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In vitro binding of bile acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), bengal gram (*Cicer arietinum*) and moth bean (*Phaseolus aconitifolins*) ☆

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Abstract

The in vitro binding of bile acids by kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), bengal gram (*Cicer arietinum*) and moth bean (*Phaseolus aconitifolins*) was determined using a mixture of bile acids secreted in human bile at a duodenal physiological pH of 6.3. Six treatments and two blank incubations were conducted, testing whole mature raw seed as substrates on an equal protein basis. Considering cholestyramine as 100% bound, the relative in vitro bile acid binding for kidney bean, black gram, bengal gram and moth bean on equal protein basis was 12%, 15%, 35% and 13%, respectively. Relative bile acid binding on equal dry matter (DM), total dietary fiber (TDF) and insoluble dietary fiber (IDF) basis was for kidney bean 3%, 11% and 14%, black gram 3%, 29% and 36%, bengal gram 7%, 27% and 29%, and moth bean 3%, 19% and 21%, respectively. Except for bengal gram where values were much higher, bile acid binding by kidney bean, black gram and moth bean appear to be related to their DM and protein content. These results point to bile acid binding by bengal gram > black gram = moth beans = kidney bean as indicative of their health-promoting potential. Data suggest that of all four kinds of beans tested, bile acid binding may be related to the anionic, cationic, physical and chemical structure, composition, metabolites, or their interaction with active binding sites. Animal studies are in progress to validate relationship of in vitro bile acid binding of various beans observed herein to lipid, cholesterol-lowering and atherosclerosis amelioration.
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1. Introduction

The population of Asian countries have a notably low risk of coronary heart disease, presumably due to their lower intake of animal protein and higher intake of various beans, which are being introduced to the Western World mainly by migrants. Daily per capita consumption of all bean products in Asia is estimated to be 110 g, whereas in the USA it is about 9 g. In the USA about 11% of the population is of Hispanic origin and they consume 33% of all bean products (Lucer, Lin, Allshouse, & Kantor, 2000). Beans consumed are pinto, kidney, black and navy as cooked dry beans or canned (cooked, baked or refried). In the Western countries, kidney bean and garbanzo are used in salads, soups and other food products. Black gram, bengal gram (black chana, variety of garbanzo) and moth beans are quite popular in Asia, however their use in the western world is limited to the Asian migrant population. Bean consumption of four times or more per week compared with less than once a week have been associated with a 22% lower risk of coronary heart disease (Bazzano et al., 2001). Beans consumed were mainly dry home cooked or canned pinto, red and blackeye. The health benefits or cholesterol-lowering properties of food fractions could be predicted by evaluating their in vitro bile acid binding, based on positive correlations found between in vitro and in vivo studies showing that cholestyramine binds bile acids and cellulose does not (Daggy,

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O'Connell, Jerdack, Stinson, & Setchell, 1997; Kahlon & Chow, 2000; Nakamura & Matsuzawa, 1994; Suckling et al., 1991). Bile acids are acidic steroids synthesized in the liver from cholesterol. After conjugation with glycine or taurine, they are secreted into the duodenum. Bile acids are actively reabsorbed by the terminal ileum and undergo an enterohepatic circulation (Hofmann, 1977). Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism by which dietary fiber lowers cholesterol (Anderson & Siesel, 1990; Lund, Gee, Brown, Wood, & Johnson, 1989; Trowell, 1975). By binding bile acids, food fractions prevent their reabsorption and stimulate plasma and liver cholesterol conversion to additional bile acids (Balmer & Zilversmit, 1974; Eastwood & Hamilton, 1968; Kritchevsky & Story, 1974; Potter, 1998). Relative to cholestyramine (a bile acid binding and cholesterol lowering drug) and on an equal protein basis, soy protein, pinto bean and black bean bound 17%, 23% and 30% bile acid mixture, respectively (Kahlon & Woodruff, 2002). Garbanzo has been shown to bind significantly more bile acids than soy bean (Kahlon & Shao, 2004). Higher bile acid binding by garbanzo (*Cicer arietinum*) suggested that bengal gram (black chana variety of *C. arietinum*) and other beans consumed by Asian populations in the world should be evaluated for their health promoting potential.

