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Antitumor and Cytotoxic Properties of Dry Beans (*Phaseolus* sp. L.): An *in vitro* and *in vivo* Model

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Abstract: The aim of this study was to examine antitumor effects of Kidney Beans (KB) on Azoxymethane (AOM) induced colon cancer and the cytotoxic effects of KB extracts on colon cancer cell lines (CaCO₂). For ACF study, 12 Fisher 344 male rats were fed AIN-93G control diet (C) (n = 6) and 20% KB (n = 6), for 13 week. In the EPTM two groups of rats (n = 14) were fed AIN-93G control diet (C) and 20% KB. All rats received two s/c injections of AOM at 7 and 8 week of age at the rate of 16 mg kg⁻¹ body weight in saline. At 17 week (ACF) and 45 week of age (End-point) all rats were killed by CO₂ asphyxiation. For lactate dehydrogenase (LDH) assay, cells were incubated (24 and 48 h) with selected concentrations (0.5-25 mg/100 mL) of KB extract. Total number of ACF was 158 and 72 for groups fed C and 20% KB. Tumor size (mm) and TBR for C and 20% KB were 6.50; 1.16 and 3.8; 1.44, respectively. LDH release (%) in CaCO₂ cells after 24 and 48 h incubation with KB extracts ranged from 13.8 to 62.8 and 23.5 to 84.1, respectively. Feeding KB significantly (p<0.05) reduced the incidence of AOM induced colon tumorigenesis and KB extracts demonstrated cytotoxic effects on colon cancer cell lines (CaCO₂).

Key words: Kidney beans, aberrant crypt foci, azoxymethane, phytochemicals

INTRODUCTION

Colon cancer is the second leading cause of cancer death for both genders in the US (American Cancer Society, 2007). Aside from genetic predispositions, the major contributor to colon cancer risk is diet (Ferguson *et al.*, 2004; Ferguson, 2005). It has been established that the principal health benefit from the frequent consumption of foods high in dietary fiber is in controlling or preventing metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Lee *et al.*, 2000; Reddy, 1999; Levi *et al.*, 2001; Peters *et al.*, 2003). In view of the results from these investigations, dietary fiber has since been among the most frequently investigated dietary factors in studies of the etiology of colorectal cancer (Kushi *et al.*, 1999).

For thousands of years, dry beans (*Phaseolus vulgaris* L.) have been a staple for millions around the world (Lee *et al.*, 2000; Perla *et al.*, 2003). They are a stable source of protein, (16-33%), vitamins (thiamine, riboflavin, niacin, vitamin B and folic acid), dietary fiber (14-19%), macro and micro minerals (Ca, Fe, Cu, Zn, P, K, Mg), they contain no cholesterol and are exceptionally high in complex carbohydrates (Reyes-Moreno and Paredes-López, 1993; Rehman *et al.*, 2001). Since half the legumes consumed worldwide are common dry beans, they represent the species of choice for the study of grain legume nutrition.

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Whereas dry beans (legumes) play a significant role in the diets of most developing countries, in contrast in western diets, beans are less consumed. In fact the daily per capita consumption of all bean products in Asia alone is 110 g compared to about 9 g in the US (Kahlon and Shao, 2004). This estimate is below that which would considerably lower the risk of colon cancer (Alabaster *et al.*, 1996). Although cereals, compared to legumes make up the bulk of diets composed of basic grains and thus supply more energy, legumes according to Welch *et al.* (2000), are more superior to cereals as sources of micronutrients.

While it is possible to presume that dietary fiber in its entirety may provide protection against colon cancer, it is imperative to note that the accompaniment of several dietary phytochemicals in foods high in dietary fiber may very well be involved in reducing colon cancer by other mechanisms other than those proposed for fiber (Ferguson, 2005). Hence, the therapeutic role of dry beans in preventing metabolic disorders may be associated with the presence of phenolic compounds (Oomah *et al.*, 2005, 2006). Polyphenols from dry beans such as flavonoids and phenolics (Hughes *et al.*, 1997) may possibly act as antioxidants, thus hindering the formation of free radicals that eventually lead to the deterioration of biological molecules.

