Efficacy of Garbanzo and Soybean Flour in Suppression of Aberrant Crypt Foci in the Colons of CF-1 Mice

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Abstract. Background: Epidemiological studies have reported a low incidence of colon cancer in countries with high legume consumption. Moreover, experimental studies have found that legumes, such as soybeans and pinto beans, have anticancer properties. While garbanzo beans are a rich source of various phytochemicals they have not been well studied. In the present study, the azoxymethane (AOM)-induced aberrant crypt foci (ACF) in CF-1 mice was utilized as a model to assess and compare the effects of garbanzo flour to that of soy flour. Materials and Methods: Twenty, 5-week-old CF-1 mice were divided into four groups of 5 animals each: 10% garbanzo, 10% soy, 10% mixed (soy and garbanzo flours), and control (rodent chow). Animals received subcutaneous injections of AOM (10-mg/kg b.w.) once a week for two weeks to induce ACF. At week ten, the animals were sacrificed and the colons were scored. Results: There was a 64% (p<0.001) suppression of ACF for animals fed the garbanzo flour, versus an inhibition of 58 and 55% (p<0.001) for the soy and mixed flour groups, respectively. Discussion: These results demonstrate that garbanzo beans possess bioactive compounds capable of inhibiting the formation of pre-cancerous lesions in mice and suggest that, like soybeans, their consumption contributes to a reduction in colon cancer incidence.

For many years, legumes have been regarded as functional foods that promote good health and have curative value (1). The chemoprotective activity of legumes in part is attributed to different anticancer agents including phytoestrogens, protease inhibitors, phytate, saponins, phytosterols, as well as other possible anticarcinogens such as fiber and omega-3 fatty acids (2).

To date, the most widely studied legume is soybean. While epidemiological data suggest that populations which regularly consume soybeans have a lower incidence of colon cancer than those ones that do not (3,4), data from experimental models on soy and soy isolavones have been conflicting (5). For example, soy foods (e.g., soy flour, full fat soy flakes, miso) have been reported to have both protective effects (6,7) as well as no effect (8,9) on colon carcinogenesis. On the other hand, while some reports have found that some soy constituents (e.g., soy protein isolate, genistein) significantly suppress the development of aberrant crypt foci (10-13), a number of reports have demonstrated either no effect (8) or even an enhancing effect in the development of colon pre-neoplastic lesions and/or tumors in experimental animals (14-16). Based on the published data, it appears that consumption of the soy foods may provide some protection against colon cancer, while isolated components of soybeans (e.g., genistein, soy protein isolate) may provide little or no protection against colon carcinogenesis.