The objective of this study was to determine potential health benefits of kidney bean (*Phaseolus vulgaris*), black gram (*Vigna mungo*), bengal gram (*C. arietinum*) and moth bean (*Phaseolus aconitifolins*) by evaluating their in vitro bile acid binding using whole mature raw seed on equal amounts of protein basis, with a bile acid mixture observed in human bile under duodenal physiological pH of 6.3.

2. Materials and methods

2.1. General

Food grade kidney bean, black gram, bengal gram and moth bean were obtained from local vendors. All the beans were ground in a Thomas–Wiley Mill No. 1 (Arthur Thomas, Philadelphia, PA) to pass a 0.6 mm screen. Samples were analyzed for total, insoluble and soluble dietary fiber by method 991.43 (AOAC Suppl. 3, 1992). Samples were enzymatically digested to remove starch and protein and soluble dietary fiber precipitated with alcohol. Nitrogen was analyzed by Method 979.09 (AOAC, 1990) for crude fat with petroleum ether by Accelerated Solvent Extractor (ASE 200 Dionex Corp., Sunnyvale, CA) as described by Barrios, Camara, Torija, and Alonso (2002), and moisture by method 935.29 (AOAC, 1990). Cellulose, a non bile acid binding fiber, was the negative control and cholestyramine, a bile acid binding anionic resin (a drug that lowers cholesterol and binding bile acids), was the positive control and both (Sigma, St. Louis, MO) were considered as 100% insoluble dietary fiber (IDF) on a dry matter (DM) basis. Eight replicate incubations, six with bile acid mixture and two substrate blanks without bile acid mixture, were run for each treatment and control. All treatments used whole dry raw seeds containing 22–23 mg protein content for each treatment (nitrogen to protein factors used were 6.25) or 24 mg dry matter for cholestyramine and cellulose.

2.2. Bile acid binding procedure

The in vitro bile acid binding procedure was a modification of that by Camire, Zhao, and Violette (1993) as previously reported (Kahlon & Chow, 2000). The stock bile acid mixture was formulated with glycocholic bile acids providing 75% and taurine-conjugated bile acids 25% of the bile acids, based on the composition of human bile (Carey & Small, 1970; Rossi, Converse, & Hoffman, 1987). This mixture contained glycocholic acid (9 mmol/l), glycochenocholic acid (9 mmol/l), glycodeoxycholic acid (9 mmol/l), taurocholic acid (3 mmol/l), taurochenocholic acid (3 mmol/l) and taurodeoxycholic acid (3 mmol/l) in pH 6.3, 0.1M phosphate buffer. This stock solution of 36 mmol/l was stored in the refrigerator and diluted to the working solution (0.72 μmol/ml) immediately prior to each assay. Six replicates of kidney bean, black gram, bengal gram and moth bean, cholestyramine and cellulose were tested using 108, 101, 114, 98, 24 and 24 mg dry matter, respectively. One substrate blank, one positive blank (2.88 μmol bile acid mixture per incubation) and six treatment replicates were weighed into 12×125 mm glass, screw-capped tubes. Samples were digested in 1 ml 0.01 N HCl for 1 h in a 37 °C shaker bath. After this acidic incubation, which simulated gastric digestion, the sample pH was adjusted to 6.3 with 0.1 ml of 0.1 N NaOH. To each test sample was added 4 ml of bile acid mixture working solution (0.72 μmol/ml) in a 0.1 M phosphate buffer, pH 6.3. A phosphate buffer (4 ml, 0.1 M, pH 6.3) was added to the individual substrate blanks. After the addition of 5 ml of porcine pancreatin (5×, 10 mg/ml, in a 0.1 M phosphate buffer, pH 6.3; providing amylase, protease and lipase for digestion of samples), tubes were incubated for 1 h in a 37 °C shaker bath. Mixtures were transferred to 10 ml centrifuge tubes (Oak Ridge 3118-0010 Nalgene, Rochester, NY) and centrifuged at 99,000*g* in a 75-Ti rotor at 39 K for 18 min at 25 °C in an ultracentrifuge (model L-60, Beckman, Palo Alto, CA). Supernatant was removed into a second set of labeled tubes. An additional 5 ml of phosphate buffer was used to rinse out the incubation tube and added to the centrifuge tube, which was vortexed and centrifuged as before. Supernatant was removed and combined with