The objective of this study was to determine the chemopreventive properties of Red kidney beans on Azoxymethane induced colon tumorigenesis in Fisher 344 (using a biomarker, Aberrant Crypt Foci (ACF) and End Point tumors) and the cytotoxic activity of red kidney bean extracts on a colon cancer cell line (CaCO₂).

MATERIALS AND METHODS

Preparation of Dry Beans

Dry beans were purchased from a local store and soaked overnight in distilled water. Beans were rinsed twice in distilled water, flash frozen in liquid nitrogen and freeze dried (Consol 24 Virtis; The Virtis Company, Gardiner, NY). Freeze dried beans were roasted in a microwave oven (Kenmore microwave oven, 1200 watts) for 8 min. The beans were ground to a fine powder in a food processor (Robot coupe, Blixer RSI, BS3) and stored in amber containers at 4°C until use.

Animal Housing and Treatment

All protocols involving rats were approved by the Institutional Animal Care and Use Committee of Alabama A and M University (2006). Animals were housed in stainless steel cages (two rats/cage) in temperature (21±1°C) and humidity (50%) controlled rooms. Daily light and dark cycles were kept at 12 h each. Animals were allowed access to food and water.

Fifty Fisher 344 male rats (Harlan, IN) were randomly assigned to 4 groups (Fig. 1). All rats were fed American Institute of Nutrition (AIN)-93G (C) based-diets (Reeves *et al.*, 1993a, b)

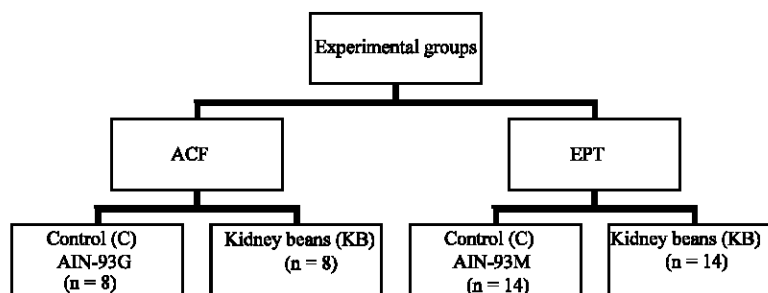


Fig. 1: Experimental design ACF- Aberrant crypt Foci, AIN-93G/M- American Institute of Nutrition Growth/Maintenance diet, EPT-End point tumor

Table 1: Composition of diets

| Ingredients (g) | AIN-93G/M ^a | AIN-93G+20% KB | AIN-93M+20% KB |
|---------------------------------|------------------------|----------------|----------------|
| Kidney beans | 0 | 200.00 | 200.00 |
| Cornstarch | 397.5/465.7 | 302.10 | 349.40 |
| Sucrose | 100/100 | 92.60 | 92.60 |
| Casein | 200/140 | 152.80 | 113.70 |
| Fiber | 50/50 | 0.00 | 0.00 |
| Soybean oil | 70/40 | 70.00 | 40.00 |
| Methionine | 3/3 | 3.00 | 3.00 |
| Common ingredients ^b | 182.5/204.3 | 182.50 | 204.30 |

^a: Formulations of diets based on AIN-93G (growth) and AIN-93M (maintenance) (Reeves *et al.*, 1993a, b); ^b: Common ingredients (g) (AIN-93G): dextrose, 132; mineral mix(AIN-93G), 35; vitamin mix, 10; L-cystein, 3; choline bitartrate, 2.5. (AIN-93M): dextrose, 155; mineral mix (AIN-93M), 35; vitamin mix, 10; L-cystein, 1.8; choline bitartrate, 2.5

(Table 1). Rats in groups 1 and 2 were fed these diets for 13 weeks (ACF experiment). In the End-point tumor study, groups 3 and 4, rats were initially fed AIN-93G (C) (n = 14) and AIN-93G+20% KB (n = 14) diets and were switched to AIN-93M at 20 weeks of age. Rats remained on AIN-93M diets until 46 week of age. Rats in a fifth group (n = 10) served as a saline vehicle. Weekly body weights and daily feed intakes were recorded. Animal feed was prepared biweekly and stored at 4°C for freshness. Ingredients for preparation of basal AIN-93 diets were obtained from ICN (Costa Mesa, CA).

