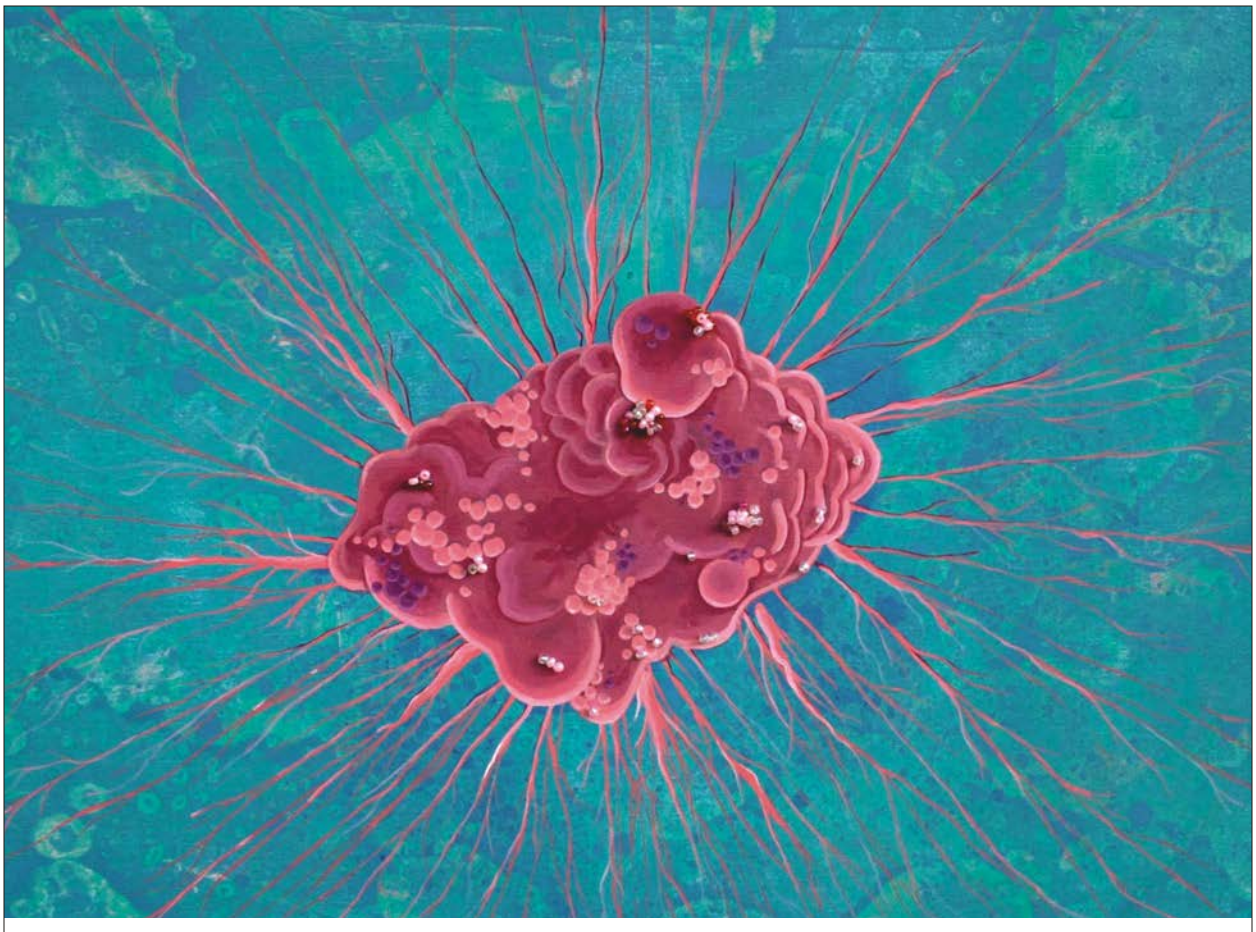

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Research Article
Modifying Effects of Pistachio Nuts on Antioxidant Enzymes in Azoxymethane (AOM)-induced Formation of Aberrant Crypt Foci

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Abstract

Background and Objectives: There is limited information on the effect of pistachio nuts in cancer prevention. Since pistachios contain several protective compounds with antioxidants properties, the aim of this study is to determine if pistachios can reduce precancerous colon cancer lesions in rats by affecting several biomarkers of oxidative stress including induction of endogenous antioxidant enzymes. **Methodology:** Thirty Fisher 344 male rats were randomly assigned to 5 groups. Rats in groups 1 and 2 were fed AIN-93G as positive (CON+) and negative control (CON-), while rats in groups 3-5 were assigned AIN-93G with 5, 10 and 15% pistachio meal (PM). Rats (except group 2), received AOM injections at 7 and 8 weeks of age and killed at 17 weeks of age by CO_2 asphyxiation. **Results:** Total ACF and crypt multiplicity, antioxidant enzymes: Glutathione-s-transferase (GST), glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) were determined. Total serum cholesterol (TC) and triglycerides (TG) were also assessed. Results showed significant ($p<0.05$) reductions in ACF in all treatment groups compared to the rats fed CON+(158). The ACF ranged from 72-119 in rats fed 5, 10 and 15% PM. Rats in group 2 developed no ACF. Significant ($p<0.05$) increase in antioxidant enzyme activities (protein μmol mg^{-1}) was observed compared to CON+. The GST (5.86-10.84), GR (4.91-7.21), CAT (1.66-3.38), GPx (4.25-7.61) and SOD (protein U mg^{-1}) (1.02-1.73). The TC and TG were significantly ($p<0.05$) decreased in the treatment groups compared to CON+. Data indicated PM reduced ACF by enhancing phase II and antioxidant enzyme activities and reducing serum lipids. **Conclusion:** The PM could be investigated as putative dietary chemoprevention in colon cancer therapy.

