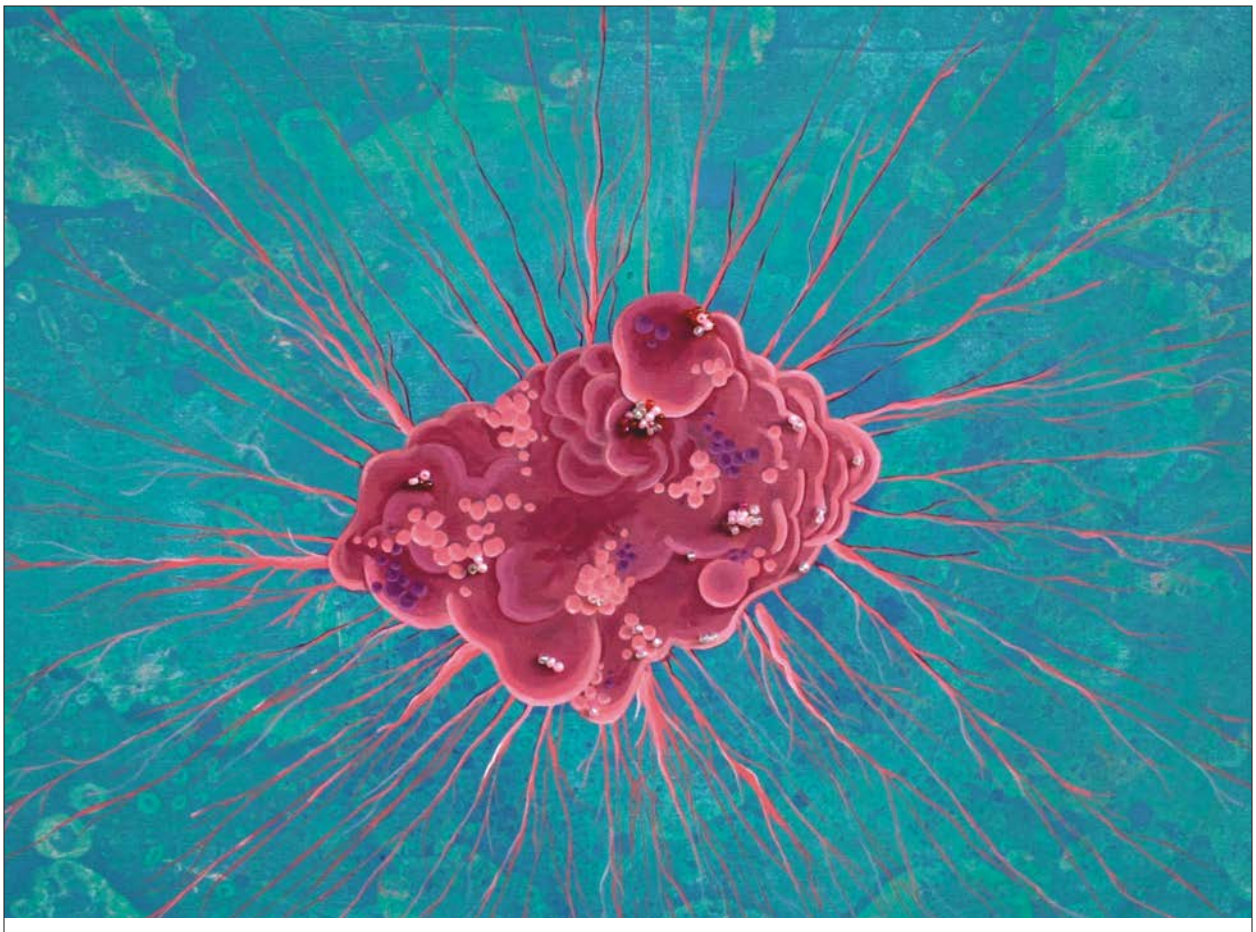

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Inhibitory Effects of Feeding Selected Levels of Peanuts on Azoxymethane-Induced Aberrant Crypt Foci in Male Fisher 344 Rats