AOM Injection and Sample Collection

ACF and colon tumors were induced by injecting rats with 2 injections of Azoxymethane (Sigma Chemicals, St. Louis, MO) s/c (16 mg kg⁻¹ body) for 2 consecutive weeks at 7 and 8 weeks of age. Rats in the ACF experiment were killed at 17 weeks of age and at 46 weeks of age for End point tumor model by CO₂ asphyxiation. Colons, liver and cecum were removed. Colons and livers were immediately rinsed in potassium phosphate buffer (0.1 M, pH 7.2) (Fisher Scientific, Suwannee, GA), colonic mucosal scrapings were flash frozen in liquid nitrogen and kept at -80°C until analysis. Cecum of rats was weighed and pH of cecal contents was noted.

Counting of Aberrant Crypt Foci (ACF)

Enumeration of Aberrant Crypt Foci (ACF) was performed as described by Bird (1995). Colons were slit open and the exposed luminal surface flushed with potassium phosphate buffer (0.1 M, pH 7.2) (Fisher Scientific, Suwannee, GA) and laid flat between two strips of filter paper placed in 10% buffered formalin (Fisher Scientific, Suwannee, GA). Colon was separated into proximal and distal portions and stained with 0.5% methylene blue solution (Sigma chemicals, St. Louis, MO). ACF and crypts per focus were scored. The analyst conducting ACF enumeration was blinded to the study.

Characterization of Colon Tumors

The colons of all rats in the End point tumor studies were removed and flushed with potassium phosphate buffer (0.1 mol L⁻¹, pH 7.2). Tumor number, size and location were characterized as described by Shackelford *et al.* (1983).

Preparation of Liver and Colonic Mucosal Tissues for Analysis of Glutathione-S-Transferase (GST) Activity

GST activity in cytosolic liver fraction and colonic mucosal scrapings were assayed following the methods described by Habig *et al.* (1974). The resulting product was analyzed using a UV/VIS dual beam spectrophotometer (Cary1/3, Varian) at 340 nm.

Preparation of Red Kidney Beans Extract for Cytotoxic Activities

Two hundred and fifty grams of ground freeze-dried sample of toasted red kidney beans were extracted with methanol overnight at room temperature, filtered and concentrated by rotary evaporation, as described by Yuan *et al.* (2005). The concentrated extracts were washed with hexane and the lower methanol phase then extracted with H₂O + ethyl acetate. The lower H₂O-methanol layer was then extracted with 1-butanol and the upper butanol layer concentrated by rotary evaporation to obtain a light brown residue. The red kidney bean extracts were solubilized in 0.1% ethanol for use in the assay. The red kidney bean extracts in 0.1% ethanol were each sonicated for 30 min and sterile-filtered prior to addition to 24 well cells.

Determination of Lactate Dehydrogenase (LDH) Release on CaCO₂ Colon Cancer Cells

CaCO₂ cells were maintained in DMEM with 10% fetal bovine serum. For assay, 100 µL 5×10⁵ cells/well were seeded to a 24 well culture plate and incubated at 37°C, 7% CO₂ until a monolayer was developed, then 400 µL of fresh serum free DMEM was added to the 24 wells. Selected concentrations (0.5-25 mg/100 mL) of Red kidney bean extracts were made with saline and 100 µL was added and incubated for 24 and 48 h. After incubation, cells were centrifuged (300 x g, 3 min) and cell supernatant fluid (0.1 mL) was placed in a 24 well microtiter plate. LDH buffer (0.1 mL) (Boehringer Mannheim, Anaheim, CA) was added to 0.1 mL of cell supernatant (CaCO₂) cells in 24 well plates and incubated at RT for 3 min. Absorbance of LDH activity was measured at 490/655 nm using a micro plate reader (Fisher Scientific, Suwannee, GA).

Statistical Analysis

Data presented in this study were analyzed using the SAS statistical program (2004). Results were performed by ANOVA and values are given as means±SEM and means were separated using Tukey's studentized range test. Differences between treatment groups were tested by student's t test and paired t-test. Unless otherwise indicated level of significance was considered at p<0.05.