Key words: Pistachio nuts, azoxymethane, antioxidant enzymes, aberrant crypt foci, crypt multiplicity, glutathione-s-transferase, cholesterol, glutathione, catalase, triglycerides

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nuts have been an integral part of the diet for many around the world and evidence of its uses in the diet dates as far back to the ancient Romans. Nuts, which include tree nuts such as almonds, pecans, pistachios, walnuts, macadamia and Brazil nuts and grounds nuts which are referred to as peanuts, contain an impressive array of health promoting nutrients unmatched by most foods. In many ways, nuts have the ideal macronutrient profile for chronic disease risk reduction. Nuts are an excellent source of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and good sources of (n-3) fatty acids[1,2].

There is substantial evidence suggesting that nuts may have significant effects on several diseases. Recent studies, such as the adventist health study have reported the protective effect of nuts on metabolic syndrome and obesity[3]. Others have reported an association with lower risk of type 2 diabetes and coronary hear disease with nut consumption[4,5]. Furthermore, Gulati *et al*.[6] also showed the beneficial effect of pistachios on the cardiometabolic profile of Asian Indians with metabolic syndrome. It is apparent from these studies that the favorable fatty acid profile of nuts (high in unsaturated fatty acids and low in saturated fatty acids) contributes to cholesterol lowering and, hence, CHD risk reduction. The favorable fatty acid composition and lipid lowering effect of nuts has been demonstrated in experimental studies with almonds[7], peanuts[8,9], pecans[10] and walnuts[11,12].

Aside from being rich sources of dietary fat, macronutrients and essential micronutrients (folate, potassium, magnesium, vitamin E, K and selenium)[2,13], nuts contain dietary fiber and are significant sources of phytochemicals/bioactive components including flavonoids and stilbenes such as resveratrol, phytoestrogens and phytosterols[14-19], all of which have been implicated in disease prevention through their anti-inflammatory, anti-proliferative, anti-angiogenic and antioxidative actions[13,20-22].

The glutathione system has been reported to play a significant role in oxidative stress. Decline in cellular levels of glutathione (GSH) and increased levels of glutathione-disulfide (GSSG) are used as an indication of oxidative stress in any organ system. The effects that Reactive Oxygen Species (ROS) may have on organs are many. First, ROS can oxidize DNA with subsequent wrong or hampered synthesis of proteins leading to malfunctioning and structural changes of these proteins[23,24]. Furthermore, they may also peroxidize membrane lipids causing dysfunction of the cell membrane. By altering the DNA bases they are able to impair the DNA repairing mechanisms and through hydrolysis lead to malondialdehyde production from deoxyribose[23,24]. Several human chronic diseases including but not restricted to cardiovascular diseases (CVD), cancer and Alzheimer's have been associated with oxidative stress.

There is a large body of evidence suggesting cardioprotective effects of nuts, however, little information exists on the potential benefits of nuts, especially pistachio nuts with respect to cancer. The World Cancer Research Fund (WCRF) and the American Institute of Cancer Research (AICR) highlighted this view in a report that even though there are reasons that seem to suggest that diets high in nuts might protect against some cancer, evidence is still lacking. Moreover, according to Falasca *et al*.[25], studies indicating the chemopreventive properties of nuts such as pistachios are warranted, especially since the possible *in vivo* role of pistachio nuts in reducing colon cancer has not been previously explored. Thus, given the important role of pistachio nuts in metabolic diseases, it is worth investigating the anti cancer properties. In this study, the aim was to evaluate the chemopreventive potential of pistachio nuts and to further investigate its effect on biomarkers of oxidative stress and phase II antioxidant enzymes activities.

