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# Combinational Effect of Green Tea, Phytic Acid and Inositol on Bone Mineralization and Mineral Balance in with Azoxymethane-Induced Colon Carcinogenesis Induced Fisher 344 Male Rats

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**Abstract:** The aim of the study was to determine the combinational effect of dietary Phytic Acid (PA), Green Tea (GT) and Inositol (I) at 1 and 2% level (in drinking water) on bone mineralization in rats with azoxymethane (AOM)-induced colon carcinogenesis. After one week period of acclimatization, 9 groups of rats (6 rats each) were fed AIN 93G (till 20 week) and later switched to AIN 93 M diets (till 45 weeks age). All rats received AOM s/c at the rate of 16 mg $kg^{-1}$ body weight at 7 and 8 weeks of age. Urine and fecal samples were collected for a 12 day period. Rats were killed by $CO_2$ asphyxiation at 46 week of age and samples (cecum, blood, tibia and femur) were collected and analyzed by ICP for selected minerals (Ca, P, Mg, Fe and Zn). Physical parameters (weight, length, circumference and volume) of tibia and femur were examined. There were no significant differences in apparent absorption, retention and serum concentrations of macro minerals (Ca, P and Mg), although apparent absorption, bone and serum levels of Fe and Zn were significantly lower in 2% combinations. Results of this study showed that combination of treatments at lower levels may be beneficial in reducing the negative effects on bone mineralization.

**Key words:** Colon cancer, mineralization, apparent absorption, retention

## INTRODUCTION

Cancer is a major public health problem in the United States and in other developed countries. Colon cancer is the third leading cause of cancer related deaths in the US. It is estimated that there will be 104,950 new cases of colon cancer and 56,290 deaths in the year 2007. The incidence of colorectal cancer is almost similar in men and women (11%) (Jemal *et al*., 2005). In a continuing effort to reduce the public health burden of cancer, there is an increasing interest in the concept of chemoprevention. Studies have suggested 20-40% of cancer deaths in the US are preventable by diet modifications.

Inositol hexaphosphate ($IP_6$) is a naturally occurring polyphosphorylated carbohydrate present in almost all plant and mammalian cells and has chemopreventive effects. Inositol is also a natural constituent possessing moderate anti-cancer potential (Vucenik and Shamsuddin, 2003). Green tea contains polyphenols which have been shown to reduce risk of a variety of chronic diseases (Khan and Mukhtar, 2007). Some concerns have been expressed regarding the mineral deficiency that results from an intake of foods high in $IP_6$ that might reduce the bioavailability of dietary minerals. Green tea catechins have the potential to affect absorption and metabolism of ions because flavonoids/polyphenols interact with a variety of minerals/metals.

A number of epidemiological and laboratory studies have shown that administration of Green Tea (GT), Phytic Acid (PA) and Inositiol (I) in drinking water can influence the occurrence and

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development of lung, intestinal tract and skin cancer (Wang *et al*., 1992; Ullah and Shamsuddin, 1990). Many tumor initiators and promoters are free radical generators with the result that antioxidants may often have anticarcinogenic effects. In addition, free radicals can be generated by redox reactions involving trace elements such as iron and copper. The gallate residues of polyphenols and tannins in GT and phosphate groups in PA are able to chelate metals thus reducing oxidation (Lopez *et al*., 2002; Fredlund *et al*., 2006; Samman *et al*., 2001). Tea ploy phenols form insoluble complexes with iron and thus inhibit iron absorption (Samman *et al*., 2001; Thephinlap *et al*., 2007). Phytic acids chelation with other divalent cations like Fe, Mg and Zn also play a significant role in suppression of tumor progression (Muraoka and Miura, 2004).

Minerals are essential factors in human nutrition. Bioavailability of minerals is greatly influenced by both dietary inhibitors and enhancers (Davidsson *et al*., 1994; Hurrell *et al*., 2000). Bone stores 99% of body's calcium and calcium salts are responsible for the hardness of bones (Hallberg *et al*., 1989). Magnesium is a critical ion in mammals, as a cofactor for many enzymes and it is also necessary for bone formation (Wolters *et al*., 1993). In complex carbohydrates, PA and associated substances bind minerals, hence possibly alter mineral bioavailability (Lopez *et al*., 2002; Fredlund *et al*., 2006). PA molecule is highly charged with six phosphate groups extending from the central inositol ring and serves as an excellent chelator of mineral ions such as Ca, Zn and Fe. The phytate content of some foods (whole wheat products, wheat bran and soy products) was reported to be responsible for the decrease in calcium and zinc balance in rats and humans (McClung *et al*., 2006; Lopez *et al*., 2003; Egli *et al*., 2004). Some researchers (Zhang *et al*., 2005; Jenab and Thompson, 2002) reported the advantage of high consumption of fiber and PA-rich dietary products in decreasing the risk of colon cancer. The insoluble fibers dilute the carcinogens thereby preventing their contact with the colonic mucosa. Soluble fibers produce Short Chain Fatty Acids (SCFA), acetate, butyrate and propionate by the action of gut bacteria where butyrate induces apoptosis (Lupton, 2004). There are no studies conducted to evaluate the effect of phytochemicals on mineralization in rats injected with azoxymethane. Most studies showing an antineoplastic effect of PA and GT failed to study the mineral status of animals. A few studies reported the serum level of minerals. However, the results were not fully convincing because serum concentration is not always the most sensitive indicator of mineral status, as serum mineral content responds to metabolic conditions. The aim of this study was to determine the combinational effect of Green Tea (GT), Phytic Acid (PA) and Inositol (I) on bone mineralization and mineral balance in colon cancer induced Fisher 344 male rats.