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Abstract: The aim of this study was to explicate the effect of feeding peanuts at 3 levels (10, 15 and 20 g 100^{-1} g of feed) on azoxymethane (AOM)-induced Aberrant Crypt Foci (ACF) in Fisher 344 male rats. Four groups of rats were fed AIN93-G control diet (n = 8) and 3 experimental diets containing 10, 15 and 20% peanuts (n = 8) for 13 weeks. All rats received 16 mg kg^{-1} body weight of AOM at 7 and 8 week of age. The rats continued to receive the assigned diets until euthanized by CO_2 at 17 weeks of age. The percent reductions in ACF in the groups consuming diets containing 10, 15 and 20% peanuts were 31.9, 48.7 and 61%, respectively. GST activity (μmol mg^{-1}) in the rats fed control diet was significantly ($p<0.05$) lower compared to the rats fed peanuts. Present results indicate that feeding peanuts significantly reduced AOM-induced ACF in Fisher 344 male rats. The phytochemicals present in peanuts may have antitumor properties and could possibly reduce colon cancer.

Key words: Peanuts, cancer, phytochemicals, azoxymethane, aberrant crypt foci

INTRODUCTION

Cancer is the leading cause of death in the United States (American Cancer Society (Jemal *et al*., 2005a). The prognosis for a patient with metastatic carcinoma of the lung, colon, breast, or prostate (four of the most common and lethal forms of cancer, which together account for more than half of all deaths from cancer in the USA), remains dismal (Jemal *et al*., 2005b). Chemoprevention is one of the most direct ways to reduce morbidity and mortality (Aziz *et al*., 2003).

Cancer of the colon is a highly treatable and often preventable disease when localized to the bowel. It is the third most commonly diagnosed cancer in the United States and the second most common cause of cancer-related deaths (Jang *et al*., 1997). Diet is the single greatest contributor to human cancer, including colon cancer and may be associated with 35-70% of the incidence of the disease (Jemal *et al*., 2005b). Colorectal cancer can take many years to develop and it usually is preceded by changes in the lining of the colon or rectum that occur over several years (Doll, 1998).

Recent epidemiological studies have shown that consuming a diet rich in plant-derived foods that are high in phenolic compounds, even while consuming high amounts of saturated fatty acids (Potter *et al*., 1993) are associated with a reduced incidence of cardiovascular mortality (Renaud and Lorgeril, 1992; Hertog *et al*., 1993, 1995; Keli *et al*., 1996). Recently, it was demonstrated that

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polyphenolic compounds extracted from red wine and black tea protected against DNA oxidative damage in rat liver and intestine (Geleijnse *et al.*, 1999) and inhibited colon carcinogenesis induced by azoxymethane (AOM) in rodents (Lodovici *et al.*, 2000). Polyphenols are powerful antioxidants and free radical scavengers (Caderni *et al.*, 2000). They have anti-inflammatory properties (Rice-Evans *et al.*, 1995) and may modulate the activity of phase I and phase II enzymes, in particular glutathione (GSH)-related enzymes (Middleton and Kandaswami, 1992). Glutathione S-Transferases (GSTs) is a multigene family of dimeric enzymes that are ubiquitously distributed and comprise approximately 2-4% of total cytosolic proteins (Steele *et al.*, 2000) Aberrant Crypt Foci (ACF) are induced specifically by carcinogens that predominantly elicit colonic tumors and are considered to be precursors of colon cancer. Multiplicity of ACF increases with time and appears to be a predictor of tumor outcome (Eaton and Bammler, 1999).

The polyphenolic compound resveratrol is a naturally occurring phytochemical and can be found in many plant species, including grapes, peanuts and various herbs (Pretlow *et al.*, 1991). Several studies have established that resveratrol can exert anti-oxidant and anti-inflammatory activities. Resveratrol has also been found to possess cancer chemopreventive activity through the inhibition of ribonucleotide reductase and cellular events associated with cell proliferation, tumor initiation, promotion and progression (Cal *et al.*, 2003; Fontecave *et al.*, 1998; Jang *et al.*, 1997). The present study was designed to explicate the effects of feeding selected levels of peanuts (10, 15 and 20%) on Azoxymethane induced aberrant crypt foci in male Fisher 344 rats and the activity of GST (a phase II detoxification enzyme) in the liver.

