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FULL ARTICLE

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Hypolipidemic and hypoglycemic potential of raw, boiled, and sprouted mung beans (*Vigna radiata* L. Wilczek) in rats

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Abstract

This study was carried out to investigate the hypolipidemic and hypoglycemic potential of raw, boiled, and sprouted mung beans in rats. Oven dried mung bean powders; raw, sprouted, and boiled were included at 30% level in the diet of seven weeks old male Wistar rats maintained for 5 weeks on high (0.5%) cholesterol diet in comparison with control diet. Low serum glucose and triglyceride concentrations ($p < .05$) in raw and processed mung bean diets fed rats were supported by low serum insulin level in both raw mung bean diet and boiled mung bean diet fed rats. Hypoglycemic effect in sprouted mung bean fed rats was supported by higher α-amylase inhibitory activity and α-glucosidase inhibitory activity of sprouted mung beans. Increase in serum non-HDL cholesterol concentration and decrease in HDL cholesterol concentration caused by high cholesterol diet were modulated ($p < .05$) by both boiled and sprouted mung bean diets.

Practical applications

Mung bean is a green legume rich in protein, fiber, antioxidants, and phytonutrients. Hypocholesterolemic and hypoglycemic potential of raw mung beans have been shown previously. It is well known that cooking and processing modulate nutritional and biochemical parameters of foods. However, very limited information is available on the effect of processing on functional properties of legumes. Results of this study showed that boiling and sprouting improved the soluble fiber content and hypocholesterolemic potential of mung beans. Thus, the processed mung beans may be more suitable for developing food supplements for patients with hypercholesterolemia.

KEYWORDS

hypocholesterolemic, mung bean, processing, serum cholesterol, serum glucose, Wistar rats

1 | INTRODUCTION

Mung bean (*Vigna radiata* [L.] R. Wilczek) is one of the major grain legumes which contains potential agents for reducing serum lipids (Tang, Dong, & Ren, 2014). Antioxidants in mung beans act as myocardial preservation agents by regulating cholesterol levels, scavenging free-radicals and reversing damage to blood vessels, and lowering inflammation (Bai et al., 2016; Chung, Yeo, Kim, & Moon, 2011). High levels of amino acids, oligosaccharides, and polyphenols, in mung beans are considered as the main contributors to their antioxidant activity (Tang et al., 2014). Vitexin and isovitexin are two types of protective flavonoids present in mung beans with high free-radical scavenging abilities (Bai et al., 2016; Tachibana, Wanezaki, Nagata, & Motoyama, 2013).

Growing prevalence of cardiovascular diseases in individuals has become a major threat to global health (Simon & Vijayakumar, 2013). Hypercholesterolemia, hyperglycemia, and hyperinsulinemia are generally accepted as major modifiable risk factors for development of coronary heart disease (Simon & Vijayakumar, 2013). Many studies have shown that elevated concentrations of total or LDL cholesterol in the blood progressively increases coronary heart disease, whereas high concentrations of HDL cholesterol may protect against coronary heart disease (Koncsos, Fülöp, & Juhász, 2016). Reduced intake of plant fiber and plant antioxidants, with increased intake of carbohydrate/fatty

foods are major causes of hyperlipidemia (Siri-Tarino, Sun, Hu, & Krauss, 2010). Hence, much attention has been paid to dietary interventions that lower plasma cholesterol concentration as a tool to prevent and treat coronary heart diseases (Kerckhoffs, Brouns, Hornstra, & Mensink, 2002). Several studies have reported that legume consumption may be linked to lower incidence of hyperlipidemia and hyperglycemia (Reynolds, Chin, & Lees, 2006; Yang et al., 2011).

It is well known that cooking and processing modulate nutritional and biochemical parameters of the foods (Nergiz & Gökgöz, 2007). However, very limited information is available on the effect of processing on functional properties of legumes (Dahiya, Linnemann, & VAN Boekel, 2015). Thus, this study was carried out to investigate the hypolipidemic and hypoglycemic potential of raw, boiled, and sprouted mung beans in an animal experimental model.