## MATERIALS AND METHODS

### Animal Housing and Diets

All the protocols involving rats were approved by Institutional Animal Care and Use Committee of Alabama A and M University Normal, AL 35762. Fisher 344 weanling rats (Harlan, IN) were housed in stainless steel wire cages at the rate of 2 rats per cage in a temperature- and humidity-controlled room (21°C and 50% relative humidity) with a 12 h light/dark cycle (Fall, 2003). Rats were given free access to AIN 93G (Reeves *et al*., 1993a, b) diet and deionized water for a 1 week adaptation period after which rats were divided into 9 groups (n = 6). Groups were assigned to combinations of 1 and 2% Green Tea (GT), Phytic Acid (PA) and Inositol (I) (in drinking water). All the rats were given free access to AIN 93 G/M diet and treatments throughout the experiment period. Mineral content of diets are given in Table 1. Dietary ingredients for preparing AIN-93 based diets are obtained from ICN (Costa Mesa, CA). All diets were prepared fresh each week and stored at 4°C until fed. Body weight was recorded biweekly and food intake was monitored daily. Urine and feces were collected from all rats on 24 h basis for a period of 12 days at 42 week of age. At the end of the

Table 1: Mineral content of AIN 93G* and AIN 93 M* diets

| Ingredients | AIN 93 G (mg $kg^{-1}$) | AIN 93 M (mg $kg^{-1}$) |
|---|---|---|
| Calcium | 5000.0 | 5000.0 |
| Phosphorous | 5161.0 | 1992.0 |
| Magnesium | 507.0 | 507.0 |
| Zinc | 30.0 | 30.0 |
| Iron | 35.0 | 35.0 |

*Reeves *et al.* (1993a, b)

45 week experiment at period, feed was withheld overnight and the rats were killed by $CO_2$ euthanasia. Blood was collected and centrifuged to separate the serum. Femurs and tibia were excised from rats.

**Carcinogen Injection**

For induction of colon cancer all rats were given two subcutaneous injections of Azoxymethane (AOM), (Sigma Chemical Company, St. Louis, MO) in saline at the rate of 16 mg $kg^{-1}$ body weight at 7 and 8 weeks of age.

**Preparation of Treatments**

Gunpowder Green tea® was obtained from Frontier Herbs (Iowa). Solutions of 1 and 2% GT solutions were brewed by boiling leaves in distilled deionized water for 15 min. PA and I (Sigma Chemical Company, St Louis, MO) were dissolved in distilled deionized water to prepare 1 and 2% solutions.

**Cecal Weight and pH**

The cecum from each rat was excised, split open, weight of cecal tissue and pH of the contents were noted.

**Mineral Balance Studies**

Feces and urine samples were pooled together for each cage. Feces was freeze dried and micropulverized. Micropulverized fecal samples and urine were ashed at 600°C for 24 h. The ashed samples were extracted for analysis using 5% HCl and concentrated $HNO_3$ solutions. The concentrations of Calcium, Phosphorus, Magnesium, Zinc and Iron in the feces and urine were determined by Inductively Coupled Plasma (ICP) spectrophotometer (Perkin-Elmer 400 Norwalk, CT, USA) at the following wavelengths (nm): 393(Ca); 214(P); 285(Mg), 213(Zn) and 238 (Fe). The absorption and retention of minerals were determined by following equations:

$$\text{Apparent absorption (\%)} = ((\text{Intake} - \text{fecal excretion})/\text{intake}) \times 100$$

$$\text{Retention (\%)} = ((\text{Intake} - (\text{fecal excretion} + \text{urinary excretion}))/\text{total intake}) \times 100$$

**Serum Analysis**

Blood from rats obtained by cardiac puncture at death was placed in heparinized tubes and centrifuged for 5 min at 800 x g to obtain serum. Serum Ca, P, Mg, Fe and Zn were analyzed by ICP (AACC Method, 1984).

**Bone Analysis**

Length, weight, circumference and volume of both tibia and femur for each rat were recorded. Bones were dry-ashed and prepared for mineral analysis. Selected minerals in the bone were analyzed by ICP (AACC Method, 1984).

**Statistical Analysis**

The data are the mean values with SD. Statistical analysis was conducted by one-way ANOVA. Tukey's test was used to determine the significantly different groups when the ANOVA indicated a significant effect. All calculations were performed using SAS version 9.1, 2004 software. Significance was assigned at $p<0.05$.