While most of the emphasis has centered on investigating the relationship between soybeans and cancer risk, other legumes have also been reported to have chemoprotective effects. Dry beans, which are the most commonly consumed non-soy legume in the world, have been reported to have chemopreventive activity against several types of cancers. For example, Correa (17) reported an inverse correlation between dry bean consumption and deaths due to breast, prostate and colon cancer. Furthermore, the efficacy of dry beans against colon carcinogenesis has also been demonstrated in experimental models (18-20). Hughes et al. (19) fed rats either pinto beans or casein and found that the incidence of colon cancer in the group that was fed the pinto beans was 50% to that of the casein-fed animals. In addition, in rats that did develop tumors, those consuming the beans had reduced multiplicity. Treatment groups had
### Table 1. Composition of experimental diets fed to mice (per kg diet).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Kcal</th>
<th>Pro (g)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
<th>Fiber (g)</th>
<th>Daidzein (mg)</th>
<th>Genistein (mg)</th>
<th>Biochanin A (mg)</th>
<th>N/a</th>
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<tbody>
<tr>
<td>Control</td>
<td>3820.0</td>
<td>230.3</td>
<td>609.0</td>
<td>42.5</td>
<td>47.0</td>
<td>123.00</td>
<td>132.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 g Chow</td>
<td>48.0</td>
<td>12.0</td>
<td>30.0</td>
<td>9.0</td>
<td>1.0</td>
<td>3.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Oil</td>
<td>157.5</td>
<td>37.0</td>
<td>37.0</td>
<td>20.0</td>
<td>17.0</td>
<td>7.0</td>
<td>7.0</td>
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<tr>
<td>Total</td>
<td>4025.5</td>
<td>262.0</td>
<td>609.0</td>
<td>60.0</td>
<td>47.0</td>
<td>123.00</td>
<td>132.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Soy flour</td>
<td>3438.0</td>
<td>225.0</td>
<td>549.0</td>
<td>38.0</td>
<td>42.0</td>
<td>110.00</td>
<td>119.00</td>
<td></td>
<td></td>
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<tr>
<td>900 g Chow</td>
<td>347.0</td>
<td>37.0</td>
<td>37.0</td>
<td>20.0</td>
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<td>7.0</td>
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<td>Corn oil</td>
<td>155.0</td>
<td>37.0</td>
<td>37.0</td>
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<td>17.0</td>
<td>7.0</td>
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<td>262.0</td>
<td>549.0</td>
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<td>59.0</td>
<td>181.20</td>
<td>215.80</td>
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<tr>
<td>10% Garbanzo bean flour</td>
<td>3438.0</td>
<td>225.0</td>
<td>549.0</td>
<td>38.0</td>
<td>42.0</td>
<td>110.00</td>
<td>119.00</td>
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<tr>
<td>100 g Garbanzo</td>
<td>383.0</td>
<td>20.0</td>
<td>60.0</td>
<td>7.0</td>
<td>17.0</td>
<td>0.06</td>
<td>0.06</td>
<td>1.78</td>
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<tr>
<td>Corn oil</td>
<td>80.0</td>
<td>20.0</td>
<td>60.0</td>
<td>7.0</td>
<td>17.0</td>
<td>0.06</td>
<td>0.06</td>
<td>1.78</td>
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<tr>
<td>Total</td>
<td>4014.0</td>
<td>262.0</td>
<td>549.0</td>
<td>90.0</td>
<td>59.0</td>
<td>110.04</td>
<td>119.06</td>
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<tr>
<td>15% Mixed flour</td>
<td>3438.0</td>
<td>225.0</td>
<td>549.0</td>
<td>38.0</td>
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<td>110.00</td>
<td>119.00</td>
<td></td>
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</tr>
<tr>
<td>900 g Chow</td>
<td>191.5</td>
<td>10.0</td>
<td>30.0</td>
<td>3.5</td>
<td>8.0</td>
<td>0.02</td>
<td>0.03</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>50 g Soy flour</td>
<td>238.0</td>
<td>19.0</td>
<td>30.0</td>
<td>3.5</td>
<td>8.0</td>
<td>0.02</td>
<td>0.03</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>65.0</td>
<td>10.0</td>
<td>30.0</td>
<td>3.5</td>
<td>8.0</td>
<td>0.02</td>
<td>0.03</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3976.0</td>
<td>262.0</td>
<td>597.0</td>
<td>90.0</td>
<td>55.0</td>
<td>145.62</td>
<td>167.43</td>
<td>0.89</td>
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</tr>
</tbody>
</table>

*Source: Harlan Teklad, Madison, WI.

*25. USDA-Iowa State University Database on the Isoflavone Content of Foods.*

An average of one tumor/rat, while animals receiving the casein diet had 2.5 tumors/rat. In a similar study, Hangen and Bennink (19) compared a casein-based diet to a diet containing black beans or a diet containing navy beans. They reported that administration of black beans or navy beans reduced the number of rats that had colon cancer by over 50% (18). Thus, these studies suggest that eating dry beans reduces the incidence of colon cancer in rodents.

Garbanzo beans, the third most common legume in the world, have found a recent increased popularity in the United States (21). The consumption of garbanzo beans in the year 2002 averaged 89 million pounds, that is nearly doubling the average amounts consumed in the 1990s (22). The increased interest in ethnic foods, such as hummus and in Mediterranean cooking in general, have been suggested to be the cause for the increased demand for garbanzo beans in the United States (22). Given the increased interest in this legume, the aim of the present study was to investigate the efficacy of garbanzo beans on the development of carcinogen-induced ACF and compare it to that of soybean, the most widely studied legume.

