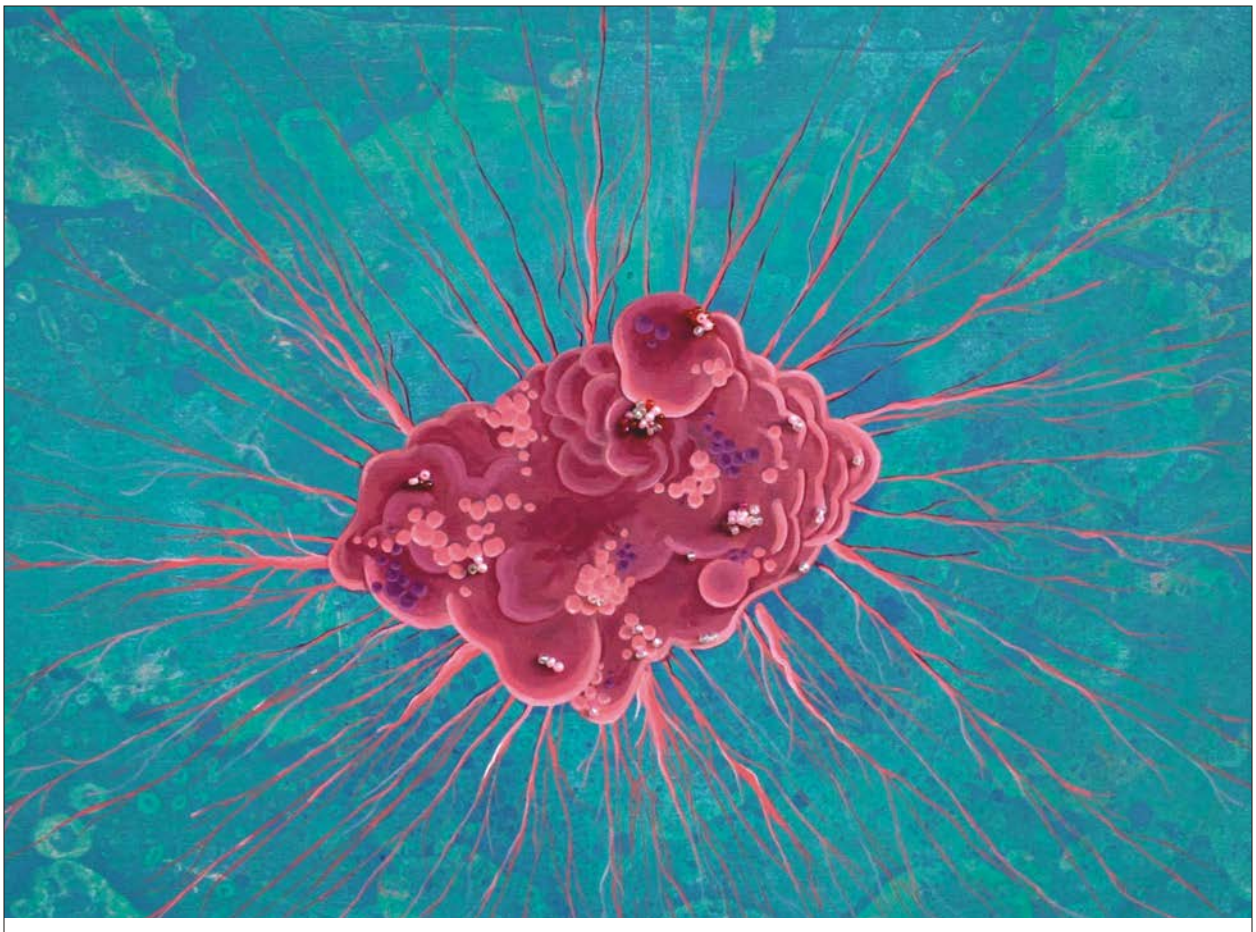

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A Comparison of Rice Bran, Corn Oil and Soybean Oil Against Azoxymethane Induced Colon Cancer in a Fisher 344 Rat Model

V. Panala, M. Verghese, J. Boateng, R. Field, L. Shackelford and L.T. Walker
Department of Food and Animal Sciences, Alabama A and M University,
P.O. Box 1628, Normal, AL 35762, USA

