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## SHORT COMMUNICATION

# Interactive suppression of aberrant crypt foci induced by azoxymethane in rat colon by phytic acid and green tea

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**Several epidemiological studies point to a strong correlation between nutrient composition of the diet and cancer of the colon. Phytic acid, present in grains, has been credited with reducing the risk of cancer of the colon. A number of reports are available indicating the benefits of green tea consumption in reducing the risk of stomach, lung and skin cancer, but little data are available on the effect of green tea in reducing the risk of colon cancer. Also, there are no studies on the combined effect of these compounds on colon tumorigenesis. Thus the primary objective of this investigation was to elucidate the combined effects of green tea and phytic acid on colonic preneoplastic lesions and the Phase II enzyme glutathione S-transferase. Fisher 344 male weanling rats were divided into nine groups of 15 rats each and fed the experimental diet for 13 weeks. Rats received two s.c. injections of azoxymethane in saline at 16 mg/kg body wt at 7 and 8 weeks of age. Rats received three levels (0, 1 and 2%) of phytic acid with three levels (0, 1 and 2%) of green tea within each phytic acid level in a 3 × 3 factorial experiment. Results indicate that while green tea had a marginal effect ($P < 0.14$), phytic acid significantly reduced the incidence of aberrant crypt foci ($P < 0.008$). The interaction between green tea and phytic acid was significant ($P < 0.029$ for distal and $< 0.0168$ for entire colon) and positive, pointing to a synergistic effect of green tea and phytic acid.**

Phytic acid (myoinositolhexakisphosphate) is widely distributed in plants, especially in cereals and legumes (1). Both *in vivo* and *in vitro* experiments have shown striking anticancer potential for phytic acid (2). In several studies phytic acid supressed colon tumorigenesis in rat and mouse models (3–7), induced reversal of malignant cells back to a normal phenotype (2), inhibited colon tumorigenesis in a dose-dependant manner (7), acted as a natural antioxidant (8) and augmented the activity of natural killer (NK) cells (9).

The polyphenolic fraction isolated from green tea (*Camellia sinensis*) (GTP) or its constituent epicatechin derivatives have been shown to inhibit cytochrome P-450 mixed function oxidase enzymes in the skin and liver (10), protect against UVB-induced photocarcinogenesis in SKH-1 hairless mice (11), inhibit TPA-induced epidermal ornithine decarboxylase activity in SENCAR mice (12) and inhibit cyclooxygenase and lipooxygenase activities (13). Further, GTP has been shown to inhibit lung cancer in mice (14) and benzo[*a*]pyrene metabolism in rat liver (15). Other mechanisms, such as induction of Phase II enzymes (16) and trapping of the ultimate carcinogens by GTP (10), have been suggested. Green tea suppresses the elevated levels of 8-OH-deoxyguanosine in lungs of 4-[methylnitroso amino]-1-[3-pyridyl]-1-butanone-treated mice (17). GTP has also been shown to possess an anti-promotional effect (12,13). Recent studies showed that both green and black tea contain flavonoids such as quercetin (10–25 mg/l), kaempferol (7–12 mg/l) and myricetin (2–5 mg/l), which are potentially anti-carcinogenic (18,19). However, there are very few published reports on the effect of green tea on colon carcinogenesis (20,21).

Very few studies in the literature have emphasized the possible synergistic effects of dietary ingredients in suppressing carcinogenesis. We have recently demonstrated that *Bifidobacterium longum* and lactulose exert an additive effect in suppressing colon tumorigenesis in rats (22). Experimental evidence has shown that part of the anti-tumorigenic effect of tea is at the initiation level, while for phytic acid the anti-carcinogenic effect is largely directed at the promotional stages of carcinogenesis. The objective of this study was, therefore, to study the effect of feeding green tea and phytic acid singly and in combination on azoxymethane (AOM)-induced aberrant crypt foci in rat colon.

Gunpowder variety green tea (Frontier Herbs, Ames, IA) was prepared fresh daily at a 2% concentration. The leaves were placed in half the required amount of boiled distilled deionized water for 15 min and then decanted through cheesecloth. The residue was extracted again with the remaining volume of boiling distilled deionized water. The filtrates were combined and placed on ice until they reached room temperature and for 1% green tea dilutions were made with distilled deionized water.