In the present study, the ACF model was used to assess the usefulness of the flours to suppress ACF in the colons of CF-1 mice. The ACF assay has been employed by numerous studies to evaluate the efficacy of chemopreventive compounds (23-26). Aberrant crypt foci have been identified on the colonic mucosal surface of rodents treated with carcinogens, and these have been shown to be one of the earliest recognizable lesions in the colon. Furthermore, it has been demonstrated that the carcinogens (e.g., AOM) that induce ACF also induce colon cancer in rodents (27). Several lines of evidence strongly suggest that ACF are good intermediate biomarkers of colon cancer, both in rodents (27) and in humans (28). Morphologically, ACF are distinguishable from normal crypts by their increased size and the more elliptical shape of the luminal opening, with a thicker lining of epithelial cells (29). The ACF contain elements of dysplasia (evident by alterations in enzyme activity) and express mutations in the APC gene and the ras oncogene, which suggests that they are part of the pathway leading to colon cancer (30,31). Longitudinal studies have shown that the areas where ACF appear correlate with tumor appearance, suggesting that these are the preferred sites for tumorigenesis (32). As a result of these findings, the ACF assay has been used to evaluate many chemopreventive agents including quercetin (13), curcumin
Start Mice \(\rightarrow\) Carcinogen \(\rightarrow\) Sacrifice

Topological Features of Aberrant Crypt Foci
- Elevated on the normal mucosal surface
- Rounded and defined with respect to the normal mucosa
- Deeper vital staining than normal mucosa
- Greater crypt area than normal
- Luminal shape polymorphism (round, serrated, elongated)

Figure 1. Experimental design to evaluate chemopreventive effects of garbanzo bean and soy flours in CF-1 mice. Features of aberrant crypt foci from AOM-treated colon of CF-1 mice stained with methylene blue (three crypt foci).

(15), saponin (16) and phenethyl isothiocyanate (16). Several agents, which have been evaluated using the ACF assay, are currently being evaluated in clinical trials. For example, piroxicam, a non-steroidal anti-inflammatory drug (NSAID) used for the treatment of inflammatory arthritis, is currently being evaluated in clinical trials as a potential chemopreventive agent for colon cancer (33). Thus, the evaluation of ACF in colon crypts provides a powerful screening tool for testing potential chemopreventive agents/foods.

Materials and Methods

Chemicals. All chemicals were purchased at the highest purity available. Azoxymethane was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals and diets. Female CF-1 mice were purchased at 5 weeks of age from Charles Rivers (Raleigh, NC, USA). The animals were quarantined for 7 days and housed 5 mice per cage, with a 12-h light-dark cycle, and a relative humidity of 50%. Drinking water and Teklad 4% Mouse/Rat Diet 7001 (Harlan-Teklad, Madison, WI, USA) were supplied to the animals ad libitum. This study was approved by the University of Illinois at Chicago Animal Use and Care Committee. After grouping by weight, the mice were randomly assigned to one of four groups consisting of 5 animals per group: control (Teklad-4% Mouse/Rat Diet, supplemented with 10% flour by weight), 10% garbanzo bean flour, 10% soy flour and 10% mixed flours (5% of soybean and garbanzo bean flours). The 10% supplementation was selected based on previous studies which have shown good tolerance by animals. Diets were adjusted for 26.2 g protein/100 g diet with casein. The fat concentration of all diets was adjusted to 6 g/100 g with corn oil. The approximate diet composition of the 4% Teklad Diet 7001 (34) together with the nutrient contributions of the garbanzo bean and soybean flours is shown in Table 1. Values for isoflavones were estimated based on the USDA-IOWA State Database (35). The isoflavone content of the rodent chow was estimated based on data obtained from Teklad. All diets were refrigerated to prevent spoilage.