MATERIALS AND METHODS

Animal housing: Fisher 344 male rats were used for the study. Rats were assigned into groups and fed AIN-93G[26,27] as control (CON). The AIN-93G containing 5, 10 and 15% pistachio meal (with skins) (PM) served as treatment diets (Table 1). All diets containing PM were made isocaloric by modifying protein, fat, dietary fiber and cornstarch contents. The animals were procured from Harlan (IN) and maintained and cared for in accordance with the AAMU guidelines for the protection and care of animals. The Institute of Animal Care and Use Committee (IACUC) committee at Alabama A and M

Table 1: Composition of experimental diets

Ingredients (g)	Control diet	PM (-5%)	PM (-10%)	PM (-15%)
Corn starch	397.5	390.5	381.5	360.5
Casein	200.0	189.0	180.0	168.0
Dextrose	132.0	132.0	132.0	132.0
Sucrose	100.0	96.0	92.0	100.0
Soybean oil	70.0	47.0	24.0	16.0
Fiber	50.0	45.0	40.0	35.0
Mineral mix	35.0	35.0	35.0	35.0
Vitamin mix	10.0	10.0	10.0	10.0
Cystine	3.0	3.0	3.0	3.0
Choline	2.5	2.5	2.5	2.5
Pistachio meal	0.0	50.0	100.0	150.0

Formulations of diets based on AIN-93G (American Institute of Nutrition, Reeves *et al*.[26,27]), PM: Pistachio meal

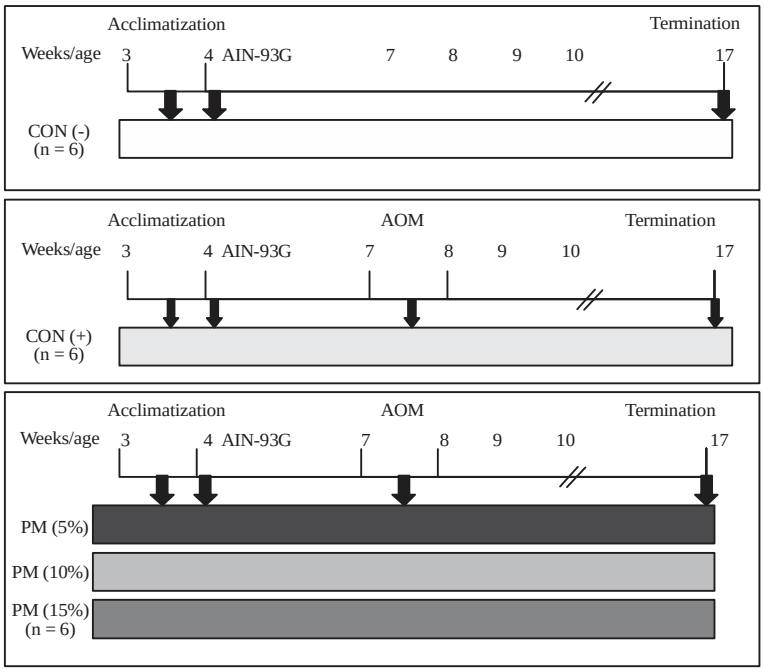

Fig. 1: Schematic representation of experimental groups, AOM: Azoxymethane, Con: Control, PM: Pistachio meal

University approved the protocol for this study. Animals were housed following standard protocols and were allowed *ad libidum* access to food and water. After acclimatization for a period of 2 weeks, the animals were divided at random into five groups consisting of six rats each (n = 6) (Fig. 1).

ACF induction and sample collection: The ACF was induced by injecting rats with Azoxymethane (AOM) s/c in saline (16 mg kg^{-1} b.wt.) at 7 and 8 weeks of age. Rats in groups 1 and 2 were the controls. Group 1 received azoxymethane and was designated as positive control (CON+) and group 2 received saline instead of AOM and was designated as negative control (CON-) (Fig. 1). At the end of the 13 weeks (17 weeks of age), rats were fasted overnight before being anaesthetized with CO_2. Blood was collected from the heart and serum separated by centrifugation (3000×g for 15 min). Colons were removed and prepped for ACF and crypt multiplicity; livers were removed immediately, flash frozen in liquid nitrogen and stored at -80°C for further analysis. Cecal pH and liver weights were recorded.

ACF determination: Enumeration of Aberrant Crypt Foci (ACF) was performed as described by Bird[28]. A 2 cm segment of colon was examined and the total ACF was categorized based on multiplicity; small (1-3 crypts/focus), medium (4-5 crypts/focus) and large (≥5 crypts/focus).

Evaluation of biomarkers of oxidative stress and antioxidant related enzymes: Liver samples were prepared for antioxidant and phase II enzyme determination as previously described[29]. The homogenate was centrifuged (10,000×g for 30 min) and the supernatant was collected and used to determine the following assays: Glutathione peroxidase (GPx) activity was spectrophotometrically measured by a coupled reaction with GRx using cumene hydroperoxide as substrate following the methods of Jaskot *et al.*[30]. Enzyme activity was calculated by the change in absorbance value at 340 nm for 10 min. The GPx was expressed as protein (nmol mg^{-1}). For GSH determination, the method of Griffith[31] was utilized. The GSH was determined by incubating samples for 3 min at 30°C in the presence of 0.3 mM NADPH 6 mM DTNB. After incubation, glutathione reductase (GR) (200 IU mL^{-1}) was added and changes in absorbance were determined at 412 nm at 25°C. Enzyme activity was calculated as protein (nmol min^{-1} mg^{-1}). For glutathione-s-transferase (GST) activities, the method of Habig *et al.*[32] was modified for the microplate reader. 1-chloro-2, 4-dinitrobenzene was used as the substrate and GST was calculated as protein (µmol mg^{-1}). Catalase (CAT) was detected based on the methods developed by Johansson and Borg[33]. Catalase (CAT) activity was assayed at 450 nm and expressed as protein (µmol mg^{-1}). Superoxide dismutase (SOD) was determined using the method outlined by