the previous supernatant tube. Aliquots of pooled supernatant were frozen at −20 °C for bile acids analysis. Bile acids were analyzed using Sigma bile acids procedure No. 450 (Sigma, St. Louis, MO) using a Ciba-Corning Express Plus analyzer (Polestar Labs, Inc., Escondido, CA). Each sample was analyzed in triplicate. Values were determined from a standard curve obtained by analyzing Sigma bile acid calibrators (Sigma 450-11) at 5, 25, 50, 100 and 200 μmol/l. Individual substrate blanks were subtracted, and bile acid concentrations were corrected based on the mean recoveries of bile acid mixture (positive blank). The effect of treatment was tested using Lavene's test for homogeneity and least square means were calculated. Dunnett's one-tailed test was used for comparison of cholestyramine as well as cellulose against all treatments, and differences among kidney bean, black gram, bengal gram and moth bean were tested for significance with Tukey's test for comparison of all possible pairs of means (SAS Institute, Cary, NC). A value of $P \leqslant 0.05$ was considered the criterion of significance.

3. Results and discussion

The composition of the kidney bean, black gram, bengal gram and moth bean is given in Table 1. Both cellulose and cholestyramine were considered as IDF being 100% DM. Protein content among these beans was quite similar, values ranging from 19% to 23%. Bengal gram contained 5% fat, whereas other beans tested contained similar and lower levels of fat (1%). There was a wide variation in the dietary fiber content of these beans. Total, insoluble and soluble fiber were kidney bean, 22%, 18% and 4%; black gram, 11%, 9% and 2%; bengal gram, 25%, 23% and 3%; moth bean, 16%, 15% 2%, respectively. Starch content of each bean could be calculated by difference. Chemical characterization of polysaccharides of various dietary fiber fractions were not conducted; neither were such data found in the literature search.

On an equal DM basis, bile acid binding was significantly higher with cholestyramine and significantly lower with cellulose than for whole mature raw seed black gram, bengal gram and moth bean (Table 2). Bengal gram bound significantly more bile acids than kidney bean, black gram and moth bean. Cholestyramine bound 96% of the bile acids. Similar in vitro bile acid binding values for cholestyramine have been previously observed (Kahlon & Chow, 2000). Cholestyramine bound glycocholate and taurocholate 87% and 93%, respectively (Sugano & Goto, 1990). In our study, cholestyramine binding to the mixture of bile acids was similar to that observed for taurocholate by Sugano and Goto (1990). Story and Kritchevsky (1976) reported 81% bile acid binding by cholestyramine using 50 mg of substrate and 50 μmol of bile acids. Higher bile acid binding by cholestyramine in our studies may be due to the use of physiological pH and/or a higher substrate to bile acid ratio. Assigning a bile acid binding value of 100% to cholestyramine, the relative bile acid binding percentages for the test samples were kidney bean 3%, black gram 3%, bengal gram 7% and moth bean 3%. Except for bengal gram, where values were much higher, data suggest that bile acid binding by kidney bean, black gram and moth bean appear to be related to their DM content. Evaluating health properties (cholesterol lowering and excreting toxic metabolites) of various foods and fractions would be desirable by testing their bile