Experimental design. aberrant crypt assay. As illustrated in Figure 1, the mice were injected subcutaneously with the carcinogen AOM (10 mg/kg body weight) once a week for two weeks. Azoxymethane was dissolved in normal saline and kept on ice throughout the procedure. Ten weeks following the initiation of the study, the animals were sacrificed by carbon dioxide asphyxiation. The colons were evaluated for ACF by the procedure previously described (39). Briefly, the colons were excised, cut open along the longitudinal axis, flushed with cold saline and fixed flat on 0.1 M phosphate-buffered 10% formalin solution (pH 7.4, 4°C) for 24 h. The colons were then transferred to 70% ethanol solution and stored at 4°C until staining with 0.2% methylene blue (dissolved in PBS) for 3 min. The number and size of ACF per colon were determined under a microscope at a magnification of x40. As shown in Figure 1, ACF were distinguished from surrounding non-aberrant crypts by their increased size, elongated luminal opening, increased distance from then luminal to basal surface of cells, thickened epithelial cell lining and enlarged pericryptal area.
Table II. Efficacy of garbanzo bean flour, soybean flour and a combination of flours on colonic aberrant crypt formation.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Initial Wt. (gm)a</th>
<th>Final Wt. (gm)b</th>
<th>Multiplicityb</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.2</td>
<td>34.0</td>
<td>1.13</td>
<td>–</td>
</tr>
<tr>
<td>10% Soybean flour</td>
<td>22.2</td>
<td>35.0</td>
<td>0.52***</td>
<td>58</td>
</tr>
<tr>
<td>10% Garbanzo Flour</td>
<td>21.6</td>
<td>34.6</td>
<td>0.41***</td>
<td>64</td>
</tr>
<tr>
<td>10% Mixed flour</td>
<td>22.6</td>
<td>32.4</td>
<td>0.51***</td>
<td>55</td>
</tr>
</tbody>
</table>

*aMean values ± SE
*bNumber of ACFs per cm² of colon
***Values statistically significant (p<0.001) from control

![Graph showing Number of Large (>4 focus) ACFs](image)

Figure 2. Analysis of ACF exhibiting four or more crypts per focus (large crypts). The mean number (±SE) of large crypts are shown for the treatment groups. Asterisks show values significantly different from those in the control group: * p<0.05, ** p<0.01 and ***p<0.001. All other values are not statistically significant relative to those of the control group.

relative to surrounding normal crypts.

Statistical analyses. All data were analyzed using GraphPad Prism V3.0 (GraphPad Software, Inc., San Diego, CA, USA). Treatment agents and schedules were compared with the AOM-only group using one-way ANOVA. If a significant difference (p<0.05) was observed, the Bonferroni t-test was used as a multiple comparison test.

Results

The effects of garbanzo bean and soybean flour on the development of AOM-induced ACF are presented in Table II. Treatment of animals with AOM resulted in a 100% incidence of ACF, whereas we have previously shown that no ACF are identified in saline-treated animals (36). The distribution of the ACF was greatest in the distal colon, with the fewest ACF found in the proximal colon. There was no evidence of toxicity in animals treated with either the flour-supplemented diet or carcinogen. Body weights of the mice were monitored weekly from day 0 until the time of termination (Table II). No significant differences (p>0.05) were observed between groups. All mice were active and healthy during the experimental period.

Supplementation with soybean flour significantly decreased the total number of ACF/cm² of colon from 1.13 in the control to 0.52 in the soybean group, that is, a 53% (p<0.001) inhibition as compared to the control group (Table II). Supplementation with 10% garbanzo flour resulted in suppression of ACF formation by 64% (p<0.001). Combining soybean and garbanzo bean flour did not show any additional or synergistic efficacy as compared to the administration of individual flours.

Since studies have shown that the ACF composed of four or more crypts per focus correlates closely with subsequent development of colon cancer (37), the number of multi-crypt clusters of ≥4 crypts per focus were evaluated. As
shown in Figure 2, the mean (±SE) number of large foci in
the control mice was 2.2±0.68 vs. 0.4±0.24, 0.0±0.0 and
0.8±0.37 for 10% soy, 10% garbanzo and 10% mixed flour,
respectively. These results demonstrate that garbanzo bean
flour, soy flour, or mixed flour effectively reduced the
number of large foci (≥4 crypts/focus) per colon by 100, 81
and 64%, respectively. These results strongly support that
consumption of legumes, especially garbanzo beans,
provides protection against colon cancer.