RESULTS

ACF Study

Body Weight Gain, Feed Intake and Cecal Weight and pH

Weight gain was significantly (p<0.05) higher in rats fed control compared with the KB fed rats (Table 2). Although weight gain was lower in KB fed rats, daily feed consumption did not differ between the groups. There were no significant differences in cecal weights between the KB fed rats and the control, although numerically rats fed KB had a higher cecal weight compared to the control. Cecal pH was however, significantly (p<0.05) lower in the KB fed rats compared with the control rats. Cecal pH is inversely proportional to cecal weight.

Table 2: Weight gain, feed intake, cecal weight and cecal pH in Fisher 344 male rats fed red kidney beans

| Treatments | ACF experiment | | EPTM experiment | |
|------------------------------------|------------------------|------------------------|-------------------------|------------------------|
| | Control | KB | Control | KB |
| Weight (g/13 weeks) | 254.0±7.1 ^a | 222.5±8.0 ^b | 316.60±9.3 ^a | 273.8±6.1 ^b |
| Feed intake (g day ⁻¹) | 16.7±0.1 ^a | 17.7±0.5 ^a | 16.70±0.2 ^a | 14.3±0.4 ^{ab} |
| Cecal weight (g) | 1.9±0.2 ^a | 2.4±0.6 ^a | 1.10±0.1 ^b | 2.6±1.1 ^a |
| Cecal pH | 7.5 ^a | 6.1 ^b | 7.72±0.01 ^a | 6.7±0.02 ^b |

Values are expressed as means±SEM. ^{ab}: Means in the same row with the same letter(s) are not significantly different by Tukey's studentized range (HSD) test (p<0.05). KB: Kidney beans; EPTM: End Point Tumor Model

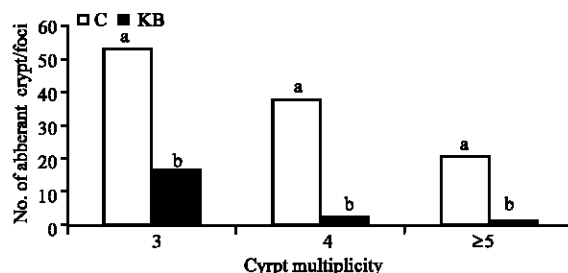


Fig. 2: Effect of diets on crypt multiplicity in Fisher 344 male rats. ACF consisting of ≤ 3 crypts have been reported to progress into tumors. ^{ab}: Means in the same column with the same letter are not significantly different by Tukey's studentized range (HSD) test ($p < 0.05$)

Table 3: No. of aberrant crypt foci in colon of azoxymethane-induced Fisher 344 male rats fed kidney beans

| Treatments | Proximal colon | Distal colon | Total ACF | Total crypts |
|-------------|---------------------|----------------------|------------------|--------------------|
| Control (C) | 40±0.9 ^a | 118±1.7 ^a | 158 ^a | 474.0 ^a |
| KB | 24±6.4 ^b | 48±9.5 ^b | 72 ^b | 111.6 ^b |

Values are expressed as means±SEM. ^{ab}: Means in the same column with the same letter(s) are not significantly different by Tukey's studentized range (HSD) test ($p < 0.05$). KB: Kidney beans

Aberrant Crypt Foci (ACF), Total Colonic Aberrant Crypts and Crypt Multiplicity in AOM Induced rats

Rats injected with vehicle (saline) did not develop any ACF. Rats fed KB had a significantly ($p \leq 0.05$) lower number of ACF in the proximal and distal colon compared to the rats fed the control diet (Table 3). Total number of ACF was significantly higher ($p \leq 0.05$) in rats fed control compared with the rats fed KB. There was about a 54% reduction in total ACF numbers in rats fed KB compared to the control. Compared to the control group, total aberrant crypts were significantly ($p \leq 0.05$) lower in the rats fed KB (Table 3). Total aberrant crypts in the proximal and distal colons were significantly ($p \leq 0.05$) higher in the control compared to rats fed KB (Table 3). Figure 2 shows crypt multiplicity in rats fed KB and control. Rats fed KB had significantly ($p \leq 0.05$) lower number of aberrant crypts with 3, 4 and 5 foci compared to rats fed control. Aberrant crypts with ≥ 4 foci will more likely progress into tumors over time.

