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 that do not (3,4), data from experimental models on soy and soy isoflavones 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 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% of that of the casein-fed animals. In addition, in rats that did develop tumors, those consuming the beans had reduced multiplicity. Treatment groups had 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, which is nearly double 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 suggest 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 may be 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 (15), saponin (16) and phenethyl isothiocyanate (16).

**Table I. 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>
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<td>3820.0</td>
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<td>609.0</td>
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<td>47.0</td>
<td>123.00</td>
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<tr>
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<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
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<td>157.5</td>
<td>--</td>
<td>--</td>
<td>17.5</td>
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<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<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>
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<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>
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</tr>
<tr>
<td>100 g Soy flour</td>
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<td>37.0</td>
<td>37.0</td>
<td>20.0</td>
<td>17.0</td>
<td>71.20</td>
<td>96.80</td>
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<td>60.0</td>
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<td>181.20</td>
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<tr>
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<td>3438.0</td>
<td>225.0</td>
<td>549.0</td>
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<td>42.0</td>
<td>110.00</td>
<td>119.00</td>
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</tr>
<tr>
<td>100 g Garbanzo flour</td>
<td>383.0</td>
<td>20.0</td>
<td>60.0</td>
<td>7.0</td>
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<td>110.00</td>
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<td>191.5</td>
<td>10.0</td>
<td>30.0</td>
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<td>8.0</td>
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<td>238.0</td>
<td>19.0</td>
<td>18.0</td>
<td>10.0</td>
<td>8.0</td>
<td>35.60</td>
<td>48.40</td>
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</tr>
<tr>
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<td>--</td>
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<td>--</td>
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<tr>
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<td>262.0</td>
<td>597.0</td>
<td>60.0</td>
<td>58.0</td>
<td>145.62</td>
<td>167.43</td>
<td>0.89</td>
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*aSource: Harlan Teklad, Madison, WI, USA.

*b(25). USDA-IOWA State University Database on the Isoflavone Content of Foods.*
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 River (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 (Harland-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), 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 I. 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.** 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 after the initiation of the study, the animals were sacrificed by carbon dioxide asphyxiation. The colons were evaluated for ACF by the procedure previously described (29). 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, the features of aberrant crypt foci in AOM-treated colons of CF-1 mice stained with methylene blue are shown.
ACF were distinguished from surrounding non-aberrant crypts by their increased size, elongated luminal opening, increased distance from the luminal to basal surface of cells, thickened epithelial cell lining and enlarged pericryptal area 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/cm\(^2\) 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%.
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 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 dimethylhydrazine (DMH)-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 no 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 then 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 DMH-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 but 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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References