Discussion

Garbanzo beans or chickpeas (Cicer arietinum) grow on a
plant native to the Middle East and are popular throughout
India, North Africa, Spain, southern France and Latin
America. Their production is roughly three times that of the
lentil, and world consumption is second only to dry
beans among the pulse crops. Garbanzo beans are an
excellent source of cholesterol-lowering fiber. In addition,
garbanzo beans have been reported to decrease blood sugar
levels from rising too rapidly after a meal, making them an
especially good choice for individuals with diabetes, insulin
resistance or hypoglycemia (1).

Despite the reported health benefits of garbanzo beans,
the cancer chemoprotective effects of this legume have not
been well studied. McIntosh et al. (38) investigated the
chemoprotective effects of extruded garbanzo beans/wheat
using a dimethylnitrosamine (DMN)-induced colon tumor rat
model (38). While garbanzo beans significantly lowered the
concentration of circulating plasma cholesterol, they failed to
lower the incidence of large intestine tumors. One
limitation with this study was that the garbanzo beans were
extruded and, as a result, it is possible that a lack of positive
findings may be in part due to a loss of the heat-sensitive
anticancer agents (e.g., protease inhibitors) (39). For the
current study, garbanzo bean flour was administered with
processing to avoid loss of crucial anticancer agents.

Soybeans have been extensively studied and have been
considered to be protective against colon cancer. The results
are often supported by epidemiological studies on
immigrants. Specifically, people consuming diets rich in soy
often have protective effects against cancer as compared to
the people migrating to countries such as the United States
where the soy consumption is limited. While the protective
role of soy has been attributed in part to its high
phytochemical content (e.g., genistein, diadzein or Bowman
Birk inhibitor (BBI) protein), studies with the isolated
phytochemicals, particularly genistein, have met with mixed
results. For example, whereas Steele et al. (12) found
protective effects of genistein against AOM-induced ACF
in F344 rats, Rao et al. (16) observed enhancing effects of
genistein under similar experimental conditions. Furthermore,
soy protein isolate depleted of isoflavones was
found to be more effective than soy protein isolate
containing isoflavones against mammary tumors in rats (40).
Based on these reports, it appears that studies on whole
legumes rather than the isolated phytochemicals may be
more significant for the prevention of cancer. This may be
because, in addition to the benefits of the non-nutritive
components, legumes contain several important agents such
as vegetable proteins, complex carbohydrates, dietary fiber,
vitamins and minerals, which also contribute to their health
promoting benefits (41).

Considering that the consumption of garbanzo beans has
been increasing in the United States, we found it timely to
investigate the efficacy of garbanzo flour in colon cancer
and compare it to that of soy, the most widely studied
legume. In the current report, no single or specific factor
was identified to account for the anticancer activity of
garbanzo and/or soybeans. Since garbanzo flour contains
biochanin A, which can be metabolized to genistein, it is
theoretically possible that the effects of garbanzo flour
could be due to genistein (42). However, it is unlikely that
the estimated 1.78 mg/kg of biochanin A in garbanzo flour
would be able to provide the effectiveness of 96-mg/kg
equivalent of genistein found in soy flour (43). An
alternative explanation is that, as shown in our previous
report, the anticancer effects of the beans could be
independent of genistein (40). Garbanzo beans contain
several other agents such as BBI and saponins that have
been shown to have chemopreventive activity in colon
cancer (22, 35, 45). Kennedy et al. (44) investigated the
effects of BBI on DMN-induced colon carcinogenesis and
found a significant reduction in incidence and multiplicity
in BBI-treated animals as compared to control animals.
Similarly, saponins have been shown to significantly reduce
the incidence of ACF in rats treated with chemical
carcinogens (45). Thus, the present study indicates that
garbanzo beans are as effective as soy flour at inhibiting
colon cancer. Based on the results shown here it is unlikely
that the efficacy of garbanzo beans or soybeans can be
attributed to one sole phytonutrient, it is more likely to be
the effect of a composite of several chemopreventive
components found in these legumes. Our results justify the
undertaking of human studies to better understand the
chemopreventive efficacy of garbanzo beans in colon cancer.

Acknowledgements

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