

Chemopreventive effect of raw and cooked lentils (*Lens culinaris* L) and soybeans (*Glycine max*) against azoxymethane-induced aberrant crypt foci

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Abstract

Although lentils (*Lens culinaris* L) contain several bioactive compounds that have been linked to the prevention of cancer, the *in vivo* chemopreventive ability of lentils against chemically induced colorectal cancer has not been examined. Our present study examined the hypothesis that lentils could suppress the early carcinogenesis *in vivo* by virtue of their bioactive micro- and macroconstituents and that culinary thermal treatment could affect their chemopreventive potential. To accomplish this goal, we used raw whole lentils (RWL), raw split lentils (RSL), cooked whole lentils (CWL), and cooked split lentils (CSL). Raw soybeans (RSB; *Glycine max*) were used for the purpose of comparison with a well-studied chemopreventive agent. Sixty weanling Fischer 344 male rats, 4 to 5 weeks of age, were randomly assigned to 6 groups (10 rats/group): the control group (C) received AIN-93G diet, and treatment leguminous groups of RWL, CWL, RSL, CSL, and RSB received the treatment diets containing AIN-93G+5% of the above-mentioned legumes. After acclimatization for 1 week (at 5th to 6th week of age), all animals were put on the control and treatment diets separately for 5 weeks (from 6th to 11th week of age). At the end of the 5th week of feeding (end of 11th week of age), all rats received 2 subcutaneous injections of azoxymethane carcinogen at 15 mg/kg rat body weight per dose once a week for 2 consecutive weeks. After 17 weeks of the last azoxymethane injection (from 12th to 29th week of age), all rats were euthanized. Chemopreventive ability was assessed using colonic aberrant crypt foci and activity of hepatic glutathione-S-transferases. Significant reductions ($P < .05$) were found in total aberrant crypt foci number (mean \pm SEM) for RSB (27.33 ± 4.32), CWL (33.44 ± 4.56), and RSL (37.00 ± 6.02) in comparison with the C group (58.33 ± 8.46). Hepatic glutathione-S-transferases activities increased significantly ($P < .05$) in rats fed all treatment diets (from 51.38 ± 3.66 to $67.94 \pm 2.01 \mu\text{mol mg}^{-1} \text{min}^{-1}$) when compared with control (C) diet ($26.13 \pm 1.01 \mu\text{mol mg}^{-1} \text{min}^{-1}$). Our findings indicate that consumption of lentils might be protective against colon carcinogenesis and that hydrothermal treatment resulted in an improvement in the chemopreventive potential for the whole lentils.

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Keywords:

Lentils (*Lens culinaris* L); Colorectal cancer; Fischer 344 rat; Aberrant crypt foci; Glutathione-S-transferases

Abbreviations:

ACF, aberrant crypt foci; ANOVA, analysis of variance; AOM, azoxymethane; CRC, colorectal cancer; CSL, cooked split lentils; CWL, cooked whole lentils; F344, Fischer 344; GST, glutathione-S-transferases; RSB, raw soybeans; RSL, raw split lentils; RWL, raw whole lentils.

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1. Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer-related deaths throughout the world [1]. More than 85% of persons with CRC have sporadic type [2]; this emphasizes the role of environmental factors, including eating practices, on the development of CRC and allows for describing CRC as a “diet-related cancer.”

Legumes and their bioactive compounds are among the foods with strong chemopreventive ability against several neoplasms, including CRC, as evidenced by epidemiological [3], in vivo [4], and in vitro [5] studies. Of the Leguminosae family, lentils (*Lens culinaris* L) are nowadays considered among the most important legumes for human nutrition [6]. Lentil seeds are rich sources of proteins [6] and bioactive peptides, including lectins [7], “defensin” protein [8], and Bowman-Birk trypsin inhibitors [9]; each of which aids in halting tumorigenesis and has gained special attention for its inhibitory effect against CRC [10,11].

Carbohydrates represent a major component of lentils [6], which include soluble and insoluble dietary fibers as well as prebiotic oligosaccharides [12]. Resistant starches represent a major fraction in lentil starches [13] and have been reported to inhibit colon carcinogenesis by different mechanisms [14]. In addition to macronutrients, lentils are an excellent source of folate [15], which was found to be involved in halting carcinogenesis [16]. Along with essential nutrients, lentils are good sources of many nonnutrient functional phytochemicals such as phytic acid and tannins [17], which are considered among the functional antioxidant ingredients [18–19]. Lentils are also good sources of the antioxidants catechins and procyanidins [20]. As a consequence of the presence of a wide spectrum of phenolic and nonphenolic antioxidants in lentils, they have shown the highest antioxidant and oxygen radical-absorbing capacities among different legumes [21].

Aberrant crypt foci (ACF) are among the most frequently used histopathological biomarkers for the experimental study of chemopreventive agents against CRCs at the early phases of carcinogenesis [22]. Aberrant crypt foci were first identified topographically in methylene blue-stained whole-mount preparations of colonic mucosa in rodents exposed to colorectal carcinogens and have long been regarded as precarcinogenic lesions [23].