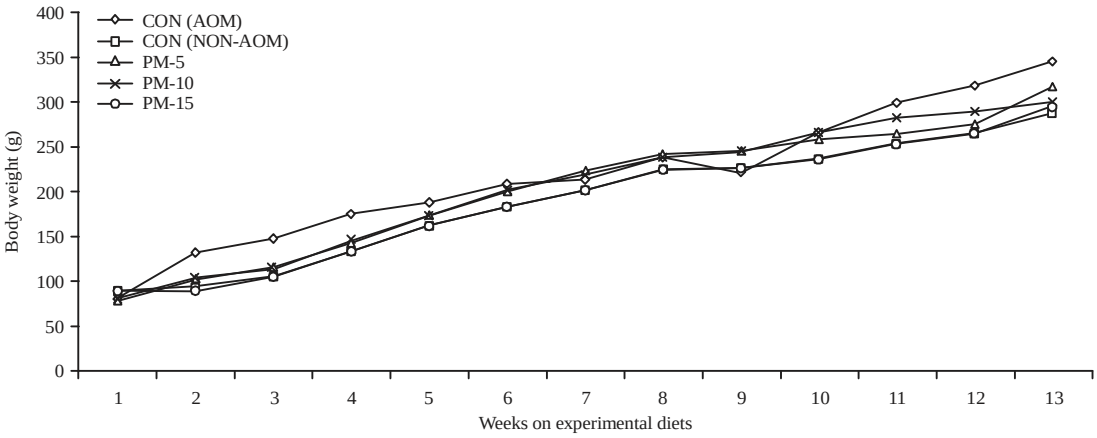

Fig. 2: Trend in collective body weights of Fisher 344 male rats fed PM and control diets (1-13 weeks), n = 6, AOM: Azoxymethane, Con: Control, PM: Pistachio meal

Table 2: Food consumption, initial weight, final weight and weight gain in Fisher 344 rats fed diets containing pistachio nuts for 13 weeks

Treatments	Feed intake (g day^{-1})	Initial weight	Final weight	Weight gain
*CON (+AOM)	16.7±0.11^{a}	80.25±2.86^{a}	347.00±11.22^{a}	266.75±6.33^{a}
*CON (-AOM)	9.4±0.17^{a}	89.00±2.34^{b}	288.50±4.32^{c}	199.50±5.38^{c}
PM-5	13.1±0.11^{a}	78.25±2.04^{a}	318.50±4.49^{b}	240.25±7.72^{ab}
PM-10	12.7±0.14^{a}	80.00±3.58^{a}	300.25±8.08^{b}	220.25±8.71^{bc}
PM-15	12.1±0.31^{a}	86.25±2.05^{b}	295.75±8.41^{b}	209.50±6.32^{bc}

Values are Mean±SEM (n = 6 per group), ^{abc}Means in a column with the same superscript do not significantly differ ($p<0.05$) using Duncan multiple range test. AOM: Azoxymethane, Con: Control, PM: Pistachio meal, *CON (+AOM): Positive control, *CON (-AOM): Negative control

Fernandez-Urrusuno *et al.*[34]. Xanthine oxidase was utilized to generate a superoxide flux and samples were measured at 440-505 nm after addition of xanthine oxidase. Values were expressed as units of SOD activity per milligram protein. Protein in liver lysate was determined using BCA protein assay kit from pierce (Rockford, IL).

Biochemical analysis of serum: Blood taken from rats from each group was allowed to clot and the serum separated and analyzed for total triglycerides and cholesterol using standard kits (Cayman Chemicals Ann Arbor, MI, USA).

Statistical analysis: All values are expressed as Mean±SEM (n = 6 per group). Statistical analyses comparing the different measures for the subgroups were performed using statistical analysis system (SAS 9.3) means were separated using duncan multiple range test. The measure for the use of the term 'Significant' in the text was that the probability value (p) for a given test was $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of PM on weight gains and feed intake: The mean weight gain (g@13 weeks) (Table 2) was higher in CON+ (266.75±6.33) group compared to rats fed PM diets and CON-AOM groups (Fig. 2). Nuts are high-fat, energy-dense foods with the possibility for contributing to increased body weight. However, there are studies that indicate nuts are not associated with high body fat[3,35,36] or for that matter weight gain[6,37,38]. Li *et al.*[39] determined that pistachios, when consumed as a portion-controlled snack, led to weight loss in obese individuals by comparison to refined carbohydrate snacks. In the current study, all diets containing PM were made isocaloric, which may have also contributed to the significant weight loss. It has been suggested that isocaloric replacement of nuts for other food items in the diet may in fact decrease body weight and fat mass[36,38]. Compared to CON+ group, weight loss in rats fed PM ranged from 9-25%.