MATERIALS AND METHODS

Animals and Housing

Male Fisher 344 weanling rats were obtained from Harlan, IN and housed in stainless steel wire cages (2 rats cage^{-1}). The temperature and relative humidity were maintained at 21 and 50%, respectively. Light and dark cycles were 12 h each and feed and water were provided *ad libitum*. Following an acclimatization period of one week, rats were randomly divided into 4 groups (8 rats each) and fed control and experimental diets for 13 weeks (Fig. 1) in September 2005. During that time, biweekly body weights and daily feed intakes were recorded. All rats received AIN 93G based diets (Reeves *et al.*, 1993a, b), for the treatment diets, modifications were made to cornstarch, casein and soybean oil (Table 1). All diets were prepared at intervals of 4 weeks or less and stored at refrigeration temperature (4°C) until fed.

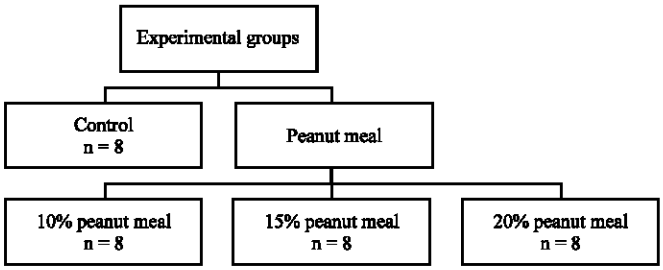

Fig. 1: Experimental design-Diets consumed by Fisher 344 male rats during the experiment. One control and 3 peanut diets were fed to the rats for the duration of the experiment (13 week). Peanuts were added into the American Institute of Nutrition (AIN 93 G) (control) diet at 10, 15 and 20% levels

Table 1: Composition of experimental diets based on American Institute of Nutrition (growth) diets

		Peanuts (%)		
Ingredients (g kg^{-1} diet)	Control	10	15	20
Cornstarch	397.48	360.00	391.50	307.00
Peanuts	0.00	100.00	150.00	200.00
Soybean oil	70.00	27.00	5.50	0.00
Casein	200.00	180.00	170.00	160.00
Common ingredients[2]	333.00	333.00	333.00	333.00

[1]Formulations of diets based on AIN-93G (American Institute of Nutrition, Reeves and others, 1993a, b), [2]Common ingredients (g): dextrose, 132; mineral mix (AIN-93G), 35; vitamin mix, 10; L-cystein, 3; choline bitatrate, 2.5

Preparation of Peanut Meal

Raw peanuts (Virginia type) were roasted in a microwave oven (Kenmore microwave oven, 1200 watts) for 8 min. The roasted peanuts were ground into a meal using a food processor (Robot coupe, Blixer RSI, BS3) and stored in plastic containers at 4°C until use.

Chemicals and Dietary Ingredients

All biochemicals including Azoxymethane (AOM) were obtained from Sigma Chemical (St Louis, MO). Dietary ingredients were obtained from ICN (Costa Mesa, CA). Peanuts were from Flavor House Products, Inc., (Dothan, Alabama).

Carcinogen Injection

For induction of ACF, all animals received s/c injection of Azoxymethane in saline @ 16 mg kg^{-1} body wt. at 7 weeks and another at 8 weeks of age.

Colon Sample Collection and Counting of ACF

The colons were removed and flushed with potassium phosphate buffer (0.1 mol L^{-1}, pH 7.2) and scored for Aberrant Crypt Foci (ACF). ACF in the colon were counted as described by Bird (1987). Total ACF as well as the number of crypts per focus was scored using a light microscope. Aberrant crypts were distinguished from the surrounding normal crypts by their increased size, significantly increased distance from lamina to basal surface of cells and the easily discernible pericryptal zone.

Cecal Weight and Cecal pH

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

Statistical Analysis

Data presented in this study were analyzed using the SAS Statistical Program (2004). Results were performed by ANOVA and values are given as Means±SEM and means were separated using Tukey's studentized range test. Differences between treatment groups were tested by student's t test and paired t-test. Unless otherwise indicated level of significance was considered at $p<0.05$.

RESULTS

Weight Gain, Feed Intake, Cecal Weight and Cecal pH

There were no significant differences in weight gain or feed intake between control and treatment groups (Table 2). Cecal weight and cecal pH were statistically ($p<0.05$) similar in all of the experimental groups. While cecal pH was slightly above neutrality in all of the groups, the rats fed high peanut diet (20%) had a cecal pH below neutrality (6.74).