Table 2
In vitro bile acid binding by kidney bean, black gram, bengal gram and moth bean on equal weight, dry matter (DM) basis[A,B]

Treatment	Bile acid binding (μmol/100 mg DM)	Binding relative to cholestyramine (%)
Kidney bean	0.28 ± 0.02^{cd}	2.5 ± 0.2^{cd}
Black gram	0.37 ± 0.02^{c}	3.3 ± 0.2^{c}
Bengal gram	0.76 ± 0.05^{b}	6.7 ± 0.5^{b}
Moth bean	0.35 ± 0.02^{c}	3.1 ± 0.2^{c}
Cholestyramine	11.2 ± 0.10^{a}	100 ± 0.9^{a}
Cellulose	0.17 ± 0.05^{d}	1.5 ± 0.5^{d}

[A] Pooled values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.

[B] Kidney bean, black gram, bengal gram, moth bean, cholestyramine and cellulose treatments contained 108, 101, 114, 98, 24 and 24 mg dry matter, respectively.

Table 1
Composition of kidney bean, black gram, bengal gram and moth bean

Source	Moisture	Dietary fiber			Fat	Protein[a]
		Total	Insoluble	Soluble		
Kidney bean	11.6	22.2	18.1	4.1	1.4	21.5
Black gram	11.3	11.4	9.2	2.2	1.1	22.0
Bengal gram	10.2	25.4	22.9	2.5	4.9	19.3
Moth bean	8.8	16.3	14.8	1.5	0.9	23.3
Cholestyramine	9.6	100	100	–	–	–
Cellulose	5.4	100	100	–	–	–

[a] Nitrogen to protein factor used was 6.25 for bean protein, starch was the remaining content and could be calculated by difference.

acid binding on a dry matter basis. Similar observations have been reported for various ready to eat breakfast cereals (Kahlon & Woodruff, 2003a) as well as for soy bean, black eye bean and lima bean (Kahlon & Shao, 2004). Previously 43% higher bile acid binding by garbanzo has been observed than with bengal gram in the study reported herein. Garbanzo and bengal gram are two varieties of *C. arietinum*. Data suggest that there appears to be large varietal differences in bile acid binding. Soy protein and soy fiber has been shown to lower plasma total and LDL cholesterol, and atherosclerosis index in hypercholesterolemic humans and laboratory animals (Anderson, Johnstone, & Cook-Newell, 1995; Anthony, Clarkson, Bullock, & Wagner, 1997; Carroll, 1991; Huff, Roberts, & Carroll, 1982; Wang, Zhao, & Chen, 1996). Data suggest that animal and human studies should be conducted to explore health benefits of kidney bean, black bean, bengal gram and moth bean.

The bile acid binding on equal total dietary fiber (TDF) basis is shown in Table 3. Cholestyramine bound bile acids significantly more and cellulose significantly less than the various beans tested. On a TDF basis, considering cholestyramine as 100% bound, bile acid binding values for kidney bean, black gram, bengal gram and moth bean were 11%, 29%, 27% and 19%, respectively. The bile acid binding values for black gram and bengal gram were significantly higher than those for moth bean and kidney bean; values for moth bean were also significantly higher compared with those for kidney bean. Bile acid binding by black gram and bengal gram may appear to be related to their TDF; however, for kidney bean and moth bean, values are not proportional to their TDF content. Previously more than twofold higher bile acid binding by garbanzo has been reported than observed with bengal gram in the study reported herein (Kahlon & Shao, 2004). Suggesting that varietal differences may have wide potential variability in bile acid binding or health promoting properties. The variability in bile acid binding between the beans tested maybe related to their flavonoid, tannin content, anionic and cationic, physical and chemical structure. Soy bean fiber has been reported to lower total and LDL cholesterol and atherosclerosis index (Wang et al., 1996). Wide variability in bile acid binding values on TDF basis among beans tested is very encouraging, suggesting that these beans need to be incorporated into foods and their full potential to lower lipids, total and LDL cholesterol, and atherosclerosis index should be explored.