With feed intake, the groups fed PM consumed significantly ($p<0.05$) less feed when compared to CON+ group (Table 2). In fact, feed intake did not significantly differ among the treatment groups. The observed effect can quite possibly be attributed to the satiety properties ascribed to nuts. It has been suggested that nuts have salutary effect which leads to reduced energy consumption and hence decreased risk for weight gain[36,40]. The satiety properties of nuts are mostly attributed to their macronutrient profile (high fiber and protein) and low glycemic index[41,42]. However, as pointed out by Martinez-Gonzalez and Bes-Rastrollo[42], the macronutrient profile of nuts is likely to be associated with

Table 3: Liver weight, liver index, cecal weight and cecal pH in Fisher 344 rats fed diets containing pistachio nuts for 13 weeks

Treatments	Liver weight	Liver index	Cecum weight	Cecum wall	Cecal pH
*CON (+AOM)	11.25±0.39[a]	4.25±0.28[a]	2.81±0.31[a]	1.36±0.15[a]	7.49±0.03[a]
*CON (-AOM)	7.30±0.33[b]	3.76±0.48[a]	2.65±0.14[a]	1.32±0.26[a]	7.52±0.04[a]
PM-5	9.12±0.72[b]	3.81±0.33[a]	2.45±0.31[a]	1.70±0.30[a]	7.46±0.02[a]
PM-10	7.75±0.69[b]	3.50±0.13[a]	2.35±0.09[a]	1.15±0.02[a]	6.81±0.02[b]
PM-15	7.65±1.13[b]	3.62±0.37[a]	2.92±0.30[a]	1.45±0.20[a]	6.11±0.05[b]

Values are Means±SEM (n = 6 per group), [abc]Means in a column with the same superscript do not significantly differ ($p<0.05$) using Duncan multiple range test. Liver index: Relative liver weight (g/100 g b.wt.), AOM: Azoxymethane, Con: Control, PM: Pistachio meal, *CON (+AOM): Positive control, *CON (-AOM): Negative control

Table 4: Incidence of ACF in Fisher 344 rats fed pistachio nuts meal for 13 weeks

Treatments	Proximal colon	Distal colon	Total ACF
*CON (-AOM)	0	0	0
*CON (+AOM)	40±0.94[a]	118±1.70[a]	158[a]
PM-5	42±2.58[a]	77±2.32[b]	119[b]
PM-10	26±4.97[b]	50±7.29[c]	76[c]
PM-15	25±1.75[b]	47±2.39[c]	72[c]

Values are Means±SEM (n = 6 per group), [abc]Means in a column with the same superscript do not significantly differ ($p<0.05$) using Duncan multiple range test. AOM: Azoxymethane, Con: Control, PM: Pistachio meal, *CON (+AOM): Positive control, *CON (-AOM): Negative control

increased release of glucagon-like protein 1 (GLP-1) and cholecystokinin (CCK) which are gastrointestinal hormones with satiety effects. Others have also documented that the satiety effects from the consumption of nuts may well be the consequence from unsaturated fatty acids, which may influence diet-induced thermogenesis by increasing resting energy expenditure[36,40]. Here, Rajaram and Sabate[36] explained that unsaturated fatty acids present in nuts are more readily oxidized than saturated or trans-fatty acids because of higher diet-induced thermogenesis, thus leading to reduced fat accumulation.

Effect of PM on cecal pH and liver weight: Modulation of PM on cecal pH shows a significant decrease ($p\leq0.05$) in PM-10 and PM-15 groups (Table 3). Studies on dietary fiber propose a beneficial effect by altering the intestinal flora[43,44]. Such modifications generate Short Chain Fatty Acids (SCFA), which play an integral role in reducing or suppressing Aberrant Crypt Foci (ACF) formation[29]. Butyrate, one of the crucial SCFAs is commended for promoting the growth of colonocytes and as a primary protective factor against colon cancer[45]. Further, SCFAs propionate and acetate are known to impact circulating cholesterol and triglycerides[46]. Notably, increased SCFA production is affiliated with lower cecal/colonic pH. Lowering of colonic pH was found to be beneficial for colon health, because it supports an unwelcome environment for the proliferation of pathogenic bacteria, reduces peptide degradation and the subsequent formation of their toxic metabolites such as ammonia, amines and phenolic compounds and decreases the activity of undesirable bacterial enzymes[47]. The low ($p\leq0.05$) pH in cecum of rats fed PM-10 and PM-15 perhaps contributed to the significant reductions in ACF, serum lipids and weight gain observed in those groups.