One of the proposed mechanisms for protection against cancer by dietary constituents may include induction of glutathione-*S*-transferases (GST). These detoxifying enzymes have the ability to detoxify the oxidized metabolites of potentially carcinogenic xenobiotics into forms that are relatively inert and more easily excreted. Thus, they are involved in preventing the initiation of carcinogenesis [24].

Because lentils constitute variable types of potentially bioactive micro- and macroconstituents and chemopreventive agents, our hypothesis for this study is that lentils may suppress colon carcinogenesis in an animal model. Hence, this study was designed to meet 2 objectives: the first was to examine the chemopreventive effect of lentils against early

colon carcinogenesis induced by azoxymethane (AOM) in Fischer 344 (F344) rat model by using histopathological (ACF) and biochemical (GST) biomarkers. Because lentils, like other legumes, are customarily eaten after culinary heat treatment, the second objective of the study was to evaluate the impact of hydrothermal treatment of lentils on the chemopreventive ability of that leguminous food.

2. Methods and materials

2.1. Animals and diets

This research was approved by Department of Nutrition and Food Technology Committee for Animal Experimentation/University of Jordan. Sixty 4- to 5-week-old male weanling-specific pathogen-free F344/NHsd inbred rats were obtained from Harlan (Indianapolis, Ind) through Product Safety Research Laboratories, Jordan. The animals were acclimatized for 1 week in the Animal Unit, Department of Nutrition and Food Technology, University of Jordan. During this week, animals were fed a standard laboratory chow that provided all the requirements of nutrients by the growing rat. Rats were randomly assigned to 6 groups. Ten animals were assigned for each of the 6 experimental groups: the control group (C) was fed AIN-93G diet [25]; the groups receiving legumes were assigned to AIN-93G+5% raw whole lentils (RWL), AIN-93G+5% cooked whole lentils (CWL), AIN-93G+5% raw split lentils (RSL), AIN-93G+5% cooked split lentils (CSL), and AIN-93G+5% raw soybeans (RSB). The latter diet group was used for the purpose of comparison with a well-studied chemopreventive leguminous food. Each animal was housed in a single plastic cage (North Kent Plastic Cages, Ltd, Dartford, UK). Powdered diet, provided in feeding cups, and water were available ad libitum. Artificial light was supplied in a 12:12-hour dark/light cycles, extending from 6:00 PM to 6:00 AM. Relative humidity was maintained at 55% ± 5%, and temperature was controlled and maintained at 22°C ± 2°C. Room conditions and health signs of animals were monitored daily.

2.2. Preparation of experimental diets

Whole and split lentils (*L. culinaris* L) as well as soybeans (*Glycine max*) used in the current research were purchased from the local market. Grains of lentils (whole and split) and soybean were processed in the Laboratory of Meal Preparation, Department of Nutrition and Food Technology/University of Jordan, according to the following sequence: 20 kg from each of the 2 lentil forms (raw whole and raw split) were cleaned by handpicking from broken stones and other foreign materials. Ten kilograms of cleansed RWL was boiled in an excess of tap water (1:5, wt/vol) for 1 to 2 hours using 20-L volume stainless-steel kitchen bowls over direct flame and boiled at 96°C until they became soft when pinched between the fingers. Ten kilograms of cleansed RSL was cooked in the same manner for less than 1 hour until they

became a thick slurry. Cooked whole lentils and thick slurry of split lentils were immediately spread flat over clean aluminum trays to be cooled at room temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$). After cooling, cooked lentils were dried in an air-circulated drying oven (Blue M, Blue Islands, Ill) at 70°C for 16 hours. Ten kilograms of each of the 2 raw lentil forms (whole and split), 10 kg of each of the 2 dried cooked lentil forms (whole and split) (total of 40 kg), and 10 kg of RSB were then ground separately to pass through a 5-mm mesh (Retsch GmbH, Haan, Germany), kept in polyethylene bags, and stored in the refrigerator at 4°C until used for preparation of experimental diets. Experimental diets were prepared twice a week and preserved at 4°C . To obtain isocaloric and isonitrogenous diets, modifications in dietary compounds were made for lentils and soybean diets according to the United States Department of Agriculture Nutrient Database for Standard Reference (SR21) [15]. Ingredients of the diets used in the animal experiment are shown in Table 1.

2.3. Study design and carcinogen administration

All of the 6 experimental groups were fed the experimental diets for 5 weeks before carcinogen administration, during the week of carcinogen injections, and 17 weeks after last carcinogen injection. All animals randomly received 2 doses of subcutaneous injections of AOM in normal saline (0.9% NaCl) of 15 mg/kg body weight at the end of the fifth and sixth weeks of feeding of the experimental diets between 9:00 and 11:00 AM. After 17 weeks next to the last injection, all animals were euthanized with chloroform (trichloromethane, CHCl_3) (Fig. 1). Rats underwent careful necropsy. At necropsy, the liver was removed and preserved at -80°C to be further analyzed for GST enzymes. Colons were

harvested separately for later ACF counting and preserved in 70% ethanol solution.

