0	100.0	0.0	11.0	11.0
I+P-20%	4/13	31.0	0.0	100.0	0.0	4.0	4.0

C = Control; I = Initiation; P = Promotion; SBO = Soybean oil, Flax seed meal was fed at 10 g/100 g, N^1 = rats with tumors; N^2 = total number of rats at killing

Table 5: Effect of feeding 7 and 14% flax seed oil on tumor incidence in azoxymethane-induced colon tumors in Fisher 344 male rats

Groups	N^1/N^2	Tumor incidence	Proximal (%)	Distal	Proximal	Distal (n)	Total
C+7% SBO	15/15	100.0	16.0	84.0	10.0	47.0	57.0
C+14% SBO	15/15	100.0	0.0	100.0	0.0	89.0	89.0
I-7%	15/15	100.0	0.0	100.0	0.0	21.0	21.0
I-14%	15/15	100.0	0.0	100.0	0.0	28.0	28.0
P-7%	8/13	61.0	10.0	90.0	1.0	9.0	10.0
P-14%	9/15	60.0	0.0	100.0	0.0	12.0	12.0
I+P-7%	7/12	58.0	0.0	100.0	0.0	10.0	10.0
I+P-14%	8/13	61.0	0.0	100.0	0.0	11.0	11.0

C = Control; I = Initiation; P = Promotion; SBO = Soybean oil, Flax seed oil was fed at 7 g/100 g, N^1 = rats with tumors; N^2 = total number of rats at killing

lower than the rats fed 14% SBO and 14% FSO (Table 3). There were no significant differences between daily feed intake, cecal weight and cecal pH among rats fed control (7 and 14% SBO) and FSO (7 and 14%) diets.

Tumor Incidence and Tumor Numbers

Tumor incidence in the colon of rats fed the control diet was 100%. The tumor incidence was lower in both the 10 and 20% FSM groups. Tumor incidences (%) in the colon of rats in I, P and I+P (10 and 20% FSM) fed groups were: 80, 66.6, 66.6 and 80, 66.6 and 31, respectively (Table 4). Greatest reduction in tumor incidence was seen in rats fed C+20% FSM (I+P) stages. Table 5 shows that tumor incidence in the colon of rats fed the C+7 and 14% SBO diets were 100%. Tumor incidences (%) in the colon of rats in I, P and I+P groups (7 and 14% FSO) were: 100, 61 and 58 and 100, 60 and 61, respectively (Table 5). Tumor incidence was lower in the P stages as compared to the I stages. Greatest reduction (58-61%) in tumor incidence was seen in rats fed C+7 and C+14% (P and I + P) stages. Tumor numbers were higher in the control group compared to the treatment groups. In rats fed FSO (7 and 14%), tumor numbers were significantly ($p<0.05$) different in treatment groups as compared to the control. Tumor numbers were lower in the P and I+P stages compared to the control.

Distal and Proximal Tumors

The incidence of tumors in the distal section of the colon was significantly ($p<0.05$) higher than in the proximal sections (Table 4, 5). These data are consistent with findings that the distal colon shows a greater incidence of colorectal cancer than the proximal colon (Gonzalez *et al*., 2001). Rats fed 20% FSM (P) and (I+P) stages showed 100% incidence of tumors in the distal section of the colon. In rats fed 14% FSO at I, P and I+P stages, tumor incidence was 100% in the distal section of the colon (Table 4). Table 5 shows that all rats in the C+7 and 14% SBO fed groups developed colon tumors. Rats fed 14% FSO (I, P and I+P) stages did not develop any tumors in the proximal colon.

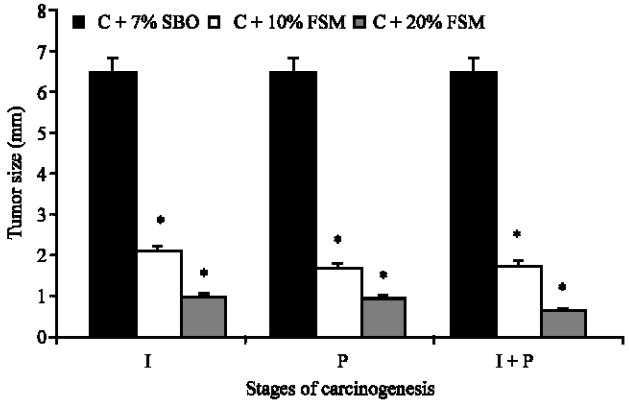

Fig. 2: Dietary flax seed meal on tumor size (mm) in azoxymethane-induced tumors in Fisher 344 male rats *: Significantly ($p<0.05$) different from control, C = control; I = initiation; P = promotion, C+7% SBO = 7 g/100 g soybean oil in AIN 93G/M, C+10% FSM = 10 g/100 g flax seed meal in AIN 93G/M, C+20% FSM = 20 g/100 g flax seed meal in AIN 93G