Abstract: The objective of this study was to compare the inhibitory effects of Rice bran oil (RBO), Corn oil (CO) and Soybean oil (SBO) at 7% (normal fat level) and 14% (high fat level) on Azoxymethane (AOM) induced Aberrant Crypt Foci (ACF). The long term effect (End Point Tumor (EPT) study) of dietary fat from the above sources on colon cancer in Fisher 344 male rats was determined. In the ACF study 2 groups of F344 rats (4 weeks old) (n = 6) received AIN-93G Control (C) diet containing 7 and 14% Soybean oil (SBO). The remaining groups were assigned treatment diets consisting of 7 and 14% RBO and CO. The rats remained on their respective diets for 13 weeks. Rats in the EPT study were fed a control (AIN-93G) diet with 7% SBO, while the treatment groups were fed diets containing 7% RBO and CO, respectively. At 20 week of age rats in the EPT study were switched to AIN-93Maintenance (M) diets. All rats received 2 s/c injections of AOM at 7 and 8 week of age @ 16 mg kg^{-1} body weight in saline. At 17 and 45 week of age all rats were killed by CO_2 asphyxiation. Total colonic ACF in the rats fed SBO, RBO and CO at 7 and 14% levels ranged from 101-189. In the EPT study, all the rats fed 7% SBO and CO developed tumors (100% tumor incidence) while tumor incidence in the groups fed RBO, was 54% while tumor size (mm) and tumor/Tumor Bearing Rat ratio (TBR) in the rats fed SBO, RBO and CO ranged from 1.3-6.86 and 1.83-5.86, respectively. Present results indicate that the type and constituents (such as n-3 PUFA, vitamin E, phytosterols) of dietary fat plays a significant role in the formation of AOM induced colonic ACF and tumors in Fisher 344 rats.

Key words: Colon cancer, azoxymethane, rice bran oil, corn oil, Fisher rat model, dietary fat

INTRODUCTION

Cancer is a leading cause of death in the United States. Colon cancer is the second most common cause of cancer related deaths. The ACS estimates that about 112,340 cases of colorectal cancer will be diagnosed and 52,180 individuals will die from the disease in 2008.

Dietary modification is one of the strategies used in the reduction or prevention of chronic diseases (Aggarwal *et al*., 2006; Doll and Peto, 1981; Se-Young *et al*., 2005; Park *et al*., 2005). Numerous epidemiological studies (Koushik *et al*., 2007; Zoran *et al*., 1997; McIntosh *et al*., 2001; Azizah and Yu, 2000; Lorraine and Suh, 2003; Khatiwada *et al*., 2006) have illustrated that bioactive compounds from plant origin such as, dietary fiber, phenolic compounds, phytic acid, tocopherol, phytoestrogens may play beneficial roles in the prevention of certain chronic diseases.

One of the factors associated with cancer risk is dietary fat. It has been suggested that dietary fat can promote the development of colon cancer and evidence involving the effects of dietary fat especially those from animal sources have been shown in animals fed high-fat diets. Epidemiological data show (Carroll, 1992; Takahashi *et al*., 1997) strong positive correlations between colon cancer incidence and mortality and level of dietary fat.

Corresponding Author: Martha Verghese, Department of Food and Animal Sciences, Alabama A and M University, P.O. Box 1628, Normal, AL 35762, USA Tel: (256) 372-5445 Fax: (256) 372-5432

Rice bran oil and wheat germ oil are rich sources of phytochemicals such as tocopherols, tocotrienols, squalene, γ-oryzanol, inositol hexaphosphate (IP_6) (Azizah and Yu, 2000; Juan *et al*., 2006; Dommels *et al*., 2003; Ghoneum and Gollapudi, 2003) and omega-3 fatty acids (n-3 fatty acids). It is one of the richest sources of phytosterols in nature. Phytosterols are found in plant foods and they are fat soluble phytonutrients present at high concentrations in vegetable oils and whole grains. It has been reported that phytosterols inhibited the growth of cancer cells (Awad and Fink, 2000).

The study of precancerous lesions in the colon is possible by the identification of Aberrant Crypt Foci (ACF) in rodent colons treated with a carcinogen. The growth, morphological and molecular features of ACF support the fact that ACF are putative preneoplastic lesions. The ACF system is used extensively to identify modulators of colon carcinogenesis. Using ACF as a model for a short term assay for colon tumorigenesis in laboratory rodents has so far proven to be a reliable biomarker (Bird, 1987). Azoxymethane, the carcinogen used to induce tumorigenesis in this study, is commonly used to determine the chemopreventive effectiveness of foods in rodent models (Corpet and Pierre, 2003). The aim in this study was to determine the effects of feeding Soybean oil (SBO), Rice bran oil (RBO) and Corn oil (CO) at 7% (normal fat) and 14% (high fat) levels on the incidence of (AOM) induced colon cancer in Fisher 344 male rats.