Liver weight was significantly ($p\leq0.05$) higher in CON+ group compared to the treatment and CON-groups (Table 3). High liver weight could be attributed to an adaptive response to hepatic enzyme induction following exposure to xenobiotic response[48]. This innate response, which allows the liver to regenerate itself and maintain normal function, could be suppressed by the rate or extent at which xenobiotics are eliminated from the body[48]. Dietary constituents such as α-tocopherol (vitamin E) and flavonoids, which are present in PM, could accelerate the detoxification of carcinogens by inducing phase II enzymes, leading to their elimination from the body. Hence, prolonged accumulation of xenobiotics such as AOM is likely to induce toxicity to the liver resulting in glutathione depletion and generation of reactive oxygen species. This could explain the reduced antioxidant activities in the CON+ group (Table 5).

Effect of PM on ACF incidence and crypt multiplicity: Table 4 and Fig. 3 show ACF incidences and crypt multiplicity in the colon of rats, respectively. The ACFs have been reported to be the earliest observable histopathologic lesion associated with malignant transformation in the colon[49,50]. Although proximal ACFs in rats fed PM-10 and PM-15 were significantly ($p\leq0.05$) lower than the CON+, no significant differences in groups fed with PM-5 (42±2.58) and CON+ (40±0.94) was found (Table 4). The number of ACFs in the distal colon of rats fed PM-5 was 35% lower compared to the rats fed CON+, indicating a significant reduction in ACF density in the distal but not in the proximal colon. When compared to CON+, ACFs in the distal colon were decreased by 58 and 60%, respectively, in groups fed PM-10 and PM-15. With regard to ACF density in the distal colon, several authors have reported similar incidences with AOM induced ACF in rodent models[29,51,52].

This results indicate that medium and large ACF, (that is ACF with ≥4 crypts), were significantly ($p\leq0.05$) higher in CON+ compared to the treatment groups (Fig. 3). The ACF consisting of four or more crypts have been

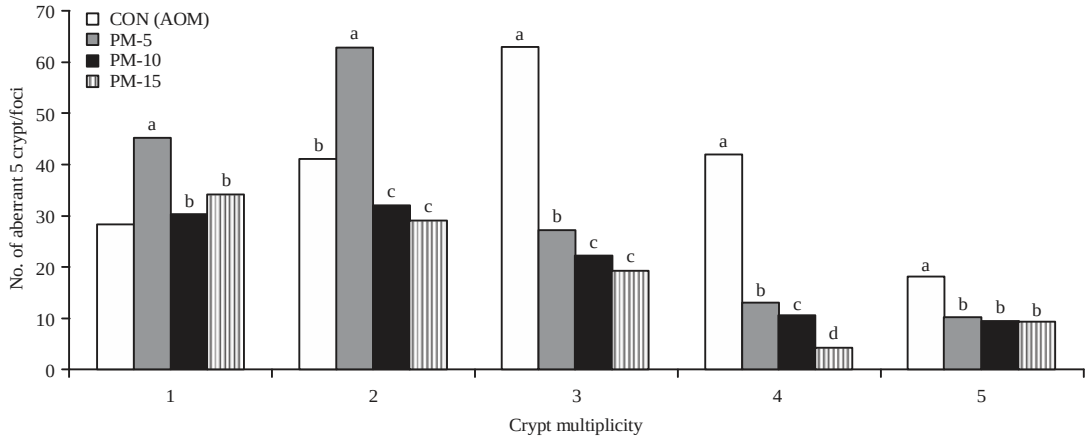

Fig. 3: ACF distribution in Fisher 344 rats fed CON+AOM and PM. Shown are crypts containing 1, 2, 3, 4, >5 crypts/ACF, i.e., number of crypt/focus. Values are Mean±SEM (n = 6 per group). [abc]Means on a bar with the same superscript do not significantly differ ($p<0.05$) using duncan multiple range test. No. of crypts ≥3 is significantly higher in rats fed control diet. ACF consisting of ≥4 crypts have been reported to progress into putative premalignant lesions. AOM: Azoxymethane, Con: Control, PM: Pistachio meal

Table 5: Effect of pistachio meal on phase II metabolizing and antioxidant enzyme activities in Fisher 344 rats

Treatments	GST (protein μmol mg^{-1})	GSH (protein μmol mg^{-1})	GR (protein μmol mg^{-1})	GPx (protein μmol mg^{-1})	SOD (protein U mg^{-1})	CAT (protein μmol mg^{-1})
*CON (+AOM)	3.39±0.38[c]	1.11±0.04[c]	2.20±0.14[d]	3.79±0.96[c]	0.98±0.01[c]	1.51±0.02[c]
*CON (-AOM)	5.10±0.54[b]	1.88±0.26[b]	3.40±0.21[c]	4.78±0.03[b]	1.71±0.06[a]	2.47±0.04[b]
PM-5	5.86±0.48[b]	2.43±0.16[b]	4.96±0.15[b]	4.25±0.02[b]	1.06±0.01[b]	1.66±0.04[c]
PM-10	7.27±1.33[b]	2.17±0.06[b]	4.91±0.19[b]	4.31±0.01[b]	1.02±0.10[b]	2.07±0.05[b]
PM-15	10.84±0.29[a]	6.06±0.49[a]	7.21±0.37[a]	7.61±0.24[a]	1.73±0.06[a]	3.38±0.10[a]