2.4. Aberrant crypt foci assessment

Colons were cut open longitudinally from cecum to anus along the main axis, flushed and washed with normal saline, placed between 2 longitudinal pieces of filter paper with their luminal surface opened, exposed, and fixed in 70% ethanol solution. Colons were then dissected into 2 to 3 portions of equal lengths and used for counting ACF. As described by Bird [23], the portions were stained with 0.2% methylene blue for 30 to 60 seconds to observe ACF, then through 70% ethanol solution to wash off excess stain. Colon segments were examined using a light compound microscope (MEIJI, Japan) at low-power magnification ($\times 40$) to score the total number of ACF as well as the number of crypts per focus. The ACF were counted only when they fulfilled the criteria described by McLellan and Bird [26].

2.5. Liver GST activity assay

Glutathione-S-transferases activity assay was carried out according to the procedure of Habig et al [27]. Livers frozen at -80°C were taken out of the deep freezer. Each solid liver was taken separately and pulverized while frozen, and 3 mL of potassium phosphate-buffered saline (pH 7.0-7.2) was added for each 1 g of the liver weight. Tissue-Buffer mixture was then homogenized using an Ultra-Turrax homogenizer while the beaker of the homogenate was submerged in an ice box (0°C). Homogenates were centrifuged for 30 minutes at $15\,000 \times g$ using temperature-controlled Eppendorf centrifuge at 4°C . Clear supernatant fluid was taken and diluted with phosphate-buffered saline by 1:10.

Table 1
Composition of the diets fed to F344 rats for 23 weeks

Ingredient (g/kg diet) ^b	Dietary treatments					
	C ^a	C+5%RWL	C+5%CWL	C+5%RSL	C+5%CSL	C+5%RSB
Lentils ^c	0	52	50	52	50	0
Soybean ^c	0	0	0	0	0	52
Cornstarch	397.5	380.5	380.0	365.5	365.5	387.0
Sucrose	100	99.0	97.0	99.0	99	96.3
Fiber ^d (α -cellulose)	50	34.6	37.6	49.6	49.6	47.0
Soybean oil ^e	70	69.4	69.4	69.4	69.5	59.6
Casein ^f	200	184.0	183.5	183.9	183.9	177.6
Other common ingredients ^g	182.5	182.5	182.5	182.5	182.5	182.5
Total	1000	1000	1000	1000	1000	1000

^a Control diet is based on the American Institute of Nutrition purified diet for experimental animals (Reeves [25]).

^b Dietary modifications for experimental diets of lentils and soybeans were made according to United States Department of Agriculture Nutrient Database (SR21): RWL (25.8% protein, 1.06% total lipids, 60.08% total carbohydrates, 30.5% total dietary fibers, 2.03% total sugars); raw pink (split) lentils (24.95% protein, 2.17% total lipids, 59.15% total carbohydrates, 10.8% total dietary fibers); raw mature soybeans (36.49% protein, 19.94% total lipids, 30.16% total carbohydrates, 9.3% total dietary fibers, 7.33% total sugars).

^c Additional 2 g (total of 52 g) of raw lentils and soybeans were added to correct for differences in moisture content between raw and dried cooked lentils.

^d α -Cellulose from Sigma Chemical Co.

^e Crude refined soybean oil without added vitamins.

^f Casein from Sigma Chemical Co. Assuming that casein is 85% or greater protein (200 g casein provides approximately 170 g protein).

^g Dextrinized cornstarch from Sigma Chemical Co, 132 g; mineral mixture (AIN-93G-Mix), 35 g; vitamin mixture (AIN-93G-Mix), 10 g; DL-methionine from Sigma Chemical Co, 3.0 g; choline bitartrate from Sigma Chemical Co, 2.5 g; tertiary butylhydroquinone (TBHQ), 0.014 g.

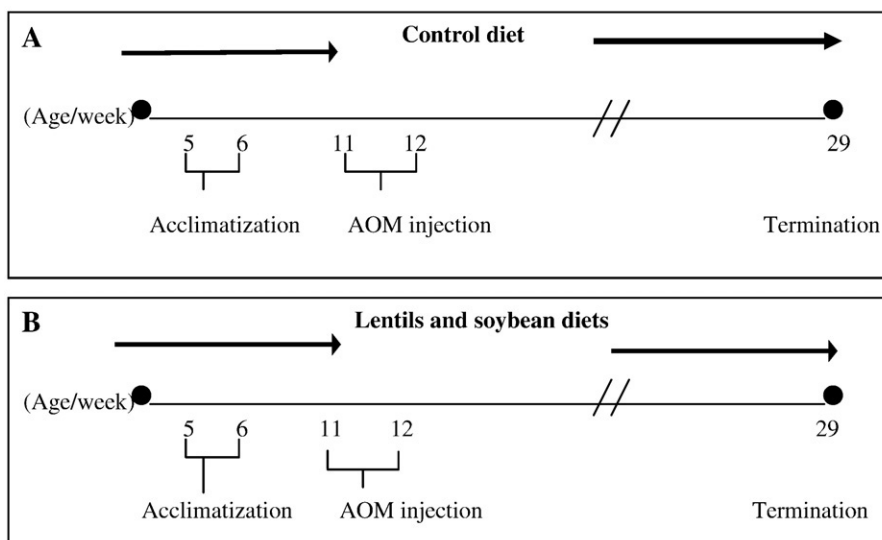


Fig. 1. Experimental design of the study. Male F344 rats at 4th to 5th week of age were obtained, acclimatized for 1 week (at 5th to 6th week of age), then all animals were put on the control and treatment diets separately for 5 weeks (from end of 6th to 11th week of age). At the end of the 5th week of feeding (end of 11th week of age), all rats received 2 subcutaneous injections of AOM carcinogen at 15 mg/kg rat body weight per dose once a week for 2 consecutive weeks. After 17 weeks of the last AOM injection, all rats were euthanized (from 12th to 29th week of age).