Table 2: Weight gain, feed intake cecal weight and cecal pH in rats fed peanuts

Treatments	Weight gain (g/13 weeks)	Feed intake (g day^{-1})	Cecal weight (g)	Cecal pH
Control (C)	203.3±3.67	21.00±0.01	1.25±0.16	7.90±0.06
10% peanut	210.9±6.33	20.18±0.55	1.00±0.01	7.63±0.04
15% peanut	207.7±5.16	20.97±1.07	1.25±0.16	7.60±0.07
20% peanut	210.6±17.6	17.81±1.69	1.25±0.25	6.74±0.09

Values are Means±SEM, n = 8

Table 3: Effect of peanuts on number of Azoxymethane-induced (AOM) Aberrant Crypt Foci (ACF) in colon of Fisher 344 male rats

Treatments	Proximal colon	Distal colon	Total colon
Control (C)	31.6±5.4^{a}	122.6±10.9^{a}	154.2^{a}
10% peanut	26.8±4.8^{b}	78.2±7.8^{b}	105.0^{b}
15% peanut	22.2±3.6^{b}	56.8±7.2^{c}	79.0^{c}
20% peanut	18.5±2.2^{b}	41.5±4.7^{d}	60.0^{d}

Values are means, n = 8. ^{abc}Means in a column with the same superscript do not significantly differ ($p<0.05$) using Tukey's studentized range test

Table 4: Percentage reductions in aberrant crypt foci compared to the control

Treatments	Reductions (%)
10% peanut	31.9
15% peanut	48.7
20% peanut	61.0

Aberrant Crypt Foci (ACF) and Crypt Multiplicity in AOM Induced Rats

The rats administered saline (vehicle) showed no evidence of ACF formation in the colon (data not shown). In all of the groups, the number of ACF in the distal colon was significantly ($p<0.05$) higher than in the proximal colon (Table 3), thus making this data consistent with reports that the distal colon shows a greater incidence of colorectal cancer compared to the proximal colon in humans. In rats fed the control, ACF was significantly higher ($p<0.05$) in both the proximal and distal colon compared to the rats fed peanut. While ACF in the proximal colon among the treatment groups, were not significantly ($p<0.05$) different, there was a dose dependant relationship seen in the incidence of ACF in the distal colon in rats fed 10% peanut and the groups fed 15 and 20% peanut. However, there was no significant difference seen in the rats fed 15 and 20% peanuts. ACF in the total colon was significantly ($p<0.05$) higher in the rats fed control compared to the groups given the treatment diets. Among the treatment groups, the rats fed 10% peanut had a significantly ($p<0.05$) higher number of ACF compared to the other groups. Total ACF reduction in rats fed treatment diets i.e., 10, 15 and 20% peanut were 31.9, 48.7 and 61%, respectively compared to the control (Table 4),

Figure 2 shows the size of the ACF expressed as the number of aberrant crypt/ ACF or crypt multiplicity. The total numbers of foci containing 1, 2, 3, 4 and = 5 crypts were counted in the proximal and distal colon. Although foci with 1 and 2 crypts did not differ among experimental groups, foci with 3, 4 >5 aberrant crypts were significantly ($p<0.05$) lower in rats fed peanuts compared to the control. ACF with 1 and 2 crypts typically dissolve over time, while ACF with 3, 4 and >5 crypts/focus will sustain and eventually develop into tumors. Among the treatment groups, crypt multiplicity was lowest in the rats fed 20% peanut and high in the group fed 10% peanut diet.

Total Hepatic Glutathione-S-Transferase (GST) Activity

Total glutathione-s-transferase (GST) activity in rats fed control and the treatment diets (peanut meal at 10, 15 and 20% levels) ranged from 7.55 (μmol mg^{-1}) to 23.35 (μmol mg^{-1}) (Table 5). In the rats fed control, GST activity (μmol mg^{-1}) was significantly ($p<0.05$) lower compared to the treatment groups. GST activity was over 50% higher in the rats fed the peanut diets compared to the control. This maybe related to the fact that peanuts contain polyphenols such as resveratrol, a plant derived phytochemical which may have increased the activity of GST, an important phase II enzyme which