2 | MATERIALS AND METHODS

2.1 | Animals and diets

Male Wistar rats were purchased from the Medical Research Institute, Colombo, Sri Lanka. They were housed individually in wire meshed cages with free access to food and water. The animal facility was maintained on a 12-hr light/dark cycle at a temperature of 23 ± 1°C and relative humidity of 60 ± 5%. Twenty rats were randomly assigned into four groups ($n = 5$). Experimental diets were prepared according to AIN 93G semipurified rodent diets (Table 1). Oven dried mung bean powders; raw, sprouted, and boiled were included at 30% level in high cholesterol (0.5%) experimental diets (RMD, SMD, BMD) in comparison with the control diet (CD). Blood samples (1 mL) were taken at the beginning and end of the 5 weeks period between 09.00 and 10.00 hr from the jugular vein of fasting rats anesthetized with sodium pentobarbital. The samples were collected without any anticoagulant, and serum was separated by centrifugation at 1,500 x g for 20 min. At the end of the experiment, all feces excreted during the last 3 days were collected and the rats were anesthetized, sacrificed, and the livers and cecum were quickly removed, washed with cold saline (9 g NaCl/L), blotted dry on filter paper, and weighed. All experimental procedures described here were approved by the Animal Experiment Committee of Faculty of Veterinary Medicine and Animal Science (VER-15–016), University of Peradeniya, Sri Lanka. All animal procedures were conducted according to the guidelines of the National Research Council (2011).

TABLE 1 Composition of experimental diets (AIN 93G Purified Rodents Diet)

	Raw mung bean (RMD)	Boiled mung bean (BMD)	Sprouted mung bean (SMD)	Control diet (CD)
Casein	101.58	118.05	141.61	210
Mung bean powder	300	300	300	–
Soya bean oil	97.90	92.26	85.96	100
Mineral mixture	35	35	35	35
Vitamin mixture	10	10	10	10
Cellulose powder	27.95	24.65	31.46	50
Corn starch	319.32	311.79	287.72	486.75
Choline chloride	2	2	2	2
Sodium cholate	1.25	1.25	1.25	1.25
Sucrose	100	100	100	100
Cholesterol	5	5	5	5

2.2 | Preparation of raw mung bean powder

Government certified mung bean cultivar, MI-6 seeds were purchased from the government seed farm, Palwehera, Dambulla, Sri Lanka. Dry mung bean seeds were visually inspected and defective seeds were discarded before preparation of the powder. Raw mung bean seeds were washed and oven dried in drying oven (YAMATO IC600, Yamato Scientific Co., Ltd., Japan) at 60°C until a constant weight was obtained and ground using a grinder (MX-151SG1, Panasonic Co., Ltd., China) to a fine consistency.

2.3 | Preparation of boiled mung bean powder

Mung bean seeds were washed and soaked overnight in distilled water and boiled for 30 min in distilled water. Boiled seeds were then air dried for 24 hr and oven dried in a drying oven (YAMATO IC600, Yamato Scientific Co., Ltd., Japan) at 60°C until a constant weight was obtained and ground using a grinder (MX-151SG1, Panasonic Co., Ltd., China) to a fine consistency.

2.4 | Preparation of sprouted mung bean powder

Mung bean seeds were soaked in distilled water overnight and allowed to germinate on wet kitchen towels for 2 days. They were then air dried for 24 hr and oven dried using YAMATO IC600 drying oven (Yamato Scientific Co., Ltd., Japan) at 60°C until a constant weight was obtained and ground using a grinder (MX-151SG1, Panasonic Co., Ltd., China) to a fine consistency.

2.5 | In vitro studies

2.5.1 | Antioxidant activity

Sample preparation

Raw mung bean extract: First, 100 g of mung bean seeds were soaked in 500 mL of distilled water overnight, ground and the filtrate was used for analysis.

Boiled mung bean extract: Soaked seeds were boiled for 20 min on a hot plate, ground, and the filtrate was used for analysis.

Sprouted mung bean extract: Soaked seeds were placed on a moist filter paper and covered with a moist cloth and kept for 48 hr for sprouting. The sprouted seeds were then ground and the filtrate was used for analysis.

Assays

The antioxidant activity of mung bean extracts was determined by the FRAP (Al-Farsi, Alasalvar, & Morris, 2005) and ABTS methods (Arnao, Cano, & Acosta, 2001). Total phenolic content and total flavonoid contents were determined by the Folin–Ciocalteu and Aluminium chloride methods adapted from Singleton and Rossi (1965) and Samatha, Shyamsundarachary, Srinivas, and Swamy (2012), respectively.