## RESULTS

**Effect of Dietary Treatments on Feed Intake, Body Weight Gain, Cecal pH and Cecal Weight**

Administration of Phytic Acid (PA), Inositol (I) and Green Tea (GT) as treatments in drinking water had no influence on cecal weight and feed intake at both 1 and 2% combinations. Weight gain in rats fed 1% PA+GT+I (376.33±13.22) was significantly ($p<0.05$) higher than control (276.50±14.29) treated rats. There were no significant differences in weight gain among all other treatment groups and the control (Table 2). The cecal pH was close to neutrality in all dietary treatments ranging from 7.31±0.09 to 7.60±0.03 with significantly ($p<0.05$) higher cecal pH (7.60±0.07) in rats fed the control diet and lower in 1% GT+PA+I (7.31±0.09) fed groups.

**Effect of Dietary Treatments on Physical Parameters and Mineral Concentrations of Tibia and Femur**

There were no significant differences in selected physical parameters among the treatment groups in the femur (Table 3). The circumference of tibia was significantly ($p<0.05$) higher in rats given 1% GT+PA (7.07±0.13) followed by 2% GT+I (6.99±0.12) and 1% GT+PA+I (6.88±0.15) groups, respectively and was significantly lower in control (6.06±0.20) rats. No significant differences ($p<0.05$) were found with regard to all other parameters measured such as weight, length and volume of tibia.

Ca content was significantly ($p<0.05$) higher in 1% GT+PA+I compared to 2% GT+PA, 2% PA+I and 2% GT+PA+I. Reduction in Ca content was higher in 2% GT+PA+I (342.14±2.09). There were however, no significant ($p<0.05$) differences among all other treatments. Calcium level in the femur showed similar pattern (Table 3). Combination treatments did not show any significant effect on levels of Mg and Zn contents of tibia and all other minerals (P, Fe and Zn) analyzed in the femur excluding Ca. P in tibia was significantly ($p<0.05$) higher 1% GT+PA+I compared to the control and all 2% combinations except 2% (GT+I). The control (125.27±1.67) rats had significantly ($p<0.05$) higher Fe content than all 2% combinations.

**Effect of Dietary Treatments on Mineral Content in Serum, Feces and Urine**

Table 4 shows the effect of 1 and 2% combinations of GT, PA, I on serum, feces and urinary minerals (Ca, P, Mg, Fe and Zn) in AOM treated rats. Other than Fe and Zn, there were no significant differences in mineral concentrations among combinations of treatments in serum. Serum Fe

Table 2: Combinational effect of GT, PA, I at 1 and 2% level on feed intake, weight gain, cecal weight and cecal pH in rats with azoxymethane induced colon carcinogenesis

| Treatments | Cecal pH | Cecal weight (g) | Feed intake (g) | Weight gain (g) |
|---|---|---|---|---|
| Control | 7.60±0.07$^{a}$ | 0.75±0.04 | 14.07±0.74 | 276.50±14.29$^{b}$ |
| 2% GT+PA | 7.47±0.03$^{ab}$ | 0.73±0.02 | 14.45±0.83 | 333.83±27.91$^{ab}$ |
| 2% GT+I | 7.47±0.03$^{ab}$ | 0.74±0.05 | 14.43±0.88 | 331.50±18.70$^{ab}$ |
| 2% PA+I | 7.48±0.02$^{ab}$ | 0.73±0.03 | 14.65±0.25 | 299.16±17.34$^{ab}$ |
| 2% GT+PA+I | 7.46±0.07$^{ab}$ | 0.74±0.02 | 14.39±0.45 | 303.00±17.71$^{ab}$ |
| 1% GT+PA | 7.57±0.05$^{ab}$ | 0.74±0.03 | 14.48±0.27 | 319.66±16.27$^{ab}$ |
| 1% GT+I | 7.50±0.05$^{ab}$ | 0.73±0.03 | 14.87±0.31 | 296.00±21.26$^{ab}$ |
| 1% PA+I | 7.55±0.05$^{ab}$ | 0.77±0.04 | 14.97±0.47 | 344.83±20.18$^{ab}$ |
| 1% GT+PA+I | 7.31±0.07$^{b}$ | 0.76±0.03 | 15.07±0.42 | 376.30±13.22$^{a}$ |

Values are Mean±SEM, n = 6. $^{ab}$: Values with same superscripts in same column are not significantly different using Tukey's test, $p<0.05$

Table 3: Combinational effect of GT, PA, I at 1 and 2% levels on physical parameters and mineral concentrations of tibia and femur in rats with azoxymethane induced colon carcinogenesis