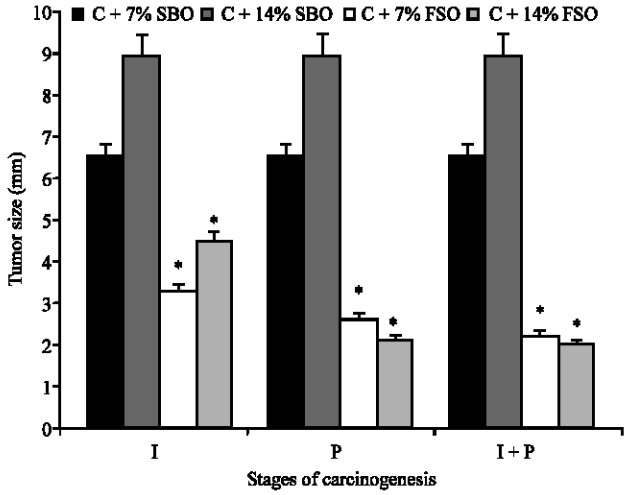

Fig. 3: Dietary flax seed oil on tumor size (mm) in azoxymethane-induced tumors in Fisher 344 male rats, *: Significantly ($p<0.05$) different from controls, C = control; I = initiation; P = promotion, C+7% SBO = 7 g/100 g soybean oil in AIN 93G/M, C+14% SBO = 14 g/100 g soybean oil in AIN 93G/M, C+7% FSO = 7 g/100 g flax seed oil in AIN 93G/M, C+14% FSO = 14 g/100 g flax seed oil in AIN 93G/M

Tumor Size

Colon tumor size was significantly ($p<0.05$) lower in groups fed FSM and FSO at the I, P and I+P stages compared to the control. Colon tumor size (mm) was 6.5, 2.1, 1.7, 1.75 and 1.0, 0.94, 0.64 for the C; I, P and I + P stages (10 and 20% FSM), respectively (Fig. 2). Tumor size was smallest in rats fed 20% FSM (P and I+P) stages. Tumor size was reduced by over 75% in rats fed diets containing FSM as compared to the control. In rats fed FSO (7 and 14%), colon tumor size was significantly ($p<0.05$) lower in the I, P and I+P stages compared to the controls (Fig. 3). Colon tumor (mm) size was 6.5, 3.26, 2.6, 2.2 and 9.0, 4.5, 2.1, 2.0 for the C; I, P and I + P (7 and 14% FSO),

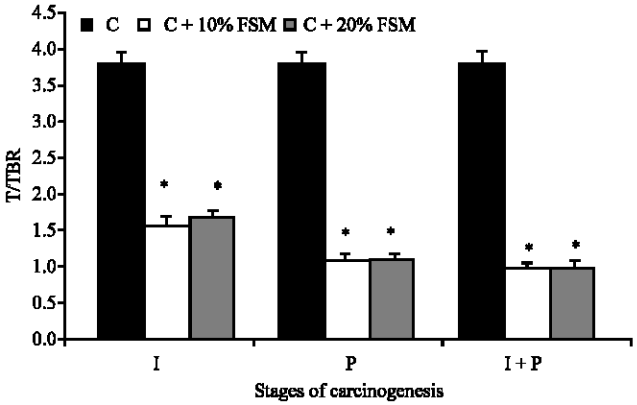

Fig. 4: Dietary flax seed meal on tumors per tumor bearing rat C = control; I = initiation; P = promotion, *: Significantly ($p<0.05$) different from control, C+7% SBO = 7 g/100 g soybean oil in AIN 93G/M, C+10% FSM = 10 g/100 g flax seed meal in AIN 93G/M, C+20% FSM = 20 g/100 g flax seed meal in AIN 93G/M

respectively. Tumor size was reduced by over 50% in rats fed diets containing FSO. Decreased tumor size may be the result of reduced tumor cell proliferation, increased apoptosis, reduced inflammation and reduced angiogenesis by feeding flax seed products.