Glutathione-*S*-transferases activity was assayed using GST assay kit (Sigma Chemical Co, Catalog Number CS0410), in which GST catalyzes the conjugation of L-glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CNDB) through the thiol group of the glutathione. The absorption of the reaction product, GS-DNB conjugate, was measured at 340 nm in 1-cm light path quartz cuvette, using Dual Beam Spectrophotometer (SpectroScan 80 D), for 5 to 6 times with 1 minute apart.

2.6. Statistical analyses

All data of ACF counting, liver enzyme activity assay, food intake, and rat body weights were analyzed by one-way analysis of variance (ANOVA; SAS, 1998, Version 7 for windows analysis package). Means were separated using Fischer protected least significant difference test. Differences were considered significant at $P < .05$, and data were presented as means \pm SEM.

3. Results

3.1. Food intake and body weights of experimental animals

Food intakes by rats fed different lentils and soybean diets were similar, and no significant differences were found when compared with the control rats (C) (Table 2). However, it was noticed throughout the experiment that food consumption declined substantially upon and after carcinogen (AOM) administration at the start of the sixth and seventh weeks of the experiment. In addition, no significant differences were found in the initial and final body weights and weight gains for the animals fed different lentils and soybean diets

throughout the 23-week study when compared with animals of the control diet (Table 2).

3.2. Aberrant crypt foci analysis

The number of small foci (foci with 1-3 aberrant crypts, AC) was not significantly different in the colons of animals fed treatment diets when compared with the control group (C). All rats fed on treatment diets exhibited a significantly ($P < .0001$) lower numbers of the large foci (≥ 4 AC) when compared with those on the control diet (C). As shown in Table 3, the colons of animals fed C+5%CWL exhibited significantly ($P < .0001$) lower numbers of the large foci than those of the animals fed C and C+5%RWL diets. However, they did not exhibit a significantly different number from those of the animals fed C+5%RSB, C+5%CSL, and C+5%RSL diets. When expressed as a percent from total ACF, the CWL group had the least value of multicrypt foci (7.9%)

Table 2

Food intake and weight gain for rats during the 23-week experimental period

Diet group (no. of rats)	Food intake (g/rat per day)	Weight gain (g)
C (10)	14.90 \pm 1.44 ^A	286.19 \pm 27.16 ^A
C+5%RWL (10)	16.38 \pm 1.28 ^A	299.97 \pm 18.46 ^A
C+5%CWL (10)	16.56 \pm 1.39 ^A	288.82 \pm 24.91 ^A
C+5%RSL (10)	15.82 \pm 1.34 ^A	275.72 \pm 13.94 ^A
C+5%CSL (9)	16.31 \pm 1.35 ^A	296.5 \pm 17.50 ^A
C+5%RSB (10)	16.76 \pm 1.27 ^A	280.6 \pm 10.54 ^A

Values are means \pm SEM. Within a column, values without a common letter differ significantly (ANOVA, $P < .05$) using Fischer protected least significant differences test.

Table 3
Aberrant crypt foci incidence and types in colons of rats fed diets containing lentils and soybean

Diet group (no. of rats)	Incidence of ACF (%)	1 AC	2 AC	3 AC	≥4 AC	Total AC/Colon (%)	Total ACF/Colon (%)	Crypt multiplicity (%)
C (10)	10/10 (100)	18.33 ± 3.6 ^A	20.33 ± 2.9 ^A	7.67 ± 1.76 ^{BC}	12.0 ± 1.04 ^A	177.89 ± 23.67 ^A (100)	58.33 ± 8.46 ^A (100)	3.40 ± 0.47 ^A (100)
C+5%RWL (10)	10/10 (100)	12.11 ± 2.5 ^A	20.89 ± 3.47 ^A	11.22 ± 1.3 ^{AB}	8.78 ± 1.97 ^B	128.78 ± 9.99 ^{AB} (72.4)	53.00 ± 18.2 ^{AB} (90.9)	2.52 ± 0.12 ^B (74.1)
C+5%CWL (10)	10/10 (100)	10.22 ± 2.06 ^A	15.00 ± 2.62 ^A	5.56 ± 0.79 ^C	2.66 ± 0.09 ^C	70.44 ± 8.36 ^B (39.6)	33.44 ± 4.56 ^C (57.3)	2.16 ± 0.10 ^B (63.5)
C+5%RSL (10)	10/10 (100)	8.22 ± 1.92 ^A	15.89 ± 2.55 ^A	6.56 ± 1.37 ^C	6.33 ± 1.06 ^{BC}	89.11 ± 15.06 ^{CD} (50.1)	37.00 ± 6.09 ^{BC} (63.4)	2.37 ± 0.09 ^B (69.7)
C+5%CSL (9)	9/9 (100)	13.11 ± 3.88 ^A	16.22 ± 3.47 ^A	7.33 ± 1.72 ^{BC}	5.56 ± 1.05 ^{BC}	94.44 ± 17.25 ^{BCD} (53.1)	42.22 ± 8.7 ^{ABC} (72.4)	2.33 ± 0.11 ^B (68.5)
C+5%RSB (10)	10/10 (100)	6.44 ± 1.45 ^A	12.11 ± 1.93 ^A	4.78 ± 1.12 ^C	4.00 ± 1.04 ^{BC}	63.00 ± 9.88 ^D (35.4)	27.33 ± 4.32 ^C (46.9)	2.25 ± 0.10 ^B (66.2)