| *Tibia* | Control | 2% GT+PA | 2% GT+I | 2% PA+I | 2% GT+PA+I | 1% GT+PA | 1% GT+I | 1% PA+I | 1% GT+PA+I |
|---|---|---|---|---|---|---|---|---|---|
| **Physical parameters** | | | | | | | | | |
| Weight (g) | 0.81±0.07 | 0.84±0.06 | 0.86±0.02 | 0.84±0.01 | 0.73±0.04 | 0.84±0.02 | 0.85±0.02 | 0.81±0.04 | 0.91±0.07 |
| Length (mm) | 42.65±1.39 | 42.54±1.15 | 42.01±1.08 | 41.90±1.36 | 41.62±1.17 | 42.19±0.95 | 42.35±0.38 | 41.32±0.92 | 41.48±0.69 |
| Circumference (mm) | 6.06±0.20$^{b}$ | 6.71±0.13$^{ab}$ | 6.99±0.12$^{a}$ | 6.68±0.25$^{ab}$ | 6.80±0.13$^{ab}$ | 7.07±0.13$^{a}$ | 6.60±0.17$^{ab}$ | 6.70±0.18$^{ab}$ | 6.88±0.15$^{a}$ |
| Volume (mL) | 0.48±0.06 | 0.50±0.03 | 0.47±0.03 | 0.50±0.03 | 0.47±0.04 | 0.48±0.02 | 0.52±0.03 | 0.46±0.03 | 0.50±0.03 |
| **Mineral concentration** | | | | | | | | | |
| Calcium (mg g$^{-1}$) | 361.16±12.25$^{ab}$ | 342.50±1.01$^{b}$ | 361.23±1.23$^{ab}$ | 343.37±2.70$^{b}$ | 342.14±2.09$^{b}$ | 349.08±3.94$^{ab}$ | 362.35±0.90$^{ab}$ | 345.80±3.65$^{ab}$ | 366.44±3.68$^{a}$ |
| Phosphorus (mg g$^{-1}$) | 127.10±3.31$^{b}$ | 127.58±0.43$^{b}$ | 130.10±1.23$^{ab}$ | 128.53±0.61$^{b}$ | 126.59±0.51$^{b}$ | 128.95±2.22$^{ab}$ | 131.12±1.26$^{ab}$ | 128.53±0.61$^{ab}$ | 134.88±0.92$^{a}$ |
| Magnesium (mg g$^{-1}$ | 4.44±0.11 | 4.38±0.02 | 4.58±0.08 | 4.47±0.06 | 4.42±0.04 | 4.45±0.01 | 4.47±0.16 | 4.47±0.06 | 4.45±0.03 |
| Iron (µg g$^{-1}$) | 125.27±1.67$^{a}$ | 107.78±0.94$^{e}$ | 117.41±0.44$^{bcd}$ | 112.45±0.89$^{cde}$ | 110.25±3.10$^{de}$ | 117.48±0.70$^{abcd}$ | 118.68±2.50$^{abc}$ | 120.42±1.84$^{ab}$ | 117.68±1.13$^{abcd}$ |
| Zinc (µg g$^{-1}$) | 233.39±23.30 | 242.28±1.86 | 251.43±2.27 | 243.97±1.43 | 233.82±1.04 | 242.99±2.85 | 251.83±2.80 | 243.97±1.43 | 242.65±1.26 |
| Femur | | | | | | | | | |
| **Physical parameters** | | | | | | | | | |
| Weight (g) | 1.12±0.03 | 1.23±0.04 | 1.27±0.05 | 1.14±0.05 | 1.08±0.07 | 1.25±0.05 | 1.23±0.03 | 1.18±0.05 | 1.30±0.03 |
| Length (mm) | 36.82±0.82 | 38.12±0.27 | 38.76±0.60 | 36.36±0.34 | 36.45±0.77 | 39.18±0.46 | 37.20±0.64 | 38.53±0.75 | 38.03±1.14 |
| Circumference (mm) | 7.36±0.42 | 7.32±0.47 | 7.63±0.38 | 7.29±0.59 | 7.83±0.48 | 7.69±0.52 | 7.68±0.26 | 7.43±0.45 | 7.30±0.41 |
| Volume (mL) | 0.71±0.04 | 0.79±0.05 | 0.84±0.03 | 0.71±0.03 | 0.69±0.06 | 0.81±0.03 | 0.71±0.06 | 0.70±0.03 | 0.73±0.04 |
| **Mineral concentration** | | | | | | | | | |
| Calcium (mg g$^{-1}$) | 323.50±14.72$^{ab}$ | 291.00±9.17$^{b}$ | 315.00±4.62$^{ab}$ | 283.50±10.63$^{b}$ | 277.66±9.17$^{b}$ | 310.33±3.80$^{ab}$ | 307.70±4.22$^{ab}$ | 298.66±13.29$^{b}$ | 348.00±5.55$^{a}$ |
| Phosphorus (mg g$^{-1}$) | 122.16±2.72 | 129.83±5.02 | 131.50±3.33 | 123.16±2.86 | 115.50±2.73 | 127.16±10.22 | 125.01±2.27 | 120.83±12.02 | 125.16±8.21 |
| Magnesium (mg g$^{-1}$ | 4.20±0.37 | 4.22±0.10 | 4.35±0.10 | 4.06±0.12 | 3.76±0.15 | 4.25±0.16 | 4.02±0.09 | 4.21±0.13 | 4.37±0.13 |
| Iron (µg g$^{-1}$) | 116.50±9.91 | 100.33±7.96 | 106.00±1.69 | 118.33±3.55 | 109.16±6.19 | 117.00±2.40 | 106.00±5.11 | 114.83±8.49 | 126.50±8.93 |
| Zinc (µg g$^{-1}$) | 213.83±12.80 | 225.16±8.18 | 210.50±8.11 | 205.00±10.60 | 196.66±17.31 | 209.66±10.39 | 231.6±10.35 | 222.50±14.73 | 244.16±11.27 |