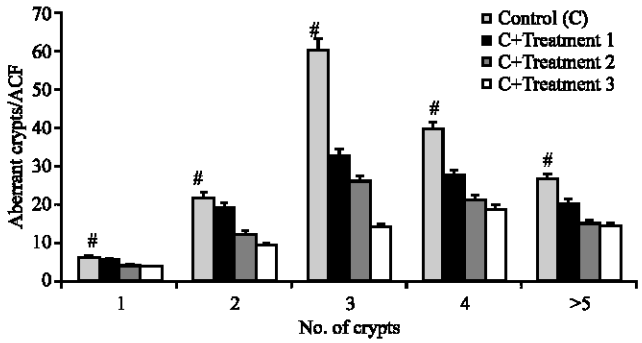

Fig. 2: Dose response effect of peanuts on number of aberrant crypt/focus/crypt multiplicity in rat colon. Values are means±SEM, n = 8/group. # significant differences among groups, ($p<0.05$)

Table 5: Total hepatic Glutathione -S- Transferase (GST) activity in AOM-induced (AOM) Fisher 344 male rats fed peanuts

Treatment	GST activity (μmol mg^{-1})
Control (C)	7.55 ± 0.73^{c}
10% peanut	18.95 ± 1.42^{b}
15% peanut	20.07 ± 1.40^{ab}
20% peanut	23.35 ± 0.73^{a}

Values are means±SEM, n = 8. ^{abc}Means in a column with the same superscript do not significantly differ ($p<0.05$) using Tukey's studentized range test

is responsible for detoxification of carcinogens and prevention of DNA damage. There was however, no significant difference in GST activity among the treatment groups.

DISCUSSION

This study was conducted to evaluate the potential inhibitory effect of peanuts against the formation of AOM-induced colonic ACF. Present data suggest that peanuts fed at selected levels (10, 15 and 20%) reduced the incidence of ACF compared to rats the fed control diet (AIN-93G). Rats fed 20% peanut in a meal form showed a marked reduction in the formation of ACF compared to rats given 10 and 15% peanut. Although the exact mechanism by which peanuts inhibited preneoplastic lesions in the colon is not fully known we can only speculate on the fact that peanuts contain several polyphenolic compounds such as resveratrol and isoflavones which studies have shown to have anticarcinogenic effects. These compounds may act as antioxidants, thereby preventing DNA damage by reactive oxidant species. In addition to polyphenolic compounds, peanut is a rich source of polyunsaturated fatty acids (PUFA) such as omega-3 (n-3 PUFA). N-3 PUFA has been shown to reduced chemically induced colon tumorigenesis in rodents (Dwivedi *et al*., 2003) due to its antiproliferative and anti-inflammatory properties.

Body weight gain of rats fed the peanut-containing diet was greater than the control diet although the difference was not significant ($p<0.05$). Verghese *et al*. (2002) reported that the body weight gain of rats fed the experimental diet (inulin) was less than those fed the control diet. Campbell *et al*. (1997) reported an initial decline in weight gain of rats after feeding the experimental diet (fructooligosaccharides, FOS), which could have been due to the change from a non-purified to a purified diet. However, there were no significant differences in body weight between groups in this study.

There were no significant differences ($p<0.05$) in cecal weight and cecal pH in rats fed peanuts. Verghese *et al*. (2002) reported a reduction in cecal weight and cecal pH after consumption of inulin.

Rowland *et al.* (1998) demonstrated a significant increase in cecal weight (20-32%) in rats fed 5 g 100^{-1} g insulin and a significant decrease in cecal pH. The authors proposed that consumption of insulin as a significant source of dietary fiber was associated with potentially beneficial changes in cecal physiology and bacterial metabolic activity in relation to tumor risk and the incidence of putative preneoplastic lesions in the colon.

GST activity in rats fed control diet was significantly ($p<0.05$) lower than the rats fed the treatment diets. The increase in GST activity may be one of the mechanisms by which peanuts may inhibit preneoplastic lesions. GST is a crucial phase II detoxification enzyme with anti-carcinogenic effects. GST catalyzes the conjugation of toxic and carcinogenic electrophilic molecules with glutathione, facilitating their clearance and thereby protecting cellular macromolecules including DNA from damage (Stoehlmacher *et al.*, 2002). This may have caused the significant reductions in ACF with feeding peanuts at 10, 15 and 20%, respectively.

CONCLUSION

In conclusion, strong relationships were seen between the reduction in ACF numbers in rat colons and levels of dietary peanuts. This may be due to the presence of non-nutritive bioactive phytochemicals in peanuts, which have been associated with a decreased risk of cardiovascular disease and certain cancers. Consumption of peanuts may reduce the chance of developing cancer and many other chronic diseases.

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