MATERIALS AND METHODS

Animal Housing and Diet

Fisher 344 male weanling rats were obtained from Harlan, IN, in January 2006 and were housed in stainless steel wire cages at 2 rats per cage. The temperature and relative humidity were maintained at 21°C and 50%, respectively. Light and dark cycles were kept at 12 h each. All rats were given free access to water and were fed AIN 93(G) (Reeves *et al*., 1993a, b) control diet during a one-week adaptation period. After this period the rats were assigned to 6 groups for the Aberrant Crypt Foci experiment (ACF) and 4 groups (12 rats each) for the End Point Tumor Experiment (EPTE) (Fig. 1). The rats fed the control diet in the ACF study (groups 1 and 2) were given free access to AIN 93 G diet containing either 7 or 14% Soybean oil (SBO) (Table 1) throughout the experimental period. The remaining groups in the ACF study were assigned treatment diets and were fed with AIN 93 G based diets containing either 7 or 14% Rice bran oil (RBO) and Corn oil (CO) (California rice oil

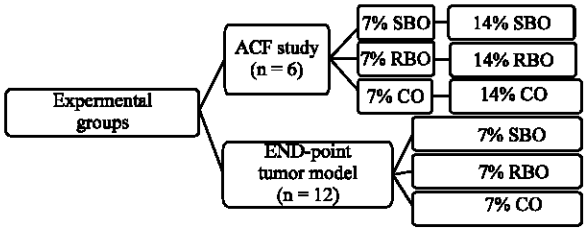

Fig. 1: Experimental design, SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

Table 1: Composition of diets

Ingredients (g)	SBO (7 and 14%)	RBO (7 and 14%)	CO (7 and 14%)
Cornstarch	397.5	397.5	397.5
Soybean Oil (SBO)	70/140	0	0
Rice Bran Oil (RBO)	0	70/140	0
Corn Oil (CO)	0	0	70/140
Common ingredients	532.5	532.5	532.5

Formulations of diets based on AIN-93G (American Institute of Nutrition, Reeves *et al*., 1993a, b), [b]Common ingredients (g): dextrose, 132: mineral mix (AIN-93G), 35: vitamin mix, 10: L-cysteine, 3: choline bitatrate, 2.5

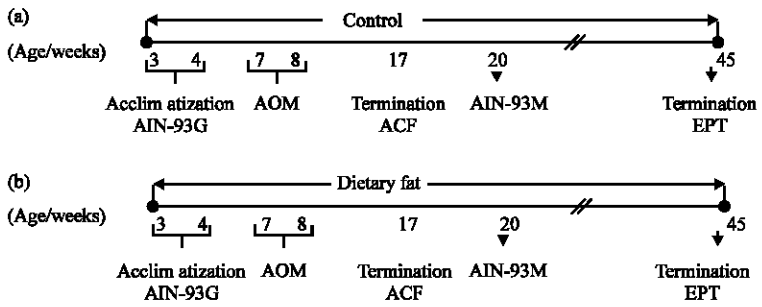

Fig. 2: Schematic representation of (a) control and (b) dietary fat diets on AOM induced colon cancer in Fisher 344 male rats. Scale is not proportional

Company, Costa Mesa , CA) instead of SBO (Table 1). In the EPT study, the rats were initially fed AIN-G with 7% SBO, RBO and CO, respectively. Rats were switched to AIN-93M at 20 weeks of age (Fig. 2). Ingredients for preparing AIN-93G/M diets were obtained from MP Biochemical's (Costa Mesa, CA). All diets were prepared fresh weekly and stored at 4°C until fed. Biweekly body weights and weekly feed intakes were recorded.

Carcinogen Injection

For induction of colon cancer all rats were given s/c injections of Azoxymethane (AOM), (Midwestern Research Institute, NCI Chemical Repository, Kansas City, MO) in saline at 16 mg kg^{-1} body weight at 7 weeks and another at 8 weeks of age.

Sample Collection

All rats were killed by using CO_2 euthanasia at 17 weeks of age for the ACF study and 45 weeks for the EPT study. Colon samples were collected for enumeration of Aberrant Crypt Foci (ACF), which are preneoplastic lesions. In the EPT study, colon tumors were collected and characterized as described by Shackelford *et al.* (1983). Liver samples and colonic mucosal scrapings were collected and stored at -80°C until analysis for Glutathione-S-Tranferase (GST) activity.