Values are Mean±SEM, n = 6. $^{abcde}$: Values with same superscripts are not significantly different using Tukey's test, $p<0.05$

Table 4: Effect of GT, PA and I at 1 and 2% combinations on serum, fecal and urinary mineral concentrations in rats with azoxymethane induced colon carcinogenesis

| | Control | 2% GT+PA | 2% GT+I | 2% PA+I | 2% GT+PA+I | 1% GT+PA | 1% GT+I | 1% PA+I | 1% GT+PA+I |
|---|---|---|---|---|---|---|---|---|---|
| **Serum** | | | | | | | | | |
| Calcium (mg $dL^{-1}$) | 12.44±0.79 | 11.31±0.96 | 11.57±1.17 | 11.34±0.67 | 10.51±1.01 | 11.34±1.14 | 11.72±0.56 | 11.43±0.98 | 11.64±0.03 |
| Phosphorus (mg $dL^{-1}$) | 6.99±1.01 | 6.43±0.77 | 6.37±0.57 | 6.50±0.66 | 6.58±1.03 | 7.67±0.92 | 6.47±0.89 | 6.52±0.58 | 6.42±0.08 |
| Magnesium (mg $dL^{-1}$) | 1.71±0.13 | 1.78±0.14 | 1.74±0.28 | 1.54±0.14 | 1.70±0.15 | 1.83±0.07 | 1.84±0.08 | 1.60±0.12 | 1.85±0.02 |
| Iron (µg $dL^{-1}$) | 258.70±6.79$^{a}$ | 221.79±1.53$^{c}$ | 225.34±3.51$^{bc}$ | 223.92±2.03$^{bc}$ | 224.59±3.81$^{bc}$ | 236.76±3.04$^{bc}$ | 239.90±0.92$^{b}$ | 231.96±2.81$^{bc}$ | 238.67±0.50$^{bc}$ |
| Zinc (µg $dL^{-1}$) | 176.24±7.56$^{a}$ | 159.17±2.53$^{b}$ | 160.61±5.24$^{ab}$ | 156.84±1.66$^{b}$ | 161.50±2.91$^{ab}$ | 166.04±2.30$^{ab}$ | 165.37±1.96$^{ab}$ | 158.55±2.17$^{b}$ | 167.70±1.26$^{ab}$ |
| **Feces** | | | | | | | | | |
| Calcium (mg $g^{-1}$) | 53.26±1.78$^{a}$ | 50.16±3.23$^{ab}$ | 41.67±2.07$^{bc}$ | 49.57±0.75$^{ab}$ | 52.73±2.68$^{ab}$ | 48.60±1.52$^{ab}$ | 38.54±1.09$^{c}$ | 48.72±2.44$^{ab}$ | 36.96±1.83$^{c}$ |
| Phosphorus (mg $g^{-1}$) | 21.54±0.89$^{a}$ | 17.09±1.14$^{b}$ | 15.14±0.55$^{bcd}$ | 17.82±0.56$^{b}$ | 16.94±0.76$^{b}$ | 16.20±0.87$^{bc}$ | 13.06±0.24$^{cd}$ | 17.40±1.08$^{b}$ | 12.12±0.62$^{d}$ |
| Magnesium (mg $g^{-1}$) | 6.13±0.28 | 5.53±0.34 | 4.46±0.71 | 5.75±0.15 | 5.50±0.22 | 5.09±0.24 | 3.86±0.11 | 5.62±0.36 | 3.85±0.26 |
| Iron (µg $g^{-1}$) | 434.69±18.59 | 388.84±27.35 | 323.68±16.23 | 389.66±8.93 | 378.05±9.53 | 353.80±22.74 | 286.78±9.49 | 373.21±22.04 | 272.10±14.25 |
| Zinc (µg $g^{-1}$) | 463.42±32.11 | 425.07±25.90 | 358.15±14.01 | 440.46±18.44 | 426.50±19.71 | 392.38±23.76 | 326.09±10.61 | 409.46±26.24 | 299.73±14.80 |
| **Urine** | | | | | | | | | |
| Calcium (mg $mL^{-1}$) | 1.47±0.11$^{a}$ | 1.24±0.11$^{ab}$ | 1.27±0.21$^{ab}$ | 1.04±0.03$^{ab}$ | 0.89±0.09$^{b}$ | 0.95±0.05$^{b}$ | 0.90±0.05$^{b}$ | 0.84±0.05$^{b}$ | 0.88±0.54$^{b}$ |
| Phosphorus (mg $mL^{-1}$) | 0.92±0.06$^{a}$ | 0.66±0.05$^{b}$ | 0.61±0.05$^{b}$ | 0.66±0.02$^{b}$ | 0.64±0.03$^{b}$ | 0.63±0.05$^{b}$ | 0.66±0.05$^{b}$ | 0.52±0.02$^{b}$ | 0.52±0.06$^{b}$ |
| Magnesium (mg $mL^{-1}$ | 0.32±0.02 | 0.28±0.02 | 0.32±0.03 | 0.26±0.01 | 0.29±0.01 | 0.28±0.02 | 0.32±0.03 | 0.26±0.01 | 0.31±0.03 |
| Iron (µg $mL^{-1}$) | 11.57±1.09$^{a}$ | 7.58±0.66$^{b}$ | 7.70±0.90$^{b}$ | 9.39±0.55$^{ab}$ | 9.96±0.78$^{ab}$ | 8.59±0.18$^{ab}$ | 9.04±0.39$^{ab}$ | 9.64±0.32$^{ab}$ | 9.82±0.77$^{ab}$ |
| Zinc (µg $mL^{-1}$) | 18.72±3.99 | 13.17±1.23 | 13.77±1.92 | 12.59±1.34 | 13.60±1.18 | 13.08±0.54 | 11.67±0.66 | 15.09±0.99 | 14.51±1.38 |