Values are Mean±SEM, (n = 6 per group), [abc]Means in a column with the same superscript do not significantly differ ($p<0.05$) using Duncan multiple range test. AOM: Azoxymethane, Con: Control, PM: Pistachio meal, GST: Glutathione-s-transferase, GSH: Glutathione, GR: Glutathione reductase, Gpx: Glutahione peroxidase, SOD: Superoxide dismutase, CAT: Catalase, *CON (+AOM): Positive control, *CON (-AOM): Negative control

reported as putative premalignant lesions and as an intermediate biomarker for colon cancer development[53]. Martinez-Ferrer *et al.*[54] reported similar findings after feeding lycopene and SBO at different concentrations to Fisher 344 rats. When differentiating the distribution of ACF and colonic carcinogenesis in a mouse model, Park *et al.*[55] reported that ACF are marker lesions for colonic neoplasms, but only in the distal colon where tumors follow the adenoma-carcinoma sequence. It has been documented that in humans and in rodents experimentally induced with a chemical carcinogen, colon tumors were predominant in the distal portion of the colon compared to the proximal section of the colon[56]. Hence, the current data indicates that treatment with PM may have suppressing effect against preneoplastic lesions of colon cancer.

Vitamin E, which is also found in pistachios at significant levels may have also lent to the reduction of ACF observed in the rats fed with PM. Indeed, several authors have suggested an anticancer property for Vitamin E. Moreover, γ-tocopherol, a predominant form of vitamin E found in pistachios has been found to have anti-inflammatory properties[57,58]. In their study Jiang *et al.*[59] reported that γ-tocopherol and its hydrophilic metabolite, γ-CEHC at physiological concentrations, reduced PGE_2 synthesis and hence COX-2 activity in two independent cell lines RAW264.7 macrophages and IL-1β-treated A549 human epithelial cells which were both lipopolysaccharide (LPS)-stimulated. This is significant, since increased levels of PGE_2 and COX-2 activity are linked to colorectal carcinogenesis[60]. The researchers indicated that γ-tocopherol contributes significantly to the antioxidant defense mechanisms of the colon. This of course is due to the fact that humans, in general, consume three times as much γ-tocopherol as α-tocopherol. Reiterating findings by Stone and Papas[61], it was noted that the biliary excretion of γ-tocopherol might contribute to the elimination of fecal mutagens and consequently a reduction in colon cancer.

Other phytonutrients in pistachios such as phytosterols and flavonoids including anthocyanins[62-64], may have contributed to the reduction of ACFs observed in the current study. Others have showed anthocyanins rich foods to be

effective in inhibiting colon carcinogenesis in chemically induced rodent models[65-67]. Some of the reported mechanisms include the suppression of the expression of matrix metalloproteinase (MMP-2) and MMP-9 and the activation of NF-κB[68]. Note that NF-κB controls the expression of COX-2, which is implicated in several tumors including colon tumors. A study by Thomasset *et al.*[69] demonstrated the inhibitory activity of anthocyanins in a human pilot study. The authors noticed that administration of mirtocyan, an anthocyanin rich extract from bilberry, for 7 days reduced proliferation and induced apoptosis in colorectal tumor cells. Furthermore, they observed physiologically active anthocyanins in colorectal tissue. These findings illustrate that consumption of nuts such as pistachios, the only nut that contains anthocyanins[64], may possibly reduce the incidence of preneoplastic lesions and hence colorectal cancer.

Effect of PM on phase II metabolizing and antioxidant enzymes: It is apparent based on several studies that tumor cells are deficient in complex enzyme systems, which exert protection by scavenging free radicals such as superoxides, hydrogen peroxides and lipid peroxides[70,71]. In this study, a significant increase in enzyme activities in rats fed PM compared to CON+ (Table 5) was observed. Furthermore, we noted that feeding PM actually enhanced enzyme activities. The SOD (protein UL mg^{-1}) and CAT (protein μmol mg^{-1}) activities in groups fed PM compared to CON+, especially in the group fed PM-15. Supplementation with PM also enhanced GPx (protein μmol mg^{-1}) activity, with rats fed PM-15 showing a 2-fold increase when compared to CON+. Studies have shown increases in hepatic CAT, GPx and SOD activities upon treatment with vitamin E in rodent models[72,73]. Important determinants of cellular antioxidant capacity are the enzymes SOD, CAT and GPx, which are responsible for the elimination of ROS. Because these enzymes act sequentially to remove ROS, the balance of the activity of these enzymes may be as critical in the defense against ROS as the activity of the enzymes alone.