2.5.2 | α-Amylase inhibitory activity

The α-amylase inhibitory activity of the extracts was determined using the glucose oxidase method (GOD) originally described by Visvanathan, Jayathilake, and Liyanage (2016). The assay system comprised the following components in a total volume of 260 μL: 40 μL of PBS (0.02 M, pH 6.9), 100 μL of GOD reagent, 40 μL of each, soluble starch (3 g/L), mung bean extract, and the enzyme solution (4 U/mL). Briefly, the enzyme solution was mixed with the mung bean extract and pre-incubated for 10 min at room temperature. The reaction was started by pipetting the starch solution into the wells and was incubated for another 15 min. Finally, 100 μL of the GOD reagent was added and the absorbance was measured at 505 nm after 15 min. As negative control, instead of sample, 40 μL of phosphate buffer was used and the absorbance was measured in parallel with samples. The results were expressed in terms of IC_{50} value.

2.5.3 | α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the mung bean extracts was assessed by the method described by Shai and Magano (2011). The α-glucosidase and substrate *p*-nitrophenyl-α-D-glucopyranoside (pNPG) solutions were prepared in 0.1 M phosphate buffer (pH 6.8). First, the sample extract (20 μL), 100 μL of 0.1 M phosphate buffer, and 50 μL of the enzyme (0.4U/mL) were added into the 96 well plate and incubated for 15 min at room temperature. After pre-incubation, 50 μL of pNPG (0.7 mM) was added and the absorbance was taken at 400 nm immediately after 30 min of incubation at room temperature. As negative control, instead of the sample, 20 μL of phosphate buffer was used and the absorbance was measured parallel with samples.

2.6 | In vivo studies

2.6.1 | Serum lipid analysis

Total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) concentrations in the serum were determined enzymatically using commercially available reagent kits (Stanbio Laboratory, TX). The non-HDL-cholesterol (non-HDL-C) concentration was calculated as follows: [non-HDL-C] = [TC]-[HDL-C].

2.6.2 | Serum glucose and insulin concentration determination

Blood glucose and blood insulin concentrations were measured using commercially available kits (Global Diagnostics Medical Solutions, Belgium and Ultra-Sensitive Rat Insulin ELISA Kit, respectively).

2.6.3 | Enumeration of cecal bacterial content

The cecal content was taken into peptone water containing tubes just after sacrificing animals. Coliform counts were carried out by inoculating diluted cecal content and incubating for 24 hr on MacConkey agar (Himedia, India) at 37°C. *Lactobacillus and Bifidobacterium* from the cecum were inoculated and incubated for 3 days on Rogosa agar (Nissui) and *Bifidobacterium* agar (Himedia, India) at 37°C by the GasPak method according to the procedure of Mitsuoka, Ohno, and Benno (1976).

2.6.4 | Statistical analysis

Data were presented as the mean and standard deviation. Completely randomized design was conducted and data were analyzed by one-way analysis of variance using the General Linear Model procedure of SAS (SAS Institute Inc., 2000) software program. The significance of differences between means was determined by Duncan's multiple range test (SAS Institute, Cary, NC). Differences were considered significant at $p < .05$.

3 | RESULTS AND DISCUSSION

3.1 | Proximate composition of mung bean powders

Data from previous publication (Chandrasiri, Liyanage, & Vidanarachchi, 2016) showed that proximate composition was different ($p < .05$) among raw (RM), boiled (BM), and sprouted (SM) mung bean powders. Various studies have shown that germination improved the nutritional value of legumes by increasing bioavailability of minerals, vitamins, increasing digestibility and reducing antinutrients and improving nutritional quality of proteins by hydrolyzing them into absorbable polypeptides and essential amino acids (Satya, Kaushik, & Naik, 2013; Subuola, Widodo, & Kehinde, 2012). Data on sprouted mung beans agreed with the above evidence showing that sprouted mung bean had higher crude protein, lower crude fat content and higher soluble fiber content than that in raw mung beans (Chandrasiri et al., 2016). Both boiling and sprouting had improved the soluble fiber content in mung beans (Chandrasiri et al., 2016) in agreement with previous findings for legumes (Benítez, Cantera, & Aguilera, 2013; Wang, Hatcher, & Tyler, 2010).