Values are Mean±SEM, n = 6. $^{abcd}$: Values with same superscripts are not significantly different using Tukey's test, $p<0.05$

Table 5: Effect of GT, PA and I at 1 and 2% combinations on apparent absorption and retention (percentage) of minerals in rats with azoxymethane induced colon carcinogenesis

| | Control | 2% GT+PA | 2% GT+I | 2% PA+I | 2% GT+PA+I | 1% GT+PA | 1% GT+I | 1% PA+I | 1% GT+PA+I |
|---|---|---|---|---|---|---|---|---|---|
| **Apparent absorption (%)** | | | | | | | | | |
| Calcium | 37.59±0.68 | 32.53±0.69 | 32.99±1.70 | 34.27±1.03 | 32.28±0.63 | 33.37±1.50 | 33.28±0.91 | 35.22±1.92 | 35.31±1.62 |
| Phosphorus | 39.01±1.25 | 42.36±1.19 | 38.78±1.23 | 40.81±0.88 | 43.05±1.19 | 40.76±0.68 | 41.55±2.29 | 40.39±1.38 | 43.54±0.87 |
| Magnesium | 31.81±1.59 | 26.62±1.32 | 29.06±1.80 | 24.79±1.33 | 27.18±1.36 | 26.70±1.56 | 32.23±2.53 | 24.36±1.76 | 29.75±2.49 |
| Iron | 18.42±0.32$^{a}$ | 13.07±1.53$^{b}$ | 13.25±2.49$^{b}$ | 13.99±0.78$^{ab}$ | 15.39±0.77$^{ab}$ | 14.37±0.26$^{ab}$ | 15.32±0.51$^{ab}$ | 15.12±0.08$^{ab}$ | 15.96±0.69$^{ab}$ |
| Zinc | 25.48±4.01$^{a}$ | 18.27±1.14$^{ab}$ | 17.69±0.62$^{ab}$ | 16.82±1.88$^{b}$ | 18.43±1.64$^{ab}$ | 18.50±0.68$^{ab}$ | 17.44±0.85$^{ab}$ | 20.29±1.15$^{ab}$ | 20.50±1.75$^{ab}$ |
| **Retention (%)** | | | | | | | | | |
| Calcium | 33.61±0.89 | 29.02±0.77 | 29.66±1.66 | 31.42±1.12 | 29.89±0.41 | 30.81±1.50 | 30.53±0.96 | 32.97±1.79 | 32.88±1.65 |
| Phosphorus | 34.26±1.94 | 37.79±1.35 | 34.69±1.26 | 36.26±1.94 | 37.05±0.79 | 33.99±0.96 | 35.59±2.15 | 35.67±1.23 | 37.45±2.04 |
| Magnesium | 24.76±2.33$^{a}$ | 18.93±0.97$^{ab}$ | 20.73±1.33$^{ab}$ | 17.57±0.96$^{b}$ | 18.49±0.97$^{ab}$ | 17.65±1.16$^{b}$ | 21.93±1.70$^{ab}$ | 16.64±1.03$^{b}$ | 19.94±2.22$^{ab}$ |
| Iron | 14.13±0.68$^{a}$ | 9.51±1.11$^{b}$ | 9.73±1.98$^{b}$ | 9.72±0.52$^{b}$ | 10.47±0.56$^{ab}$ | 9.44±0.24$^{b}$ | 10.58±0.13$^{ab}$ | 10.45±0.35$^{ab}$ | 10.69±0.77$^{ab}$ |
| Zinc | 19.50±3.05$^{a}$ | 13.13±0.78$^{b}$ | 12.53±0.48$^{b}$ | 11.91±1.33$^{b}$ | 12.57±1.07$^{b}$ | 12.35±0.61$^{b}$ | 11.96±0.68$^{b}$ | 14.00±0.85$^{ab}$ | 13.83±1.58$^{ab}$ |

Values are Mean±SEM, n = 6. $^{ab}$: Values with same superscripts are not significantly different using Tukey's test, $p<0.05$

levels were significantly ($p<0.05$) higher in control compared to all combinations of treatments. Significantly lower Fe content was observed in 2% GT+PA+I compared to 1% GT+I. All 1% combinations had higher serum Fe contents than their 2% counterparts. Serum Zn ($\mu g\ dL^{-1}$) ranged from a high of 176.24±7.56 in control rats to a low of 158.55±2.17 in rats fed 1% PA+I. Serum Zn levels were significantly ($p<0.05$) different among control and 1 and 2% (PA+I) and 2% (GT+PA).