Tumors/Tumor Bearing Rat (T/TBR)

Tumors/Tumor Bearing Rat (T/TBR) ratios were higher in the control group compared to the treatment groups (Fig. 4). TBR gives a precise picture of tumor burden thus demonstrating the effect of chemopreventive potential of dietary phytochemicals. TBR for C; I, P and I + P (10 and 20% FSM) were 3.80; 1.58, 1.10 and 1.00 and 1.67, 1.10 and 1.00, respectively. Figure 4 shows that oral administration of FSM (10 and 20%) significantly ($p<0.05$) reduced the TBR ratio compared to the control. TBR for I, P and I + P (10 and 20% FSM) were reduced by: 58, 71, 74, 56, 71 and 74%, respectively, compared to controls. Figure 5 shows T/TBR values for rats fed FSO. In rats fed FSO (7 and 14%) TBR ratios for C; I, P and I+P were: 3.80, 1.4, 1.25 and 1.43 and 5.93; 1.87, 1.33 and 1.38, respectively. Tumors/TBR ratios (%) for C; I, P and I + P (7 and 14% FSO) were reduced by: 63, 67, 62, 51, 65, 64, 76, 79, 76, 69, 78 and 77%, respectively.

Effects of FSO and FSM on GST Activity

The effect of oral administration of FSM and FSO on the total activity of GST in the liver is shown in Table 6. Consumption of FSM at I, P and I+P stages significantly ($p<0.05$) increased the total activity of the phase II detoxification enzyme in the liver of rats as compared to the control. GST ($\mu mol\ mg^{-1}$) activity was significantly ($p<0.05$) higher (20.04±1.24, 30.18±0.24, 34.68±0.64, 35.89±1.05, 40.02±0.26 and 43.51±0.67) in the rats fed FSM (10 and 20%) I, P and I+P stages as compared to the control (18.31±1.21). GST activity was increased in rats fed C+10% FSM (I) and C+20% FSM (P) by 38 and 64%, respectively compared to the control. GST activity was significantly ($p<0.05$) higher (33.22±2.56, 30.21±1.66, 39.45±2.80, 29.48±1.02, 32.46±1.89 and 40.12±2.86) in the rats fed 7 and 14% FSO (I, P and I+P) groups as compared to the controls (7 and 14% SBO) (18.31±0.24 and 16.26±1.04). The same trend was observed in the colonic mucosal scrapings. However, GST activity was significantly ($p<0.05$) higher in hepatic tissues compared to colonic mucosal scrapings.

Table 6: Total Hepatic Glutathione S-Transferase (GST) activity and Colonic Mucosal Scrapings (CMS) in Fisher 344 male rats

Groups	Hepatic GST (μmol mg^{-1})	(CMS) GST (μmol mg^{-1})
C+7% SBO	18.31±1.21^{c}	0.38±0.04^{e}
C+14% SBO	16.26±1.04^{c}	0.32±0.03^{e}
C+10%FSM (I)	20.04±1.24^{b}	1.04±0.16^{e}
C+20% FSM (I)	35.89±1.05^{b}	2.61±0.74^{d}
C+10% FSM (P)	30.18±0.24^{b}	3.04±0.20^{e}
C+20% FSM (P)	40.02±0.26^{a}	3.31±0.03^{b}
C+10% FSM (I+P)	34.68±0.64^{b}	3.12±0.04^{a}
C+20% FSM (I+P)	43.51±0.67^{a}	3.61±0.04^{a}
C+7%FSO (I)	33.22±2.56^{b}	2.76±0.06^{cd}
C+14% FSO (I)	29.48±1.02^{b}	2.72±0.08^{cd}
C+7% FSO (P)	30.21±1.66^{b}	2.96±0.04^{c}
C+14% FSO (P)	32.46±1.89^{b}	2.84±0.03^{c}
C+7% FSO (I+P)	39.45±2.80^{a}	3.69±0.07^{a}
C+14% FSO (I+P)	40.12±2.86^{a}	3.70±0.09^{a}

Values are means±SEM. ^{abcde}: Means in a column without a common letter differ, $p<0.05$. GST = Glutathione-S-Transferase; CMS = Colonic Mucosal Scrapings, AOM = Azoxymethane; C = Control; I = Initiation; P = Promotion; I+P = Initiation + Promotion; FSM = Flax Seed Meal; FSO = Flax Seed Oil