One hundred and thirty five male Fisher 344 weanling rats were obtained from Charles River Breeding Inc. (Wilmington, MA) and housed in stainless steel wire cages at 2 rats/cage. The light and dark cycles in the animal room were kept at 12 h each. Relative humidity and temperature were 50% and 21°C respectively. Groups of 15 rats each were assigned in 3×3 factorial experiments to nine dietary treatments (0, 1 and 2% phytic acid with 0, 1 and 2% green tea under each phytic acid level) based on the AIN 76A diet for 13 weeks. Green tea was given as drinking water and phytic acid was added to the feed at the expense of cellulose. The diet consisted of 20% casein, 52% corn starch, 13% dextrose, 5% corn oil, 5% alphacel (cellulose), 0.3% methionine, 1% AIN 76 vitamin mix, 0.2% choline bitartarate and 3.5% AIN 76 mineral mix. All the diets were mixed on a weekly basis and stored at 4°C until used.

All animals received s.c. injections of AOM (Sigma Chemical Co., St Louis, MO) in saline at 16 mg/kg body wt, one dose at 7 weeks and another dose at 8 weeks of age. At 17 weeks of age all animals were killed using $CO_2$. The livers were removed and stored at –80°C. The colons of all rats from each group were removed and flushed with 0.1 M potassium phosphate buffer, pH 7.2. Colons from 10 rats from each

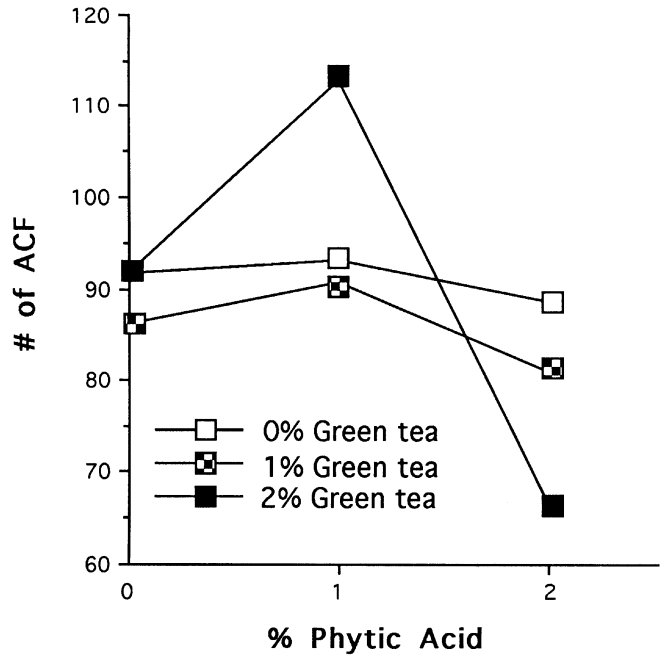


**Fig. 1.** Interactive effect of phytic acid and green tea on the number of ACF in the distal colon. Each point on the graph represents the number of ACF in the distal colon resulting from interaction between the given concentrations of phytic acid (as shown on the *x*-axis) and 0, 1 or 2% green tea (as indicated in the legend).

**Table I.** Effect of feeding phytic acid and green tea on the incidence of ACF in rat colon induced by azoxymethane[a]

| Dietary component (%) | *n* | Proximal colon | Distal colon | Total colon |
|---|---|---|---|---|
| Phytic acid | | | | |
| 0 | 30 | 79.8[b] | 89.7[bc] | 169.5[b] |
| 1 | 30 | 65.9[c] | 98.8[b] | 164.8[b] |
| 2 | 30 | 67.7[c] | 78.4[c] | 146.3[c] |
| Green tea | | | | |
| 0 | 30 | 76.7[b] | 91.1[b] | 167.7[b] |
| 1 | 30 | 67.9[b] | 85.8[b] | 153.8[b] |
| 2 | 30 | 68.9[b] | 90.1[b] | 159.0[b] |
| Pooled SEM[d] | 30 | 3.4 | 3.9 | 5.4 |

[a]Rats were fed phytic acid in the diet and green tea in the drinking water for 13 weeks and injected s.c. with AOM at 7 and 8 weeks of age at 16 mg/kg body wt.
[b,c]Means in the same column with the same letter are not significantly different by Tukey's Studentized range test. Comparisons are within green tea and phytic acid.
[d]Pooled SEM, pooled standard error of least square means.

dietary group selected at random were prepared for counting aberrant crypt foci (ACF) as described by Bird (23); the colons of the remaining five rats were split open longitudinally and the colonic mucosa scraped and stored at –80°C for glutathione S-transferase (GST) assay using the procedure of Habig *et al.* (24). Data were analyzed using the SAS statistical program (25).