One percent GT+PA and PA+I, had significantly ($p<0.05$) lower calcium compared to 1% GT+I and GT+PA+I. Rats fed 1% GT+PA+I had significantly ($p<0.05$) lower fecal P compared to the groups fed 1% PA+I and GT+PA. The rats in the control group had significantly ($p<0.05$) higher fecal P compared to the treatment groups. Fecal concentration of all analyzed minerals followed a similar trend with significantly ($p<0.05$) higher levels in the control group compared to 1 and 2% combinations. Rats fed 1% GT+I and 1% GT+PA+I had significantly ($p<0.05$) lower fecal Ca ($mg\ g^{-1}$) compared to the control. Rats fed the control diet had significantly ($p<0.05$) higher fecal phosphorus ($mg\ g^{-1}$) compared to the treatment groups.

Administration of treatments did not show any significant effect on urinary Mg and Zn concentrations among 1 and 2% combinations of treatments (Table 4). Urinary Ca content was significantly ($p<0.05$) higher in control (1.47±0.11) fed rats compared to all 1% combination treated rats. A significantly ($p<0.05$) higher urinary P concentration was found in control (0.92±0.06) treated rats compared to all combinations of treatment groups. Iron concentration in urine was significantly ($p<0.05$) lower in 2% GT+PA (7.58±0.66) and 2% GT+I (7.70±0.90) groups compared to the control (11.57±1.09) treated group. There was no consistent pattern in concentrations of urinary minerals among treatment groups.

**Effect of Dietary Treatments on Apparent Absorption and Retention (Percentage) of Selected Minerals**

There were no significant differences in apparent absorption (%) of Ca, P and Mg and retention of Ca and P among control and treatment groups (Table 5). A significant increase (%) in apparent absorption of Fe was seen in the control group (18.42±0.32) compared to 2% GT+PA (13.07±1.53) and 2% GT+I (13.25±2.49) fed rats. Control fed rats had significantly ($p<0.05$) higher percentage of apparent absorption (%) of Zn compared to the 2% PA+I (16.82±1.88) fed group. Percentage of Mg retention was significantly ($p<0.05$) higher in the control (24.76±2.33) group compared to 1 and 2% (PA+I) (16.64±1.03; 17.57±0.96, respectively) and 1% GT+PA (17.65±1.16). Significantly ($p< 0.05$) higher reduction in Fe retention (%) was observed in 1% GT+PA (9.44±0.24) and all 2% combinations except 2% GT+PA+I compared to the control (14.13±0.68) group. There were no significant ($p<0.05$) differences among control and 1% PA+I and 1% GT+PA+I in Zn retention (%). All other 1 and 2% combinations (1 and 2% GT+PA, 2% GT+I and 2% PA+I) of treatments had significantly ($p<0.05$) lower Zn retention compared to the control. Combination of treatments did not show any consistent patterns with apparent absorption and retention (%) of Fe and Zn.

## DISCUSSION

The present study was conducted to determine the effect of PA, GT and I in combinations (1 and 2%) on bone mineralization and mineral balance in rats with Azoxymethane-induced colon carcinogenesis. The results of the study showed that administration of the selected treatments did not have any significant adverse effects on the absorption of macro minerals (Ca, P, Mg) in Fisher 344 male rats.

The weight gain and feed intake throughout the study period was comparable to animal studies conducted to test the chemopreventive potential of several bio-active food ingredients using Fisher 344 male rats as models (Verghese *et al*., 2002). Weight gain in rats fed control was significantly

($p<0.05$) lower than the other groups. There were no significant differences in feed intake among the groups. A study conducted feeding GT (2.7% level) showed reduced weight gain (Hamdaoui *et al*., 1997), while, Record *et al*. (1996), reported no difference in weight gain and food efficiency (Yang *et al*., 2001).

Research has reported that dietary PA can have an inhibitory effect on Ca absorption (Fredlund *et al*., 2006; Rimbach *et al*., 1995; Sandberg *et al*., 1993) and the reduction of PA may significantly increase Ca availability (Bedford and Schulze, 1998). The ratio of PA to Ca is probably too low to observe a negative effect of PA on Ca absorption in the present study. Similar results were reported by other researchers who failed to observe any effect of PA on Ca absorption (Miyazawa *et al*., 1996; Nickel *et al*., 1997; Lopez *et al*., 2000). Recent studies have demonstrated that the antinutrient effect of PA can be manifested only when large quantities of PA are consumed with a diet poor in oligo elements (Sandstrom *et al*., 2000). A long term intake of PA in pure form did not affect mineral absorption in humans and in rats (Ullah and Shamsuddin, 1990). A study conducted using Sprague-Dawly rats reported an increased but not significant bone mineral concentration when 15 mM PA and I were given in drinking water singly and in combinations for 40 week (Vucenik *et al*., 1995). The results of the present study are consistent with these published results with regard to combinations of 1% treatments. A significant reduction in Ca level in tibia was seen in 2% GT+PA+I and 2% PA groups. Tea contain tannins, which are actually phenol-rich polymer mixtures and which may increase the fecal Ca excretion thereby reducing Ca absorption and decreasing Ca content in the tibia.