Total Hepatic Glutathione-S-Transferase (GST) Activity in ACF Study

GST is a crucial phase II enzyme which plays a role in detoxification of carcinogens. Rats fed KB had significantly ($p \leq 0.05$) higher GST activity ($\mu\text{mol mg}^{-1}$) in liver tissues compared with rats fed control (Table 6). There was over a two-fold increase in GST activity ($\mu\text{mol mg}^{-1}$) in the KB fed rats (48.84) compared to the control (20.38).

End Point Tumor Study

Body Weight Gain, Feed Intake and Colonic pH

Daily feed intakes (Table 2) were significantly ($p \leq 0.05$) lower in the rats fed kidney beans (KB) compared to the control fed group. KB fed rats had a significantly ($p \leq 0.05$) lower weight gain compared to the control. Cecal pH and cecal weight were significantly ($p \leq 0.05$) higher in the KB fed rats (Table 2). Cecal weight in rats fed KB was 57% higher and cecal pH was lower compared to rats fed the control diet.

Distal and Proximal Tumors

The incidence of tumors in rats fed KB and control were significantly ($p \leq 0.05$) higher in the distal section of the colon compared to the proximal section (Table 4). Rats fed the control diet had higher tumor induction in the proximal and distal colons compared to the rats fed KB. The percent (%) tumor

incidence was 16% in the proximal colon and 100% in the distal colon in rats fed control diet. No tumors were induced in the proximal colon of the rats fed KB but the incidence of tumors in the distal colon was 100%. All the rats in the control group developed tumors (14/14) compared to only 9 (9/15) in the rats fed KB (Table 4).

Tumor/Tumor-Bearing Rat Values and Tumor Size

A significant ($p \leq 0.05$) decrease in Tumor/tumor-Bearing Rat (TBR) ratio was observed in the group fed KB (Table 5). Rats fed the control diet showed the highest TBR ratio (3.8). Tumor size (mm) was significantly ($p \leq 0.05$) larger in rats fed control (6.50) compared to the KB fed rats (1.16). The rats fed control also had higher number of tumors in the proximal (0.66) and distal (3.44) compared to those fed KB ((0.11) proximal and (1.44) distal colon). There was a 77% reduction in the total number of tumors and a 82% reduction in tumor size in the rats fed KB compared to the control. Rats fed KB had a reduction of 63% in TBR compared to the control. The number of tumors induced in the proximal and distal colon of rats was significantly ($p \leq 0.05$) higher in the control fed group compared to rats fed KB.

Total GST Activity in Liver and Colonic Mucosal Tissues

There were significant ($p \leq 0.05$) differences in hepatic and colonic mucosal scraping (CMS) GST activities in rats fed KB and control diets (Table 6). There was 46 and 45% increase in GST activity in hepatic and CMS in KB fed rats compared to the control. GST activity in CMS was lower than the hepatic GST activity; as the liver is the primary site for detoxification and CMS GST activity reflects residual or site specific activity (Table 6).

Cytotoxic Activities

LDH (Lactate Dehydrogenase) Activity of KB Extracts on CaCO_2 Cells

There was a dose response release of LDH following incubation with KB extracts for 24 and 48 h (Fig. 3). LDH release was higher after 48 h incubation compared to the 24 h release. LDH release ranged from 13.8-62% after 24 h and 24-84% after 48 h of incubation with KB extracts (0.5-25 mg/100 mL).