Enumeration of Aberrant Crypt Foci

Colons of rats from each group were removed and flushed with PBS (0.1M, pH 7.2). These were cut open then fixed in buffered formalin. Each colon was cut into 2 equal sections, distal and proximal and stained with methylene blue. ACF and crypts per focus were enumerated as described by Bird (1987).

Characterization of Colon Tumors

Colons of all rats in the EPT study were removed and flushed with potassium phosphate buffer (0.1 mol L^{-1}, pH 7.2). Tumors were characterized based on number, size and location as described by Shackelford *et al.* (1983).

Glutathione-S-Transferase (GST) Activity

GST in the liver and colonic mucosal scrapings were assayed by the procedure outlined by Habig *et al.* (1974). The assay mixture (1 mL) contained potassium phosphate buffer (0.1 M, pH 6.5), 1-chloro 2, 4-dinitrobenzene (1 mM) and glutathione (1 mM). Reactions were started by the addition of 100 µL of sample and change in absorbance at 340 nm as a function of time was monitored in a Cary 1/3 UV/VIS dual beam spectrophotometer. Total enzyme activity was measured at the end of 5 min.

Statistical Analysis

Results are presented as Means±SEM. ANOVA was used to determine significant differences among the treatment groups. Where significant ($p<0.05$), means were separated using Tukey's Studentized Range Test. Statistical analysis was conducted using SAS.

RESULTS

Aberrant Crypt Foci (ACF Study)

Feed Intake and Weight Gain

Rats fed the high fat diet (14% SBO and CO) which is typical of a western diet had higher weight gains compared to those fed normal fat diet (7% SBO, RBO and CO) and high (14%) RBO (Table 2). Among the rats fed the high fat diet, weight gain was significantly ($p<0.05$) higher in SBO and CO groups compared to their lower fat counterparts. However, no significant differences were noted among the rats fed normal fat diets except CO. Feed intakes were not significantly different among the rats fed the control (SBO) and the treatment groups fed RBO and CO at both high and normal fat levels.

Aberrant Crypt Foci (ACF) and Total Crypts in Colon of Rats Fed Dietary Fat

Among all the groups, ACF was significantly ($p<0.05$) higher in the distal colon compared to the proximal colon. The rats fed SBO (7 and 14%) and 14% RBO and CO (7 and 14%) had significantly ($p<0.05$) higher numbers of ACF in the proximal colon compared to the group fed 7% RBO. ACF induction in the proximal colon ranged from 35-62. The incidence of ACF in the distal colon was significantly ($p<0.05$) higher in the rats fed the high fat control diet (14% SBO) and 14% CO compared to the rats fed normal fat control diet (7% SBO) and the treatment groups (RBO (7 and 14 and 7% CO). ACF in the rats fed the high fat diet was significantly ($p<0.05$) higher compared to their normal fat (7%) counterpart. Increasing fat from 7-14% resulted in an increased incidence of total ACF in rats fed SBO, RBO and CO by 16, 25 and 32%, respectively (Table 3).

Total aberrant crypt were significantly ($p<0.05$) higher in the groups fed 7 and 14% SBO and 14% CO compared to the treatment groups. Even though total aberrant crypt in the group fed 14% CO was comparable to the control (14% SBO), the rats fed normal CO (7%) had significantly lower number of aberrant crypt compared to the rats fed normal fat control diet (7% SBO), indicating perhaps that CO is much more effective in preventing the incidence of ACF at normal levels.

Table 2: Weight gain and feed intake in Fisher 344 male rats

Groups	Weight gain (g)	Feed intake (g)
Control (SBO 7%)	207.40±2.56^{c}	12.64±0.74^{a}
Control (SBO 14%)	253.40±5.18^{a}	13.12±0.96^{a}
RBO (7%)	195.40±6.40^{c}	13.21±0.41^{a}
RBO (14%)	228.60±11.23^{b}	13.20±0.37^{a}
CO (7%)	220.46±5.42^{b}	13.02±1.16^{a}
CO (14%)	261.04±7.26^{a}	13.02±1.16^{a}

Values are Means±SEM, n = 6, Means in column without a common letter(s) differ ($p<0.05$) using Tukey's studentized range test. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

Table 3: Aberrant crypt foci and total aberrant crypt foci in colon of AOM-induced Fisher 344 male rats