Table 3
In vitro bile acid binding by in vitro bile acid binding by kidney bean, black gram, bengal gram and moth bean on equal total dietary fiber (TDF) basis[A,B]

Treatment	Bile acid binding (μmol/100 mg TDF)	Binding relative to cholestyramine (%)
Kidney bean	1.28 ± 0.12^{d}	11.4 ± 1.2^{d}
Black gram	3.24 ± 0.12^{b}	28.9 ± 1.2^{b}
Bengal gram	2.97 ± 0.12^{b}	26.5 ± 1.2^{b}
Moth bean	2.15 ± 0.12^{c}	19.2 ± 1.2^{c}
Cholestyramine	11.2 ± 0.12^{a}	100 ± 1.2^{a}
Cellulose	0.17 ± 0.12^{e}	1.5 ± 1.2^{e}

[A] Pooled values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] Kidney bean, black gram, bengal gram and moth bean, cholestyramine and cellulose treatments contained 24, 12, 29, 16, 24 and 24 mg TDF.

Table 4
In vitro bile acid binding by in vitro bile acid binding by kidney bean, black gram, bengal gram and moth bean on equal insoluble dietary fiber (IDF) basis[A,B]

Treatment	Bile acid binding (μmol/100 mg IDF)	Binding relative to cholestyramine (%)
Kidney bean	1.57 ± 0.14^{e}	14.0 ± 1.4^{e}
Black gram	4.02 ± 0.14^{b}	35.8 ± 1.4^{b}
Bengal gram	3.30 ± 0.14^{c}	29.4 ± 1.4^{c}
Moth bean	2.37 ± 0.14^{d}	21.1 ± 1.4^{d}
Cholestyramine	11.2 ± 0.14^{a}	100 ± 1.4^{a}
Cellulose	0.17 ± 0.14^{f}	1.5 ± 1.4^{f}

[A] Pooled values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.
[B] Soy bean, black eye bean, garbanzo, lima bean, cholestyramine and cellulose treatments contained 20, 9, 26, 15, 24 and 24 mg IDF.

In vitro bile acid binding by kidney bean, black gram, bengal gram and moth bean on an equal IDF basis is shown in Table 4. There were significant differences in the bile acid binding among the four beans tested: black gram > bengal gram > moth bean > kidney bean. Bile acid binding relative to cholestyramine on an equal IDF basis was black gram 36%, bengal gram 29%, moth bean 21%, and kidney bean 14%. Variability in bile acid binding between the treatments may relate to differences in chemical and physical structure. These observations suggest that bile acid binding by the beans tested do not relate to their IDF content. Previously, bile acid binding of peanut oil was evaluated as about 4% (Kahlon & Chow, 2000). Higher amounts of oil present in bengal gram (Table 1) would only contribute to a minor portion of its bile acid binding.

Relating the soluble fiber to the bile acid binding with the beans, gave unrealistic values. Bowles, Morgan, Furneau, and Coles (1996) observed no chemical binding between isolated barley β-glucan and bile acids. Previously it has been reported that bile acid binding of high soluble fiber cereals (oat bran and β-glucan enriched barley) were not proportional to soluble fiber content (Kahlon & Woodruff, 2003b). The chemical composition of TDF and IDF of various beans tested was not found in the literature search nor was it determined; therefore it is not possible to speculate that variability in binding bile acids may relate to the differences in polysaccharides present in their dietary fiber

Table 5
In vitro bile acid binding by in vitro bile acid binding by kidney bean, black gram, bengal gram and moth bean on equal protein basis[A,B]