Values are means ± SEM. Within a column, values without a common letter differ significantly (ANOVA, $P < .05$) using Fischer protected least significant differences test.

among all of the treatments groups (range, 13.2%–17.1%) and was substantially lower than the control group (20.6%) (Table 3).

As presented in Table 3, the total number of AC was reduced significantly ($P < .0001$) in all dietary treatments when compared with the control (C) group. However, the colons of the rats fed C+5%CWL showed a statistically significant reduction in total AC number when compared with C and C+5%RWL ($P < .0001$), whereas no such significant reductions were observed when compared with colons of the rats fed other diets. When expressed as percent of reduction from the reference control group (C) (considered as 100%), feeding rats with C+5%RSB and C+5%CWL resulted in 64.6% and 60.4% reductions, respectively, whereas other treatments exhibited lower percent reductions.

Regarding total ACF, the pattern of difference from the control group did not run in parallel with that of total AC, and only the colons of the rats fed C+5%CWL, C+5%RSL, and C+5%RSB diets exhibited a significant reductions in total ACF number ($P = .0073$) when compared with the control (C) group, with percent reductions for these three groups range from 36.6% to 53.1%.

Results of crypt multiplicity (CM) are shown in Table 3. As expected, the colons of the control animals without any chemoprotective agent exhibited significantly the highest CM value ($P < .0024$). Colons of the treatment groups did not exhibit significant differences among their CM values; even those for animals fed C+5%CWL were apparently different and lower than those of other treatments, and percent reduction in CM value for animals fed C+5%CWL was higher than other treatments (Table 3). It is apparent that the lowest value in CM reduction tended to be for RWL.

3.3. Glutathione-S-transferases activity

Compared with the control group, hepatic cytosolic GST enzymes for liver of all animals fed on lentils and soybean diets exhibited significantly higher activities at $P < .0001$ (Fig. 2), with the livers of animals fed C+5%CWL exhibiting the highest activity value. Generally, all tested legume diets resulted in an approximately 2-fold increase in GST activity.

The relatively high hepatic GST activity for the animals fed CWL, as well as soybean diets, was parallel to or concomitant with the relatively high values of percent reductions in the total ACF, total AC, and crypt multiplicity. Furthermore, the lack of significant differences between activities for GST enzymes in animals of different lentils is in accordance with the lack of such significant differences in mean values for crypt multiplicity.

4. Discussion

Many studies have primarily focused on the potential benefits of the individual phytochemical compounds and their roles in early and late carcinogenesis prevention [28]. The cumulative synergistic effect of antioxidant and

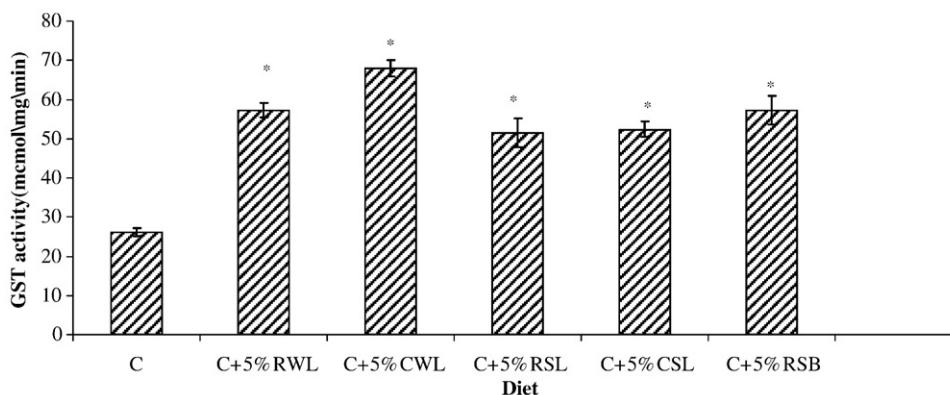


Fig. 2. Hepatic GST activity for rats fed control, lentils, and soybean diets. The data shown are means \pm SEM. *Significant difference from the control group at $P < .05$.

nonantioxidant chemopreventive phytochemicals was found to be higher when investigating the whole food material rather than a single phytochemical [29]. Because lentils are a prime source for many antioxidant phytochemicals [30], they are now looked at as a promising functional food. As thermal treatments have been shown to affect the antioxidant capacity of treated pulses [21], the study tried to elucidate the effect of thermal culinary treatments on the previously mentioned potential chemopreventive effect of lentils.