Apparent absorption of P in 1% combinational groups was similar between the phytate free basal diets and groups fed PA, which may have resulted because the nonphytate-P in the phytate supplemented diets was absorbed at the same extent as the P in the Phytate free basal diet. These findings concur with the previous finding reported in rats (Anke *et al*., 1970) and mice (Pallauf, 1982). All diets contained the dietary requirement for Mg and the addition of 1 and 2% treatments in drinking water did not show any significant effect on apparent absorption of Mg although there was a decreased trend with an increase in dose. This effect was probably caused by formation of intestinal Mg-Ca-Phytate complexes resulting in a decrease of soluble Mg in rat intestine. The results of this study were consistent with Rimbach *et al*. (1995) and Lopez *et al*. (2000). Some researchers found an inverse relationship between feeding PA and Mg absorption in rats (Miyazawa and Yoshida, 1991; Roberts and Yukin, 1960). Feeding tannins isolated from black tea did not significantly alter apparent Mg absorption (Hamdaoui *et al*., 1994). Tea decoction significantly raised tissue Zn and Mg concentrations and storage by 29.4 and 48.7%, respectively (Hamdaoui *et al*., 1997). The moderate effect of treatments on Zn and Mg concentrations and the reduction of Fe concentrations in the serum, tibia and apparent Fe absorption in this study may have resulted from the interaction between minerals in animal intestine. Zn and Mg may have mutual affinity to the carrier protein-transferrin and consequently increased the transepithelial transport of these elements across the intestinal cell wall and therefore decreased the availability of Fe (Gibson, 1994).

PA has a marked inhibitory effect on the absorption of Fe in humans (Gillooly *et al*., 1983). Only small amounts of PA (5-10 mg) in a meal are sufficient to reduce the Fe absorption by 50% (Hallberg *et al*., 1989). In this study 2% GT+PA and GT+ I treatment groups showed significant reduction in apparent Fe absorption although all 1% combinations did not show a significant difference from the control. Serum and tibia Fe were reduced in most of the 2% combination treatments. These results were in accordance with Record *et al*. (1996). Some investigators did not observe any inhibitory effects of GT or Black Tea (BT) on iron absorption and iron deposition in tissues (Record *et al*., 1996; Greger and Lyle, 1988). In contrast, research has shown that GT and BT dramatically decreased the non-heme iron bioavailability *in vitro* and in growing rats (Hamdaoui *et al*., 1994, 1995; Brune *et al*., 1989; Brown *et al*., 1990).

Zn is important in skeletal development. A moderate deprivation of dietary Zn in monkeys resulted in retardation of skeletal growth, maturation and mineralization (Golub *et al*., 1996). In addition PA has been reported to alter Zn equilibrium in several monogastric species including man. The major effects of PA on Zn deprivation is due to reabsorption of endogenously secreted Zn (Oberleas, 1996). The inhibition of Zn absorption by PA can be predicted by the molar ratio of PA to Zn. Molar ratios in excess of 15:1 progressively inhibit Zn absorption and even molar ratios as low as 5:1 may have some negative impact, resulting in lower liver and bone Zn contents. In the present study all diets contained 35 mg $kg^{-1}$ Zn and 0-2% PA. A decrease in apparent absorption of Zn content in serum was noted in 2% PA+I groups. Chelating effect of PA on Zn has been reported by Saha *et al*. (1994) and Wise (1995). Green tea had a significant influence on Zinc absorption resulting in a decrease in the apparent absorption of Zn. This study showed that the retention of Zn was significantly ($p<0.05$) reduced in all combinations of treatments. The results were in accordance with Zeyuan *et al*. (1998) and Greger and Lyle (1988). Tea consumption had a small but statistically insignificant adverse effect on zinc availability in humans (Gangi and Kies, 1994). Reddy *et al*. (1990) found that infused teas significantly decreased $^{65}Zn$ absorption by 15%. Iron, Zn and copper have been reported to induce tumors in one study (Swierenga *et al*., 1987). Conversely, Zn and Fe deficiencies have both been associated with increased incidence of esophageal cancer in humans (Nelson, 1987). Some researchers (Kuo *et al*., 2002) have found elevated trace element levels in cancer tissues and plasma.

## CONCLUSION

The combination of PA, I and GT at 1 and 2% levels did not show a significant negative effect in bone mineralization in AOM-induced Fisher 344 male rats. Combinations of treatments at lower levels seemed to be beneficial in reducing the negative effects of these mineral chelating agents.

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