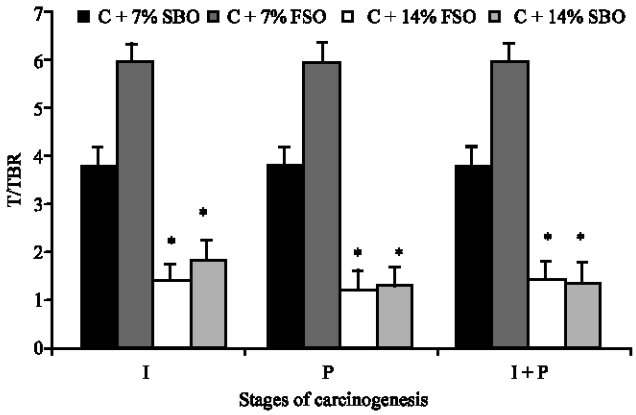

Fig. 5: Dietary flax seed oil on tumors per Tumor Bearing Rat (TBR) in azoxymethane-induced tumors in Fisher 344 male rats C = control; I = initiation; P = promotion, *: Significantly ($p<0.05$) different from controls, C+7% SBO = 7 g/100 g soybean oil in AIN 93G/M, C+14% SBO = 14 g/100 g soybean oil in AIN 93G/M, C+7% FSO = 7 g/100 g flax seed oil in AIN 93G/M, C+14% FSO = 14 g/100 g flax seed oil in AIN 93G/M

DISCUSSION

Studies have demonstrated a reduced risk of colon cancer when populations with diets high in total fat and low in fiber switched to a diet low in saturated fats and high in total fiber and whole-grain foods. Omega-3 PUFAs have emerged as bioactive compounds of potential benefit in colon cancer. Flax seed is a rich source of the plant lignan, secoisolariciresinol diglycoside (SDG), a type of phytoestrogen. Lignans are biologically active phytochemicals that have been shown to exhibit anticancer and antioxidant properties. A role for flax in the prevention of colon cancer is plausible because the colon is the region where mammalian lignans are produced from plant lignans.

The purpose of this study was to evaluate the anticarcinogenic effect of Flax Seed Meal (FSM) (10 and 20%) and Flax Seed Oil (FSO) (7 and 14%) on azoxymethane (AOM)-induced colon tumors in Fisher 344 male rats during initiation (I), promotion (P) and initiation + promotion (I+P) stages of carcinogenesis. Recent studies in our lab showed that feeding rats FSM and FSO reduced the incidence

of aberrant crypt foci in the distal colon by 88 and 77%, in the proximal colon by 86 and 87% with a total reduction of 87.5 and 84%, respectively (Williams *et al*., 2006). In a recent study by Sheng *et al*. (2006) where animals were fed dietary sesaminol glucosides containing small amounts of lignans; there were no significant differences in body weights among experimental groups. In another study conducted by Reddy *et al*. (1997), the body weights of AOM and vehicle treated animals fed the control and experimental diets containing 10% inulin or oligofructose were comparable throughout the study. In our study, feeding rats control (7% SBO) and experimental diets containing FSM (10 and 20%), also showed no significant differences in weight gain (Table 2). There were no significant ($p<0.05$) differences in weight gain among rats fed FSO and SBO (7%) and rats fed FSO and SBO (14%). Although, there were significant differences in weight gain between rats fed 7 and 14% SBO and FSO (Table 3).

Oral administration of FSM (10 and 20%) and FSO (7 and 14%) significantly ($p<0.05$) reduced the incidence of colon tumors in Fisher 344 male rats (Table 4, 5), as compared to the controls. Colon tumor incidence in rats fed the control diets was higher than those fed the treatment diets. Tumor incidence in the colon of rats fed the control diets was 100%. In the present study, the greatest reduction (27%) in tumor incidence in the rats fed FSM was seen in the group fed 20% FSM (I+P) stage. Chen *et al*. (2003) conducted a study using pregnant Sprague-Dawley rats supplemented with 10% flax seed or the lignan, SDG during lactation. At week 21 post-DMBA administration, compared with the basal diet group, the FS and SDG groups had significantly lower ($p<0.05$) tumor incidence (31.3 and 42.0%, respectively). In another study conducted by Chen *et al*. (2004), it was reported that feeding flax seed in combination with tamoxifen (a well known adjuvant therapy for breast cancer) in athymic mice with or without 17-β estradiol (E2) supplementation, reduced tumor volume/weight by 62 and 39%, respectively compared with those in the tamoxifen group alone. This indicates the potency of flax seed in reducing tumor incidence even in breast cancer. In the present study, the greatest reduction in tumor incidence was seen in rats fed 20% FSM (I+P) stage, with a total reduction of 73%. Flax seed meal is the richest source of the phytoestrogen secoisolariciresinol diglycoside (SDG), a plant lignan that can be metabolized by bacteria in the animal or human colon to the mammalian lignans enterodiol and enterolactone.