Treatment	Bile acid binding (μmol/100 mg protein)	Binding relative to cholestyramine (%)
Kidney bean	1.32 ± 0.09^{c}	11.8 ± 0.8^{c}
Black gram	1.68 ± 0.09^{c}	15.0 ± 0.8^{c}
Bengal gram	3.91 ± 0.25^{b}	34.9 ± 2.4^{b}
Moth bean	1.51 ± 0.09^{c}	13.4 ± 0.8^{c}
Cholestyramine	11.2 ± 0.09^{a}	100 ± 0.8^{a}
Cellulose	0.17 ± 0.09^{d}	1.5 ± 0.8^{d}

[A] Pooled values (means ± SEM) within a column with different superscript letters differ significantly ($P \leqslant 0.05$), $n = 6$.

[B] Kidney bean, black gram, bengal gram and moth bean contained 23, 22, 22 and 23 mg protein; cholestyramine and cellulose contained 24 mg dry matter. Nitrogen to protein factor used for bean protein was 6.25.

fractions. Since beans were tested as uncooked, and cooking may alter binding sites or fiber molecules, such effects should be explored to evaluate health benefits of various beans as normally consumed by humans.

In vitro bile acid binding by kidney bean, black gram, bengal gram and moth bean on an equal protein basis is shown in Table 5. The values for cholestyramine and cellulose are listed for comparison and are the same as in Table 4. Bile acid binding values for bengal gram were significantly higher than those for kidney bean, black gram and moth bean. Considering cholestyramine as 100% bound, bile acid binding values were kidney bean 12%, black gram 15%, bengal gram 35% and moth bean 13%. Except for bengal gram where values were 2–3 fold higher, similar bile acid binding by kidney bean, black gram and moth bean may suggest that their bile acid binding may relate to the protein content. However, it may be coincident that bile acid binding appears to be related to their protein content, since protein was digested with pancreatin. Soy protein has been shown in hamsters to significantly reduce hepatic cholesterol and increase bile acid excretion and lower plaque formation in the aortic arch (Kahlon, Chow, & Wood, 1999; Wright & Salter, 1998). Previously bile acid binding by soy protein has been observed to be 6–17% (Kahlon & Woodruff, 2002; Kahlon & Shao, 2004). Similar bile acid binding values for kidney bean, black bean and moth bean, and even higher values for bengal gram compared to soybeans suggest that these beans may have similar or even higher health promoting or degenerative disease amelioration potential, which should be explored. Much higher bile acid binding reported for garbanzo (Kahlon & Shao, 2004) than for bengal gram reported herein (both are different varieties of *C. arietinum*) suggest varietal differences may lead to significant differences in the health promoting potential among beans. Each variety or cultivar and various beans are unique and should be evaluated for their bile acid binding and health promoting potential.

4. Conclusions

Relative to cholestyramine, in vitro bile acid binding on an equal DM and protein basis were kidney bean, 3 and 12%; black gram, 3% and 15%; bengal gram, 7% and 35%; moth bean, 3% and 13%. Except for bengal gram, where values were over twofold higher, bile acid binding appears to be related to DM and protein content for kidney bean, black gram and moth bean. Relative bile acid binding on TDF and IDF basis were kidney bean, 12% and 14%; black gram, 29% and 36%; bengal gram, 27% and 29%; moth bean, 19% and 21%. Data suggest that bile acid binding was not related to TDF or IDF content of the beans tested. The difference in bile acid binding between various beans tested may relate to the variability in their phytonutrients (flavonoid, tannin, estrogenic content), non-protein composition, structure, hydrophobicity of undigested fractions, anionic or cationic nature of the metabolites produced during digestion or their interaction with active binding sites. Similar bile acid binding for kidney bean, black gram and moth bean to those previously observed for soy bean (with reported health benefits) and even higher binding for bengal gram suggest that animal and human studies should be conducted to explore their potential for lowering blood lipids, lipoprotein and atherosclerosis risk and other properties (excretion of toxic metabolites) of the beans studied herein.

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