Rats fed on lentils and soybean diets had significantly ($P < .05$) fewer colonic total AC and CM when compared with the control group, indicating that all leguminous treatments may have suppressing activity against early carcinogenesis. Suppressing agents against tumorigenesis have been found to exert their effect by several mechanisms, including disrupting the cell cycle, inducing apoptosis, modulating hormonal activity, modulating nuclear receptors, and suppressing gene expression by DNA methylation [31].

In this study, rats fed different lentil and soybean diets had 26.8% to 77.8% fewer number of large ACF (≥ 4 AC) as compared with rats fed the control diet, indicating a substantial chemopreventive ability. For lentil diets, CWL had a striking 77.8% reduction in large ACF, whereas the reduction was only 26.8% in rats fed RWL.

The reduction in the high multiplicity ACF (≥ 4 AC) had been found to be associated with lowered expression of inducible nitric oxide synthase and cyclooxygenase-2 enzymes [32] and was found to be associated with suppression of proliferation, production of prostaglandin E2 and cyclin D1 protein expression, up-regulation of apoptosis, and reduction in proliferation cell nuclear antigen [33]. Therefore, further research is required to determine the molecular changes associated with suppression of the precarcinous lesions shown in this study.

Because lentils were given before the initiation phase of carcinogenesis, the effect of lentils could be a “blocking” effect on AOM-induced ACF formation, and the suggested mechanisms of action would be directed toward the role of lentils and their compounds in modulating the metabolism of

the carcinogen. According to Chen and Kong [31], the blocking agents that inhibit colon carcinogenesis could exert their preventive effect by several mechanisms, including enhancement of detoxification of carcinogens, inhibition of cytochrome P450-mediated activation of carcinogens, scavenging free radicals and halting oxidant activity, and finally trapping the carcinogens and preventing their interaction with DNA.

Legumes contain several phenolic compounds, in addition to glutathione, soluble proteins, and tocopherols, which are considered to be natural antioxidants [34]. Among them, these nonphenolic antioxidant compounds may exert a synergistic effect, together with phenolic ones, which could be the origin of the differences in the antioxidant activity of different leguminous seeds. Consequently, total antioxidant measurements for lentil seeds were reported to be significantly ($P < .05$) higher than those of other legumes by using different antioxidant capacity indicators [21].

Legumes, including lentils and beans, are significant sources of resistant starches [13], which have been reported to be fermented by colonic bacteria to short chain fatty acids, and thus for improving the colonic health [14]. Inhibition of early and late carcinogenesis by resistant starches had been shown by several in vivo [35] and in vitro [36] studies.

Azoxymethane is a hydrophilic carcinogen and is poorly adsorbed by fiber [37]. In addition, fiber content of all experimental diets is nearly similar; thus, the effect of fiber in preventing early carcinogenesis is negligible, and if present, it may not be directed toward the effect on AOM metabolism and inactivation but rather to other possible mechanisms on colonic epithelial tissue.

Bowman-Birk trypsin inhibitors are present at higher concentrations in cotyledons of lentils as compared with other plant families and tissues [9]. Bowman-Birk trypsin inhibitors have been considered among the novel functional food ingredients. The biochemical and functional properties of Bowman-Birk trypsin inhibitors have been critically reviewed by Losso [9], with particular attention given toward the ability to prevent CRC [11].

In lentils, the slight difference in ACF inhibitory effect between whole and split lentils may be ascribed principally to the difference in polyphenolic compounds, which are aggregated mainly in the seed coats of whole lentils [38]. Furthermore, the evidenced inhibitory effect of split lentils indicates that antioxidant polyphenolics of lentils seed coats are not the only responsible factors for the inhibitory effect against early carcinogenesis. Such a finding has been reported by Lala et al [39], who found that although raspberry and bilberry diets had the lowest antioxidant capacity value among different tested diets, they were the most effective in inhibiting large ACF development and cell proliferation.

Our results show that CWL imposed a better inhibition pattern against ACF formation than RWL. This desirable effect of thermal treatment could be explained by the following 3 factors: first, phenolics can still be present in cooked seeds at a level comparable to that of raw seeds; second, approximately 50% of total phenolic compounds might be bound to insoluble fraction of other compounds possibly formed during cooking of seeds; third, the lipophilic phenolic compounds, which may be bound to the cell walls, are released during cooking [34]. In fact, significant increases in the total phenolic content and antioxidant capacity were detected in the cooked lentil seeds as compared with the raw ones [34].

In this study, significant increases ($P < .05$) in the GST activities in rats fed legume (lentils and soybean) diets were seen as compared with those of a control diet. The GST value for the control group is close to the corresponding value reported by Khatiwada et al [40]. It should be noted that the significant ($P < .05$) increase in the hepatic GST levels was concomitant with a corresponding significant reduction in total ACF number only for CWL, RSL, and RSB diet groups, but not for the other treatments. This could lead to a speculation that the other 2 diets (RWL and CSL) may have affected other drug-metabolizing enzymes of the cytochrome P450 group that could counteract the inhibitory effect of the detoxifying enzyme GST.