The incidence of tumors in the distal section of the colon was significantly higher than in the proximal sections. An important characteristic of colorectal cancer is its anatomical site of origin. These data are consistent with findings that the distal colon shows a greater incidence of colorectal cancer than the proximal colon (Gonzalez *et al*., 2001). Chang *et al*. (1997) investigated whether there were protective effects of fish oils mediated through changes in proliferation, differentiation or apoptosis, all intermediate biomarkers of colon tumor development during the promotion phase of tumorigenesis. They reported that there were a greater number of apoptotic cells/crypt columns in both proximal and distal colon after feeding fish oil versus corn oil. Various mechanisms have been postulated to explain the enhancing effect of a high fat fish oil diet during the promotion phase of carcinogenesis. The chemopreventive effects of omega-3 fatty acids have been attributed to the inhibition of oxidative metabolism of arachidonic acid (AA) through the cyclooxygenase (COX) pathway (Crawford *et al*., 2000). The possible mechanism for enhanced tumor development in the corn oil group may have been due to the metabolism of the AA to prostaglandins, which act as tumor promoters.

Tumor/tumor Bearing Rat (TBR) values are considered better indicators for studying the effect of phytochemicals on end point tumor models because they give a more precise picture of tumor inhibition (specifically, the number of tumors induced per TBR). In this study, the number of tumors per rat was also calculated for purposes of comparison (Fig. 4, 5). A decrease in TBR values was seen in rats fed FSM and FSO (10 and 20%) and (7 and 14%) at I, P and I+P stages compared to rats fed the C diet. In a study done by Bartoli *et al*. (2000), where rats were fed isocaloric 5% fat diets

consisting of olive oil (omega-9), fish oil (omega-3), or safflower oil (omega-6) for 19 weeks, tumor multiplicity was lower for the rats fed the olive oil and fish oil diets as compared to the safflower fed animals. Results showed that rats on the omega-6 diet were found to have colonic aberrant crypt foci and adenocarcinomas more often than those consuming either the omega-9 or omega-3 diets. One mechanistic approach in tumor multiplicity inhibition might be due to the levels of the inducible isoform of Nitric Oxide Synthase (iNOS) which are increased in preinvasive colon neoplasias in animal models and invasive neoplasias in the human colon (Lagares-Garcia *et al*., 2001). Its product, nitric oxide is known to stimulate COX-2 expression and activity and to post-translationally impair the activity of caspases, p53 and DNA repair enzymes (Jaiswal *et al*., 2000). Raised levels of iNOS could promote tumor development.

Glutathione-S-Transferase (GST) is a family of dimeric phase II enzymes involved in the bioactivation and detoxification system which plays an important role in carcinogenesis. The physiologic function of phase II enzymes such as glutathione (GSH) and GST is to catalyze the conjugation of small water-soluble molecules to xenobiotics and facilitate their excretion (Pickett and Lu, 1989). The effect of FSM (10 and 20%) at I, P and I+P stages of and FSO (7 and 14%) at I, P and I+P stages of carcinogenesis on the total activity of GST in the liver is shown in Table 6. Oral administration of FSM and FSO significantly ($p<0.05$) increased the total activity of GST. GST activity was significantly ($p<0.05$) higher in the rats fed 7 and 14% FSO (I, P and I+P) groups as compared to the controls. The same trend was seen in the colonic mucosal scrapings. However, GST activity was significantly ($p<0.05$) higher in hepatic tissues compared to colonic mucosal scrapings. A possible mechanism to explain the anticancer role of omega-3 PUFAs during the initiation phase of colon carcinogenesis could be related to the ability of omega-3 PUFAs to positively influence the metabolic activation and detoxification of AOM. In the current study we showed that feeding Flax Seed Meal (FSM) at 10 and 20% levels and Flax Seed Oil (FSO) at 7 and 14% levels in an AIN-93M based diet was effective in reducing the incidence of colon tumors, tumor size and tumor/TBR ratio in Fisher 344 male rats. The results of this study indicate that bioactive phytochemicals such as dietary fiber, essential fatty acids and lignans such as those found in flax seed meal and flax seed oil, suppress colon tumors, particularly at the promotion stage and may therefore, be an effective chemopreventive agent.

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