Table 4: Incidence (%) of colon tumors in Fisher 344 male weanling rats fed kidney beans

| Treatments | N ¹ /N ² | Colon tumors (%) | Proximal tumors (%) | Distal tumors (%) |
|-------------|--------------------------------|------------------|---------------------|-------------------|
| Control (C) | 14/14 | 100 | 16 | 84 |
| KB | 9/14 | 64 | 0 | 100 |

N¹ represents the No. of rats with tumors; N² is total number of rats at the end of the experiment

Table 5: Distribution and characterization of AOM-induced colon tumors in Fisher 344 male rats

| Treatments | N ¹ /N ² | Proximal tumors rat ⁻¹ | Distal tumors rat ⁻¹ | Tumor size (mm) rat ⁻¹ | Total No. of tumors | Tumor Bearing Ratio (TBR) |
|-------------|--------------------------------|-----------------------------------|---------------------------------|-----------------------------------|---------------------|---------------------------|
| Control (C) | 14/14 | 0.66 ^a | 3.44 ^a | 6.50 ^a | 57 | 3.80 ^a |
| KB | 9/14 | 0.11 ^b | 1.44 ^b | 1.16 ^b | 13 | 1.44 ^b |

Values are means \pm SEM, ^a: Means in a column with the same superscript do not significantly differ ($p < 0.05$) by Tukey's studentized range (HSD) test ($p < 0.05$). N¹ represents the number of rats with tumors; N² is total number of rats at the end of the experiment

Table 6: Specific activity of Glutathione-S-Transferase (GST) in livers and colonic mucosal tissues of AOM induced Fisher 344 male rats

| Treatments | Liver GST ($\mu\text{mol L}^{-1} \text{min}^{-1} \text{mg}^{-1}$) | | Colonic GST ($\mu\text{mol L}^{-1} \text{min}^{-1} \text{mg}^{-1}$) |
|-------------|---|-------------------------------|---|
| | ACF | EPTM | EPTM |
| Control (C) | 20.38 \pm 1.04 ^b | 15.86 \pm 1.04 ^b | 3.14 \pm 0.1 ^b |
| KB | 48.84 \pm 2.70 ^a | 29.90 \pm 0.9 ^a | 5.72 \pm 0.3 ^a |

Values are expressed as means \pm SEM. ^a: Means in the same column with the same letter are not significantly different by Tukey's studentized range (HSD) test ($p < 0.05$)

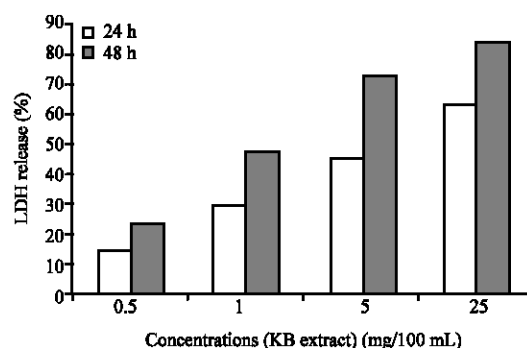


Fig. 3: Lactate Dehydrogenase (LDH) release after 24 and 48 h from CaCO₂ cells incubated with KB extract

DISCUSSION

Studies suggest that diets rich in legumes such as dry beans might be associated with a decreased incidence of many different types of cancers such as colon, breast and prostate (Potter and Steinmetz, 1996; Goodman *et al.*, 1997; Armstrong *et al.*, 2000). In this study, we examined the effects of KB on AOM-induced colon tumorigenesis and the cytotoxic effects of crude KB extracts on colon cancer cell line (CaCO₂).

We have showed that feeding KB significantly ($p \leq 0.05$) reduced the number of ACF and colon tumors compared with the rats fed the control diet. One of the protective and beneficial effects of dietary fiber (soluble/fermentable) in reducing the number of ACF and frequency of colon adenocarcinomas may be due to the production of Short Chain Fatty Acids (SCFA), specifically butyrate. Rats fed diets rich in dietary fiber such as flaxseed (Jenab and Thompson, 1996; Williams *et al.*, 2007); wheat bran (Reddy *et al.*, 1981; Alabaster *et al.*, 1996), rice bran (Kawakawa *et al.*, 1999; Katyama *et al.*, 2002) and oat bran (Zoran *et al.*, 1997) had lower number of ACF and colon tumors. Fermentable dietary fibers such as those present in dry beans have shown to be high butyrate producers (Goodlad and Mathers, 1990; Henningson *et al.*, 2001). Henningson *et al.* (2001) reported a high butyrate yield for red kidney beans in the colon of rats. Similarly, increased intake of peas was associated with an increase in the proportion of butyrate production (Goodlad and Mathers, 1990).