	ACF			Total aberrant crypts		
Dietary treatment	Proximal	Distal	Total	Proximal	Distal	Total
SBO (7%)	55±9.21^{ab}	105.0±11.63^{b}	160^{b}	152.0±24.89^{b}	310±40.53^{b}	462^{b}
SBO (14%)	56±9.87^{ab}	131.0±12.64^{a}	187^{a}	198.0±7.14^{a}	458±6.98^{a}	656^{a}
RBO (7%)	35±9.46^{c}	93.0±4.92^{b}	155^{b}	131.0±26.53^{ab}	265±22.34^{c}	396^{c}
RBO (14%)	62±9.16^{a}	81.0±10.6^{c}	116^{c}	77.0±22.07^{d}	205±25.6^{b}	279^{d}
CO (7%)	40±2.26^{b}	80.0±5.08^{c}	120^{c}	127.0±3.24^{ab}	261±5.46^{c}	388^{c}
CO (14%)	46±3.80^{b}	132.0±6.12^{a}	178^{a}	159.0±6.10^{b}	457±9.14^{a}	616^{a}

Values are Means±SEM, n = 6, Means in column without a common letter(s) differ ($p<0.05$) using Tukey's studentized range test. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

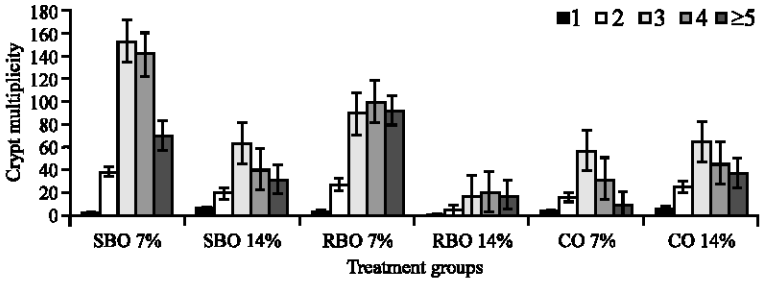

Fig. 3: Crypt multiplicity in Fisher 344 male rats fed dietary fat. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

Table 4: Effect of dietary fat on hepatic Glutathione S-Transferase (GST) activity in Fisher 344 male rats

Groups	GST activity (μmol mg^{-1})
Control (SBO 7%)	10.58 ± 0.32^{b}
Control (SBO 14%)	10.14 ± 1.02^{b}
RBO (7%)	33.57 ± 2.07^{a}
RBO (14%)	34.01 ± 1.67^{a}
CO (7%)	14.58 ± 1.60^{b}
CO (14%)	12.36 ± 0.98^{b}

Values are Means±SEM, n = 6, Means in column without a common letter(s) differ ($p<0.05$) using Tukey's studentized range test. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

Total Number of Crypts/Focus or Crypt Multiplicity

Figure 3 shows the total number of ACF with 1, 2, 3, 4 and =5 crypts per focus in rats fed SBO, CO and RBO (7 and 14%). The rats fed SBO (7 and 14%) and CO (7 and 14%) had significantly ($p<0.05$) higher numbers of ACF with 3, 4 and >5 crypts/focus compared to the groups fed RBO (7 and 14%). Foci with 1 and 2 crypts were higher in the RBO (7 and 14%) fed groups compared to their SBO and CO counterparts. RBO offered some protection against colon tumorigenesis as ACF with 1 and 2 crypts typically dissolve over time, although ACF with 3, 4 and >5 crypts/focus will sustain and eventually develop into tumors. The rats fed CO (14%) had the greatest number of ACF with 3, 4 and >5 crypts. RBO (14%) also offered a greater protection compared to RBO (7%) showing lower number of ACF with 1, 2, 3, 4 and >5 crypts. Rats fed CO (7%) had lower number of 1, 2, 3, 4 and >5 crypts/focus compared to the rats fed CO (14%).

Glutathione S-Transferase (GST) Activity

GST (a crucial detoxification enzyme) activity (μmol mg^{-1} in rats fed the control diets (SBO 7 and 14%) were significantly ($p<0.05$) lower than in the rats fed the treatment diets except for the group given CO. There were however, no significant differences in the GST activities (μmol mg^{-1}) among the treatment groups (Table 4). GST activity (μmol mg^{-1}) in the treatment groups (RBO) were over 50% higher than in the control fed rats. Rats fed the high fat control diet (SBO 14%) had significantly ($p<0.05$) lower GST activity compared to their low fat counterparts fed RBO.

Endpoint Tumor Study (EPT)

Feed Intake and Weight Gain

Weight gains and feed intakes in the groups fed (SBO and CO) were statistically lower compared to RBO. No statistical differences were observed in feed intake among the experimental groups (Table 5).