Antioxidant polyphenolics are among the monofunctional inducers that increase the activity of xenobiotic detoxifying enzymes only. The finding that hepatic levels of antioxidant enzymes such as GST were significantly greater in rats fed chemopreventive diets than in rats fed the control diet implies an increased antioxidant capacity triggered by chemopreventive agents to protect from oxidative stress [41]. The induced GST in the livers of rats fed split lentils could be ascribed partly to the presence of appreciated quantities of phytic acid in the cotyledons. Phytic acid has been reported to be implicated in elevating hepatic GST levels [40].

However, because of the following limitations in our current study, further investigation is necessary. First, the chemopreventive ability of lentils was studied in the early stages of carcinogenesis using precarcinous lesions (ACF), and this may not be sufficient to fully support our hypothesis.

Studying the chemopreventive ability against late carcinogenesis may allow for a more clearly chemopreventive effect of lentils, which requires more than 30 weeks of study period. This is, however, the first in vivo study on the chemopreventive effects of lentils against CRC induced in an animal model of human sporadic CRC, and as such, it provides preliminary findings. Second, the use of GST as a sole biomarker for interpreting ACF inhibition is not sufficient to clarify the full mechanisms of chemoprevention. Other biomarkers, such as molecular and genetic biomarkers, rather than the currently used biochemical one, are needed to fully elucidate the full chemopreventive mechanisms. Inducible nitric oxide synthase, cyclooxygenase-2 enzymes, production of prostaglandin E2, cyclin D1 protein expression, regulation of apoptosis, and proliferation cell nuclear antigen and other molecular biomarkers may be helpful in this context.

In conclusion, lentils, particularly the CWL, and soybeans had a chemopreventive effect against early carcinogenesis. This implies that lentils and soybean could play a role in preventing late colorectal carcinogenesis and that thermal treatment for whole lentils had an effect in improving their chemopreventive ability. Although the full mechanisms involved in the protective effects against cancer initiation by lentils were not clearly examined, the inhibitory action of lentils could be explained in part by the induction of the xenobiotic detoxifying enzymes (GST), by their putative antioxidant activity, and by the presence of other chemopreventive agents rather than the antioxidant phytochemicals, as was the case in the rats fed the split lentils.

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References

- [1] Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide. IARC Cancer Base No. 5. Version 2.0. Lyon, France: IARC Press; 2004. Available: <http://www.dep.iarc.fr>. Accessed on 12/16/2008.
- [2] Lynch HT, Watson P, Smyrk TC, Lanspa SJ, Boman BM, Boland CR, et al. Colon cancer genetics. *Cancer* 1992;70:1300-12.
- [3] Kushi LH, Meyer KA, Jacobs DR. Cereals, legumes, and chronic disease risk reduction: evidence from epidemiologic studies. *Am J Clin Nutr* 1999;70(Suppl):451S-8S.
- [4] Bobe G, Barrett KG, Mentor-Marce RA, Saffiotti U, Young MR, Colburn NH, et al. Dietary cooked navy beans and their fractions attenuate colon carcinogenesis in azoxymethane-induced *Ob/Ob* mice. *Nutr Cancer* 2008;60(3):373-81.
- [5] Steele VE, Periera MA, Sigman CC, Kelloff GJ. Cancer chemoprevention agent development strategies for genistein. *J Nutr* 1995;125:713S-6S.