Perhaps one of the most important endogenous non-enzymatic antioxidant is glutathione (GSH). It is implicated in several biological processes including cell proliferation, apoptosis, inflammation etc. According to Ramos *et al.*[74], GSH levels offer critical biological mechanisms for protection against toxic effects of endogenous Reactive Oxygen Species (ROS) as well as exogenous carcinogens such as AOM and their reactive intermediates. Our data suggests GSH (protein μmol mg^{-1}) levels, were significantly ($p<0.05$) increased by 2-5 fold in the rats fed PM diets compared to CON+AOM. The GST (protein μmol mg^{-1}) and GR (protein μmol mg^{-1}) activities also showed significant ($p<0.05$) increases (1.5-3 fold increase for GST activity and 2-3 fold increase for GR activity) in PM fed rats compared to CON+ (Table 5). Rats supplemented with PM-15 showed significant ($p<0.05$) increases in enzymatic and non-enzymatic activities compared to both controls (AOM and non-AOM). This clearly indicates the potential that PM can enhance the activities of critical endogenous enzymes. Serum antioxidants were reported to increase upon supplementation with pistachios, while oxidative markers were significantly reduced[75]. Studies have shown that rodents exposed to carcinogens exhibit significant reductions in endogenous antioxidant activities, whereas treatments with dietary antioxidants seem to counteract these reductions[76-79]. Because AOM, an ultimate carcinogen, is an electrophilic diazonium ion these enzymes, specifically the glutathione-dependent enzymes, play an important role in carcinogen detoxification[80]. More importantly, Sander *et al.*[81] elucidates that antioxidant enzyme activities is crucial, since altered levels could contribute to alterations in cancer susceptibility. The colon, as the author stress is known to produce increased amount of Reactive Oxygen Species (ROS) due to extended contact with oxidized food particles, toxins, redox active minerals as well as ROS generated by bacterial microflora. In order to minimize these incidences, it is imperative to consume diets that can significantly attenuate these processes. In this instance, the consumption of pistachios could alter the antioxidant system and possibly alleviate incidence of colon cancer.

Effect of PM on total serum cholesterol and triglycerides: The current data showed a significant ($p \leq 0.05$) decrease in total serum triglycerides and cholesterol in treatment groups compared to the control (Table 6). In addition to facilitating weight loss, regular intake of nuts has been shown to have beneficial effect on lipid profile[6,39,75,82-85]. This is important since dyslipidemia is a known hallmark for metabolic syndrome, which is implicated in the etiology and progression of colorectal carcinogenesis[86-88]. For example, there appears to be evidence suggesting that dietary fat, depending

Table 6: Effect of feeding pistachios on total serum cholesterol and triglycerides in Fisher 344 rats

Treatments	Total cholesterol	Triacylglycerides
*CON (+AOM)	123.86±1.25[a]	171.21±3.09[a]
*CON (-AOM)	104.16±1.68[b]	133.33±3.09[b]
PM-5	100.08±1.57[b]	106.81±3.78[c]
PM-10	72.59±1.12[c]	93.55±1.89[c]
PM-15	62.21±1.41[d]	84.09±4.88[c]

Values are Means±SEM, (n = 6 per group), [a-d]Means in a column with the same superscript do not significantly differ ($p<0.05$) using Duncan multiple range test. AOM: Azoxymethane, Con: Control, PM: Pistachio meal, *CON (+AOM): Positive control, *CON (-AOM): Negative control

on the source, (i.e., animal or vegetable), quantity and fatty acid composition (saturated, monounsaturated and polyunsaturated) is likely to influence the incidence of colon cancer[29,89,90]. This occurs through several signaling pathways, including oxidative stress, inflammation, cell proliferation, apoptosis and angiogenesis[91]. In previous studies, it is shown that dietary fats from vegetable sources did not significantly promote chemically induced carcinogenesis. In those studies we indicated that rats fed diets containing flax seed oil, red palm oil and rice bran oil showed reduced incidences of ACF[29,92,93]. Thus, it might be safe to suggest that the modulatory effects of PM may be partly attributed to the fatty acid composition. When considering the positive impact of pistachios on serum lipid one cannot discount the presence of polyphenols, which are noted to inhibit fat and cholesterol synthesis.

CONCLUSION

This preliminary study indicated that pistachio nut, as a whole food supplementation, significantly reduced the incidence of Aberrant Crypt Foci (ACF) and crypt multiplicity. Pistachio nuts also enhanced endogenous antioxidant enzyme activities. It is observed that pistachio nut supplementation, in addition to enhancing enzymatic activities and reducing the incidence of ACF, also led to significant reductions in total serum triglycerides and cholesterol levels. When all these are taken into consideration, it is plausible that pistachio nuts may have the potential to reduce the incidence of colon cancer. Future research will include long-term studies to better discern the possible mechanisms of action for pistachio nuts and to hopefully identify individual components responsible for the observed inhibition of colorectal cancer growth. Furthermore, this study can lead to a larger clinical trial that may provide another method nutritional therapy for colon cancer patient.

SIGNIFICANCE STATEMENT

- Pistachio meal reduced AOM induced ACF
- Pistachio meal supports the antioxidant capacity by significantly enhancing endogenous antioxidant enzyme activities
- Pistachio meal reduced serum triglycerides and total cholesterol levels
- Pistachios could be investigated as functional food product for dietary chemoprevention

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