In general, legumes are a significant source of Resistant Starch (RS) and this may have played a role in the antitumorigenic effect of KB observed in this study. RS is similar to soluble Non-Starch Polysaccharides (NSP) in increasing the rate of fermentation. Additionally, RS unlike NSP has the propensity to increase the formation of short-chain fatty acids, with a predominantly high butyrate fraction in the proximal colon (Govers *et al.*, 1999; Bauer-Marinovic *et al.*, 2006). Present results showed a decreased number of ACF and tumors in the proximal colon.

Other possible mechanisms for the protective effect of butyrate producing fibers could be stimulation of colonocyte immunogenicity; elevated immune cytotoxicity which may contribute to inhibition of AOM induced ACF in rats (Perrin *et al.*, 2001). Zoran *et al.* (1997) reported findings by Reddy *et al.* (1975), that show that differences in microbial populations within the colon can alter the effect of carcinogens, including AOM. Therefore, since microbial populations according to Maciorowski *et al.* (1997) and Zoran *et al.* (1997) respond to dietary changes the potential exists for a diet rich in dietary fiber to influence the metabolism of AOM via this mechanism.

It is important to make mention of other components besides fiber that may have played a part in the reduction of AOM-induced colon tumorigenesis. The various phytochemicals found in dry beans

have not been well studied. KB has been reported to contain relatively high levels of phenolics and flavonoids (Luthria and Pastor-Corrales, 2005; Heimler *et al.*, 2005), these compounds are strong antioxidants (Heber and Bowerman, 2001) and may activate liver detoxification enzymes and destroy harmful ROS by blocking carcinogens and suppressing malignant cells (Pennington, 2002).

Crude KB extracts rich in phytochemicals showed cytotoxic effects as measured by percentage LDH release against CaCO₂ cells after exposure periods of 24 and 48 h in a dose-dependent and time-dependent manner. Our findings provide evidence indicative of a potential inhibitory effect of the combination of bioactive constituents present in KB, however further evaluations are warranted before any definitive mechanisms can be suggested. In the meantime, some of the constituents of KB such as flavonoids have been reported in several studies to induce apoptosis in cancer cell lines (Paschka *et al.*, 1998; Yang *et al.*, 1998; Shen *et al.*, 2003). Thus, LDH release in CaCO₂ after incubation with KB extracts may be attributed to these non-nutritive bioactive components.

Weight gain was significantly lower in KB fed rats compared to the control. This may be due to the satiety effects associated with legumes. Furthermore, the intake of diets high in fiber decreases the intake of energy. Previous studies have shown a positive correlation between high-energy intakes and cancer risk (Slattery *et al.*, 1997; Furberg and Thune, 2003).

In this study, we observed a positive correlation between a lower cecal pH and reduction in number of ACF or tumors. Cecal pH was lower in rats fed KB compared to the control. Dietary fibers undergo fermentation to yield SCFA which may cause an acidic environment in the cecum. Previous studies have reported acidic cecal pH in rats fed fermentable dietary fibers (Campbell *et al.*, 1997; Wijnands *et al.*, 1999; Verghese *et al.*, 2002a, b). According to Verghese *et al.* (2002a, b), an acidic cecal pH is indicative of fecal pH reduction and fecal pH has been suggested to be a possible factor in suppression of colon tumorigenesis.

KB increased GST activity in hepatic tissues as well as colonic mucosal scrapings. The ability of butyrate to increase phase II detoxifying enzymes such as Glutathione S-Transferase (GST) (Beyer-Sehlmeyer *et al.*, 2003; Knoll *et al.*, 2005) may contribute to the detoxification of dietary carcinogens. Induction of GST is one of the chemopreventive effects of phytochemicals (Jung *et al.*, 2006) and this may occur at either transcriptional or post-transcriptional levels (Mandlekar *et al.*, 2006).

CONCLUSIONS

Diet contributes significantly to the maintenance of a healthy life. Dry beans contain essential nutrients and phytochemicals that have the potential to ameliorate our health. It has potential beyond just merely improving the health of the gastrointestinal tract. Recent studies have shown that dry beans contain important bioactive components that may rival those from some well known fruits and vegetables, thus, it is imperative to recognize its beneficial and unexploited uses.

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