- [6] Iqbal A, Khalil A, Ateeq N, Khan MS. Nutritional quality of important food legumes. *Food Chem* 2006;97:331-5.
- [7] Cuadrado C, Hajos G, Burbano C, Pedrosa MM, Ayet G, Muzquiz M, et al. Effect of natural fermentation on the lectin of lentils measured by immunological methods. *Food Agric Immunol* 2002;14:41-4.
- [8] Finkina KI, Shramova EI, Tagaev AA, Ovchinnikova TV. A novel defensin from the lentil (*Lens culinaris*) seeds. *Biochem Biophys Res Commun* 2008;371:860-5.
- [9] Losso JN. The biochemical and functional food properties of the Bowman-Birk inhibitor. *Crit Rev Food Sci Nutr* 2008;4:94-118.
- [10] Mejia EGL, Prisecaru VI. Lectins as bioactive plant proteins: a potential in cancer treatment. *Critic Rev Food Sci Nutr* 2005;45:425-45.
- [11] Clemente A, Gee JM, Johnson IT, MacKenzie DA, Domoney C. Pea (*Pisum sativum*, L.) protease inhibitors from the Bowman-Birk class influence the growth of human colorectal adenocarcinoma HT29 cells in vitro. *J Agric Food Chem* 2005;53:8979-86.
- [12] Vidal-Valverde C, Frias J, Sierra I, Blazquez I, Lambein F, Kuo YH. New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *Eur Food Res Technol* 2002;215:472-7.
- [13] Bednar GE, Patil AR, Murray SM, Grieshop CM, Merchen NR, Fahey GCJ. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. *J Nutr* 2001;131:276-86.
- [14] Niba LL. Resistant starch: a potential functional food ingredient. *Nutr Food Sci* 2002;32(2):62-7.
- [15] United States Department of Agriculture (USDA). USDA National Nutrient Database for Standard Reference, Release 21. <http://www.ars.usda.gov/services/docs.htm> 2008 Accessed on 03/01/2009.
- [16] Milner JA, McDonald SS, Anderson DE, Greenwald P. Molecular targets for nutrients involved with cancer prevention. *Nutr Cancer* 2001;41:1-16.
- [17] Vidal-Valverde C, Frias J, Estrella I, Gorospe MJ, Ruiz R, Bacon J. Effect of processing on some antinutritional factors of lentils. *J Agric Food Chem* 1994;42:2291-5.
- [18] Scalbert A, Manach C, Morand C, Reme'sy C, Jime'nez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* 2005;45:287-306.
- [19] Vucenik I, Shamsuddin AM. Protection against cancer by dietary IP6 and inositol. *Nutr Cancer* 2006;55:109-25.
- [20] Auger C, Al-Awwadi N, Bornet A, Rouanet JM, Gasc F, Cros G, et al. Catechins and procyanidins in Mediterranean diet. *Food Res Int* 2004;37:233-45.
- [21] Xu BJ, Chang SKC. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chem* 2008;110:1-13.
- [22] Rafter J, Govers M, Martel P, Pannemans D, Pool-Zobel B, Rechkemmer G, et al. PASSCLAIM (process for the assessment of scientific support for claims on foods)—diet-related cancer. *Eur J Nutr* 2004;S 2(43):II/47-84.
- [23] Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett* 1987;37:147-51.
- [24] Sharma RA, Manson MM, Gescher A, Steward WP. Colorectal cancer chemoprevention: biochemical targets and clinical development of promising agents. *Eur J Cancer* 2001;37:12-22.
- [25] Reeves PG. Compounds of the AIN-93 diets as improvements in the AIN-76 diet. *J Nutr* 1997;127:838S-41S.
- [26] McLellan EA, Bird RP. Aberrant crypts: potential preneoplastic lesions in the murine colon. *Cancer Res* 1988;48:6187-92.
- [27] Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974;249(22):7130-9.
- [28] Tanaka T, Shimizu M, Kohno H, Yoshitani S, Tsukio Y, Murakami A, et al. Chemoprevention of azoxymethane-induced rat aberrant crypt foci by dietary zerumbone isolated from *Zingiber zerumbet*. *Life Sci* 2001;69:1935-45.
- [29] Boateng J, Verghese M, Walker LT, Shackelford LA, Chawan CB. Inhibitory effects of selected dry beans (*Phaseolus spp.*, L.) on azoxymethane-induced formation of aberrant crypt foci in Fisher 344 male rats. *Nutr Res* 2007;27:640-6.
- [30] Amarowicz R, Karamac M, Shahidi F. Antioxidant activity of phenolic fractions of lentils (*Lens culinaris*). *J Food Lipid* 2003;10(1):1-10.
- [31] Chen C, Kong ANT. Dietary cancer-chemopreventive compounds: from signaling and gene expression to pharmacological effects. *Trends Pharmacol Sci* 2005;26(6):318-26.
- [32] Kwon Y, Magnuson BA. Effect of azoxymethane and curcumin on transcriptional levels of cyclooxygenase-1 and -2 during initiation of colon carcinogenesis. *Scand J Gastroenterol* 2007;42:72-80.
- [33] Tanaka T, Kohno H, Murakami M, Shimada R, Kagami S, Sumida T, et al. Suppression of azoxymethane-induced colon carcinogenesis in male F344 rats by mandarin juices rich in β -cryptoxanthin and hesperidin. *Int J Cancer* 2000;88:146-50.
- [34] Fernandez-Orozco R, Zielinski H, Piskula MK. Contribution of low-molecular-weight antioxidants to the antioxidant capacity of raw and processed lentil seeds. *Nahrung/Food* 2003;47(5):291-9.
- [35] Liu R, Xu G. Effects of resistant starch on colonic preneoplastic aberrant crypt foci in rats. *Food Chem Toxicol* 2008;46:2672-9.
- [36] Fassler C, Gill CIR, Arrigoni E, Rowland I, Amad'o R. Fermentation of resistant starches: influence of in vitro models on colon carcinogenesis. *Nutr Cancer* 2007;58(1):85-92.
- [37] Davies MJ, Bowey EA, Adlercreutz H, Rowland IR, Rumsby PC. Effects of soy or rye supplementation of high-fat diets on colon tumour development in azoxymethane-treated rats. *Carcinogenesis* 1999;20(6):927-31.
- [38] Duenas M, Hernandez T, Estrella I. Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chem* 2006;9895-103.
- [39] Lala G, Malik M, Zhao C, He J, Kwon Y, Giusti MM, et al. Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. *Nutr Cancer* 2006;54(1):84-93.
- [40] Khatiwada J, Verghese M, Walker LT, Shackelford L, Chawan CB, Sunkara R. Combination of green tea, phytic acid, and inositol reduced the incidence of azoxymethane-induced colon tumors in Fisher 344 male rats. *LWT* 2006;39:1080-6.
- [41] Pool-Zobel B, Veeriah S, Bohmer FD. Modulation of xenobiotic metabolizing enzymes by anticarcinogens—focus on glutathione-S-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis. *Mutat Res* 2005;591:74-92.