Tumor Incidence and Tumor Size

In the rats fed SBO and CO, there was 100% tumor induction in the distal colon while tumor induction in the proximal colon varied depending on the treatment (Table 6). The group fed RBO had

Table 5: Weight gain and feed intake in Fisher 344 male rats fed dietary fat diets

Treatment groups	Weight gain (g)	Feed intake (g)
Control (SBO 7%)	291±7.20^{b}	15.26±0.53
RBO (7%)	325±6.40^{a}	15.57±0.41
CO (7%)	300±5.80^{b}	15.42±1.12

Values are Means±SEM, Means in column without a common letter(s) differ ($p<0.05$) using Tukey's studentized range test. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

Table 6: Percent incidence (%) of colon tumors in Fisher 344 male rats

Dietary treatments	N^1/N^2	Colon tumors (%)	Proximal tumors (%)	Distal tumors (%)
SBO (7%)	12/12	100.0	31.2	100.0
RBO (7%)	6/11	54.5	33.3	54.5
CO (7%)	12/12	100.0	40.0	100.0

N^1: No. of rats with tumors N^2: No. of rats at the end of the experiment. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

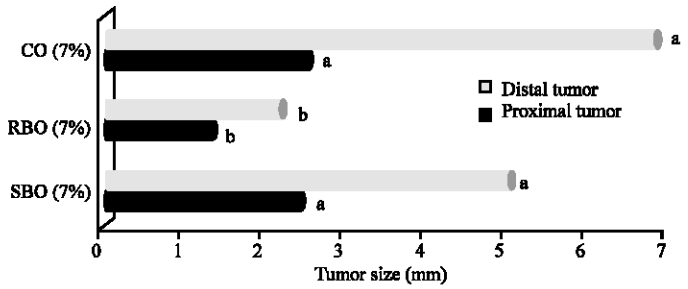

Fig. 4: Effect of dietary fat on tumor size in Fisher 344 rats Values are Means±SEM, Bars without a common letter(s) differ ($p<0.05$) using Tukey's studentized range test. SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

the lowest (54.5%) induction of tumors in the proximal colon while rats fed CO had 100% tumor incidence with 40% occurring in proximal colon. All rats fed SBO developed tumors (100% incidence) with 31% having proximal colon tumors.

The tumor size in experimental groups was significantly ($p<0.05$) larger in the distal colon compared to the proximal colon. Tumor size (mm) ranged from 1.3-2.52 in the proximal colon and 2.21-6.86 in the distal colon (Fig. 4). In the treatment groups fed RBO, tumor size was significantly smaller ($p<0.05$) compared to the control (SBO). There was however, no significant difference in tumor size (mm) between the treatment group fed CO compared to the control (SBO).

Tumor Numbers and Tumors/Tumor Bearing Rat Ratio (TBR)

Tumors numbers were 2-4 times higher in the distal colon compared to the proximal colon (Table 7). In rats fed RBO, tumor numbers in the proximal and distal colon were significantly ($p<0.05$) lower compared to the groups fed CO and SBO. Total tumors in the SBO fed groups was over 5 and 6 times greater than in the group fed RBO. However, tumor numbers (proximal and distal colon) in rats fed CO was not significantly different compared to the control.

There was a significant ($p<0.05$) decrease in tumor/Tumor Bearing Rat ratio (TBR) when rats were fed RBO compared to the groups fed SBO and CO. TBR was over 3 times greater in CO fed rats compared to the RBO fed group.

Glutathione S-Transferase

Hepatic GST activity (μmol mg^{-1}) was significantly ($p<0.05$) lower in the control (SBO) (18.31) and CO (15.86) fed groups compared to the group fed RBO (27.70). GST activity (μmol mg^{-1})

Table 7: Distribution and Characterization of AOM- induced colon tumors in Fisher 344 male rats

Treatment groups	N^1/N^2	Proximal tumors/rat (n)	Distal tumors/rat (n)	No. of tumors (n)	Tumors/tumor bearing ratio (TBR)
SBO (7%)	12/12	22^a	42^a	64^a	5.33^a
RBO (7%)	6/11	2^b	8^b	10^b	1.83^b
CO (7%)	12/12	26^a	44^a	70^a	5.86^a

Values are Mean±SEM, Means in a column with the same superscript do not significantly differ ($p \leq 0.5$) by Tukey's studentized range test ($p<0.05$). N^1: No. of rats with tumor: N^2 No. of rats at the end of the experiment. SBO: Soybean oil, RBO: Rice bran oil, CO: corn oil