Analysis of the main effects showed that phytic acid significantly reduced the number of ACF in the colon ($P < 0.008$) at the 1 and 2% levels (Table I). However, phytic acid tended to have a greater effect at the 2% level. The number of ACF in the distal colon was higher than that in the proximal region of the colon. A paired *t*-test showed a significant difference ($P < 0.05$) between the ACF levels in the distal and proximal colon. These data are consistent with reports that the distal colon shows a greater incidence of colorectal cancer than the proximal colon in humans (26). It should be noted that main effect analysis for phytic acid included data on green tea built into the design. Green tea caused a marginally significant reduction ($P < 0.14$) in the number of ACF (Table I). Feeding 2% phytic acid significantly ($P < 0.05$) reduced the weight gains of the animals compared with the control and animals receiving 1% phytic acid. A similar observation was noted for animals receiving 2% green tea.

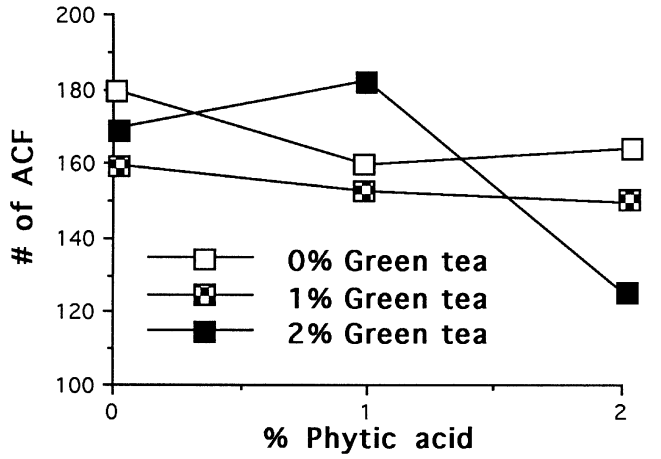


**Fig. 2.** Interactive effect of phytic acid and green tea on the number of ACF in total colon. Each point on the graph represents the number of ACF in the total colon resulting from interaction between the given concentrations of phytic acid (as shown on the *x*-axis) and 0, 1 or 2% green tea (as indicated in the legend).

**Table II.** Effect of feeding phytic acid and green tea on GST activity in rats[a]

| Dietary component (%) | *n* | Liver | | Colonic mucosa | |
|---|---|---|---|---|---|
| | | (U/g tissue) | (U/mg protein) | (U/g tissue) | (U/mg protein) |
| Phytic acid | | | | | |
| 0 | 15 | 58.9[b] | 0.57[b] | 2.10[b] | 0.06[b] |
| 1 | 15 | 60.5[b] | 0.59[b] | 2.02[b] | 0.05[b] |
| 2 | 15 | 73.3[c] | 0.71[c] | 2.25[b] | 0.05[b] |
| Green tea | | | | | |
| 0 | 15 | 65.65[b] | 0.67[b] | 2.07[b] | 0.052[b] |
| 1 | 15 | 61.55[b] | 0.61[b] | 2.16[b] | 0.054[b] |
| 2 | 15 | 65.56[b] | 0.61[b] | 2.14[b] | 0.054[b] |
| Pooled SEM[d] | | 3.2 | 0.03 | 0.10 | 0.002 |

[a]Rats were fed phytic acid in the diet and green tea in the drinking water for 13 weeks and injected s.c. with AOM at 7 and 8 weeks of age at 16 mg/kg body wt.
[b,c]Means in the same column with the same letter are not significantly different by Tukey's Studentized range test. Comparisons are within green tea and phytic acid.
[d]Pooled SEM, pooled standard error of least square means.