Table 8: Glutathione-S-Transferase (GST) activity in liver and Colonic Mucosal Scrapings (CMS) in Fisher 344 male rats

Treatment groups	Hepatic GST (μmol mg^{-1})	(CMS) GST (μmol mg^{-1})
SBO (7%)	18.31 ± 1.21^b	0.38 ± 0.04^c
RBO (7%)	27.70 ± 1.39^a	5.10 ± 0.04^b
CO (7%)	15.86 ± 1.04^b	0.30 ± 0.06^c

Values are Mean±SEM, Means in a column with the same letter(s) do not significantly differ ($p \leq 0.5$) by Tukey's studentized range test ($p<0.05$). SBO: Soybean oil, RBO: Rice bran oil, CO: Corn oil

in the colon (CMS) was also similar in the control (SBO) (0.38) and CO (0.30) fed groups but significantly ($p<0.05$) higher in the RBO fed group (5.10). In the RBO group, GST activity in the colon was significantly higher compared to either the control (SBO) or CO. The lower GST activity in the liver and colon in CO fed rats may have contributed to the increased incidence of colon tumors compared to the rats fed RBO (Table 8).

DISCUSSION

ACF Study

This study was conducted to evaluate the possible inhibitory effects of selected dietary fat (RBO, CO and SBO) on AOM-induced ACF and colon tumorigenesis. Weight gains were significantly similar in all of the experimental groups. A similar study conducted by Boateng *et al.* (2006) also observed no significant differences in body weights in rats fed normal and high fat diets. Feed intake in rats fed 7 and 14% dietary fat were not significantly ($p<0.05$) different among the groups.

Rats fed RBO (7%) and CO (7%) showed a reduced incidence of ACF compared to the control group fed SBO at 7 and 14% levels. These results are comparable to other studies conducted in our laboratory where red palm oil (7 and 14%) (Boateng *et al.*, 2006) and flax seed oil (7 and 14%) (Williams *et al.*, 2007) which are also rich sources of vitamin E, significantly ($p<0.05$) reduced the number of ACF and total crypts in Fisher 344 male rats. The mechanisms by which vitamin E may have reduced the number of ACF and total crypts are by neutralizing reactive oxygen species and other free radicals that may cause DNA damage (Jacobs and Steffen, 2003) and also by possibly inhibiting Cyclooxygenase (COX-2) activity (O'Leary *et al.*, 2004). Total aberrant crypts in all the treatment groups except 14% CO were significantly ($p<0.05$) lower compared to the control groups fed SBO at 7 and 14% levels. The enhancing effect of high fat CO and SBO diet on ACF and total aberrant crypt formation may be that these dietary fats are rich sources of n-6 polyunsaturated fatty acids (n-6 PUFA) which have been shown to have tumor enhancing effect in animal models during the post initiation phase. In animal studies, diets rich in n-6 PUFAs have been reported to increase secondary bile acids and/or PGE_2 synthesis through its production of Arachidonic Acid (AA). Increased secondary bile acid production induces tissue ornithine decarboxylase (ODC) activity and cell proliferation.

Crypt multiplicity, which is the number of crypts per focus, is a good predictor of tumor incidence with ACF containing≥ 3 crypts/foci correlating to a >50% tumor incidence and <3 crypts/foci correlating to <30% tumor incidence (Alabaster *et al.*, 1996). The lower number of ACF with ≥3

crypt/foci in rats fed RBO (7 and 14%) perhaps indicates that RBO phytochemicals such as tocotrienols, gamma oryzanol and other plant sterols were involved in inducing apoptosis in the colonic epithelial cells. Studies have showed that tocotrienols are effective in inducing apoptosis by increasing NK cells and β-Lymphocytes; NK cells have been associated with having cytotoxic activity against tumor cells (Guthrie *et al*., 1997; Nesaretnam *et al*., 1998, 2002).

One of the protective effects of a phytochemical in carcinogenesis is explained by its ability to modulate biotransformation enzymes that are involved in the carcinogenesis process. Glutathione-S-Transferase (GST) is among the principal detoxifying enzymes involved in conjugating reactions of phase II metabolism and also used as a marker enzyme to monitor the severity of carcinogenesis. The results showed that GST activity was significantly ($p<0.05$) induced in rats that consumed RBO compared to CO and SBO groups. CO and SBO being rich sources of n-6 PUFA failed to induce GST activities hence decreasing the defense capacity towards potential carcinogens. The n-6 PUFA (AA) was shown to be an ineffective inducer of electrophile-responsive element (EpRE)-regulated gene which regulates genes encoding phase II detoxification enzymes (Van-Beelen *et al*., 2006). Even though RBO is also a source of n-6 PUFA, antioxidants such as Vitamin E, may have exerted specific effects on phase II enzymes, which resulted in increased detoxification and reduced rates of activation by altering the amounts and activities of oxidative Phase I and conjugative Phase II xenobiotic metabolizing enzymes (Dommels *et al*., 2003).

End Point Tumor Study

The End Point Tumor (EPT) study was to determine the long term effect of feeding dietary fat at normal fat levels (7%) on AOM-induced colon cancer. While no significant differences were observed in feed intake among the experimental groups, body weights were significantly ($p\leq 0.05$) higher in the treatment group fed RBO compared to SBO fed rats. Since caloric intakes in all the groups were similar, it can be assumed that the lower weight gain in SBO and CO fed groups was due to the higher tumor incidence thereby reducing nutrient absorption sites and leading to weight loss.

In all the experimental groups, tumor numbers were significantly ($p<0.05$) higher in the distal colon compared to the proximal colon. As was observed in the ACF study, the distal colon of rats fed selected dietary fat developed significantly ($p<0.05$) higher number of ACF in the distal colon compared to the proximal colon. Similar results were reported in other studies where the number of tumors was higher in the distal colon compared to the proximal colon (Bommareddy *et al*., 2006; Hughes *et al*., 1997). In the present study feeding CO and SBO resulted in significantly ($p<0.05$) higher number of tumors compared to the group fed RBO.

SBO and CO which are rich source of n-6 polyunsaturated fatty acids promote colon tumorigenesis, as shown in the number of rats that developed tumors. Many studies indicate that dietary fats containing high amounts of n-6 PUFAs such as CO and SBO enhanced chemically induced colon tumorigenesis (Carroll, 1992; Narisawa *et al*., 1991; Singh *et al*., 1997). The high concentration of phytochemicals in RBO may have led to induction of apoptosis thereby decreasing cell proliferation and malignant cell growthin the colonic mucosa. This may explain the low number of tumors induced in these rats.

SBO and CO significantly enhanced cell growth proliferation in colonic epithelial cells as seen in tumor size. It is possible to suggest that dietary fat affects proliferation of colon cells through alteration of growth factor activation of intracellular signals. Growth factors such as insulin-Like Growth Factors (IGF) may be involved in the regulation of cellular growth and differentiation. *In vitro* experiments have indicated that increased levels of IGFs may stimulate the development of cancer by regulating cell proliferation, replication, inhibiting apoptosis and stimulating DNA synthesis by causing cells to navigate through the successive phases of the cell cycle (Dunn *et al*., 1997a, b; Jones and Clemmons, 1995; Khandwala *et al*., 2000). According to Zhang *et al*. (1998), dietary fat type and

quantity may affect the expression of IGF receptors in the colon, which thus influences colon cell proliferation and thereby colon cancer risk. A high Arachidonic Acid (AA) content of rat colonic mucosal phospholipid has been shown to be associated with increased rates of cell proliferation.

There is evidence suggesting that genes involved in the control of cell proliferation, apoptosis and inflammation (p27, Bcl-2, PPARγ, Il-2, tropomyosin and CTGF) and genes involved in vitamin E metabolism (*-TTP, Cyt P-450) are upregulated by one or more tocopherols, while numerous other genes with tumor-promoting activity are downregulated (Azzi *et al*., 2004; Traber, 2005; Betti *et al*., 2006). This may be one of the mechanisms behind the significantly smaller tumors in the group fed RBO, as this dietary fat is a rich source of vitamin E.

The number of tumors induced per rat is critical in determining the efficacy of a chemopreventive agent or phytochemical on end-point tumors because they give a more precise picture of tumor inhibition (i.e., the number of tumors induced in rats that developed tumors) (Verghese *et al*., 2002a, b). We observed a correlation between Tumor Bearing Ratios (TBR) and number of tumors induced in experimental groups. Rats fed SBO and CO had higher TBR compared to rats fed RBO.

GST activity was similar with those from the ACF study which shows that GST activity in rats fed RBO was significantly ($p<0.05$) induced compared to CO and SBO groups. The residual activities in the colon were similarly enhanced in treatment groups fed RBO.

The results of this study indicate that even though high intake of dietary fat has a tumor promotional effect, the type and constituents of fat may play an important role in the development of colon cancer.

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