The effects of phytic acid and green tea on the total activity of GST in the liver and colonic mucosa are shown in Table II. Feeding of 2% phytic acid significantly increased activity of the enzyme ($P < 0.0059$) in the liver compared with the control and 1% phytic acid groups. There was no significant difference in the levels of enzyme activity between animals in the control group and those receiving 1% phytic acid. The enzyme activity in colonic mucosa was not influenced. Green

tea did not affect the activity of GST in either the liver or colonic mucosa.

Factorial analysis of variance showed a significant interactive effect of phytic acid and green tea on distal ($P < 0.0295$) and total ($P < 0.0168$) colonic ACF (Figures 1 and 2). Interactive effects of phytic acid and green tea on weight gain and feed intake were not significant ($P < 0.729$). Thus it appears that the interactive effect on ACF was independent of weight gain. In fact, the correlation between ACF and weight gain, although positive, was very weak ($r^2$ for total colonic ACF 0.018). To the authors' knowledge, studies on interactive effects of various dietary components on carcinogenesis have been rare (27,28). A similar significant ($P < 0.0447$) interaction was noticed in GST specific activity in the colonic mucosa, though green tea and phytic acid by themselves did not significantly alter activity of the enzyme ($P < 0.86$ and $P < 0.27$ respectively).

In this study green tea at 1 and 2% only marginally reduced the total number of ACF in the rat colon; GST was unaffected. This was a little surprising, since in many studies (29) dietary antioxidants elevated levels of GST and it has been demonstrated that GTPs are antioxidants (30). Furthermore, GTPs were shown to inhibit binding of carcinogens to DNA (31). It is, however, important to note that in factorial experiments when the interaction is significant, the main effects are likely to be masked.

Phytic acid at the 1% level in this study significantly decreased the number of ACF in the proximal colon and at the 2% level reduced ACF number in proximal, distal and total colon. Results also show that 2% phytic acid plus 2% green tea have a synergistic effect, showing a total of ~30% reduction in ACF when given in combination, as compared with a total ACF reduction of 20% for phytic acid plus green tea when given individually at the 2% level. Thus, while green tea itself was without a statistically significant effect, it enhanced the effect of phytic acid, pointing to possibly different mechanisms of action. Thus phytic acid may have acted at the signal transduction and cell proliferative stages (promotional stages) in reducing tumorigenesis, while the effect of green tea seems to be primarily prevention of 8-OH-deoxyguanosine adduct formation (2,10). These results clearly emphasize the fact that dietary components should not be studied individually for their anti-tumorigenic effect. This is especially evident in the enhancing effect of phytic acid by green tea while green tea by itself is ineffective. A reduction in the number of ACF in rat colon by phytic acid was reported by Pretlow *et al.* (4). However, in their study phytic acid was administered in drinking water, as opposed to being incorporated in the diet, as in this study. Further, Pretlow *et al.* (4) also reported the presence of higher numbers of ACF containing 4 crypts/focus. However, in this study it was observed that the ACF containing 2 crypts/focus were significantly higher in number than ACF with 1, 3, 4 or $\geqslant$5 crypts/focus. This difference may be due to the length of the experiments.

The majority of the studies on the anti-carcinogenic effect of phytic acid have administered phytic acid to the animals via drinking water, because it is believed that it forms insoluble complexes with proteins and other macromolecules and thus would be rendered less available (4,32). However, contrary to the results observed by Hirose *et al.* (33), who did not observe a significant inhibition of colon tumors by dietary phytic acid, in this study phytic acid administered via the diet significantly reduced the number of ACF in the colon as well as increased the levels of GST in the liver when administered at the 2% level. The difference observed could be due to the fact that Hirose *et al.* used tumors as the end point. While colonic ACF have become an accepted intermediate marker, recently Hardman and Cameron (34) pointed out that ACF may not necessarily correlate with end point models.

Though phytic acid at the 2% level significantly reduced the weight gain of the animals, it does not seem to be of major consequence, since the interaction between green tea and phytic acid overall and at the 1% green tea + 1% phytic acid level, while significantly reducing the number of ACF, did not have any significant effect on weight gain. Earlier studies also point to the positive effect of phytic acid in inhibiting the development of AOM-induced colonic tumors in rats at 2% w/w (4,5,35).

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