3.2 | Antioxidant activity, α-amylase inhibitory and α-glucosidase inhibitory activity of raw, boiled, and sprouted mung beans

Total phenolic content and antioxidant activity and total flavonoid content were higher ($p < .05$) in RM than BM and SM (Table 2). In vitro α-amylase and α-glucosidase inhibitory activities were higher ($p < .05$) in SM compared to RM and BM. α-amylase and α-glucosidase inhibitory activities were different among raw, boiled, and sprouted mung bean powders. However, there was no difference in serum triglycerides, serum glucose, and serum insulin level among rats fed mung bean incorporated diets. Boiling had reduced the total phenol, total flavonoid, and total antioxidant activities in mung beans which could explain the low α-amylase and α-glucosidase inhibitory activity in boiled mung

TABLE 2 Antioxidant activity, α-amylase inhibitory, and α-glucosidase inhibitory activity of raw, boiled, and sprouted mung beans

	TPC mg GAE/g	TFC mg CE/g	FRAP (mM Fe2+/g)	ABTS mM TE/100 g	α-Amylase inhibitory activity (IC_{50}) mg/mL	α-Glucosidase inhibitory activity (% inhibition at 0.2 g/mL)
Raw (RM)	401.08 ± 5.65^{a}	82.62 ± 1.37^{a}	48.87 ± 1.51^{a}	27.06 ± 1.09^{a}	50.58 ± 1.82^{b}	14.33 ± 0.85^{b}
Boiled (BM)	70.53 ± 2.49^{c}	37.92 ± 2.17^{c}	14.46 ± 1.80^{c}	20.16 ± 0.14^{b}	199.73 ± 2.55^{a}	23.10 ± 0.30^{a}
Sprouted (SM)	339.92 ± 6.99^{b}	65.35 ± 2.12^{b}	31.96 ± 1.89^{b}	12.11 ± 1.82^{c}	15.64 ± 0.65^{c}	7.97 ± 0.52^{c}

Values are expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p < .05$.

beans compared to raw and sprouted mung beans as suggested previously (Ademiluyi, Oboh, & Aragbaiye, 2015).

3.3 | Initial and final body weights, body weight gain, and feed intake

Differences ($p < .05$) in average initial body weight (data not shown) and average food intake among experimental groups were not observed throughout the experimental period. The average final body weight of rats fed the RMD and SMD diets was lower ($p < .05$) than CD fed groups showing that raw and sprouted mung beans modulated hypercholesterolemic diet induced body weight gain.

3.4 | Serum total cholesterol, HDL-C, non-HDL-C, TG, serum glucose, and insulin concentration

There was no difference ($p < .05$) in serum total cholesterol concentration among the groups at the beginning and end of the experiment. Increase in serum non-HDL cholesterol concentration and decrease in HDL-C concentration caused by high cholesterol diet were modulated ($p < .05$) by boiled and sprouted mung bean diets (Figure 1a). Furthermore, the elevated TG level (Figure 1a) by high cholesterol diet was lowered ($p < .05$) by all three mung bean incorporated diets. Lower serum non-HDL-C and higher serum HDL-C concentrations in the boiled (BMD) and sprouted (SMD) mung bean powder diet-fed rats showed that processing improved the hypocholesterolemic ability of mung beans. Hypercholesterolemia is associated with elevation of non-HDL-C and reduction of HDL-C level, and thus modulating both cholesterol levels in processed mung bean diet-fed rats may be useful as a therapeutic treatment.

Serum non-HDL and HDL-C level in both BMD and SMD diet-fed rats were supported by higher soluble dietary fiber content (Chandrasiri et al., 2016) in boiled and sprouted mung beans and lower liver weight (Table 3) in SMD diet-fed rats and higher fecal weight (Table. 3) in rats fed mung bean incorporated diets (Bazzano, Thompson, & Tees, 2011). Hypocholesterolemic effect of soluble fibers has been reported in experimental animal models and humans (Pande, Platel, & Srinivasan, 2012; Sood, Baker, & Coleman, 2008). Soluble fibers physically bind to bile acids and entrap cholesterol resulting in lowered cholesterol absorption which leads to increased bile acid synthesis, reduced hepatic cholesterol, upregulated LDL receptors, and increased LDL clearance (Brown, Rosner, Willett, & Sacks, 1999). Processed mung beans, by virtue of their rich soluble fiber content, may be of therapeutic value for control of hypercholesterolemia. The reduction of non-HDL-C and TG levels in mung bean incorporated diets may be attributed to the inhibition of hepatic cholesterogenesis by decreasing the HMG-CoA reductase activity and catabolic conversion of cholesterol to bile acids in the liver as reported previously (Yao, Zhu, & Ren, 2014).

Tachibana et al. (2013) reported that mung bean protein isolates showed hypolipidemic and hypoglycemic potential in rats suggesting that dietary proteins with favorable amino acid composition and sequence reduce the serum cholesterol level in rats. Sulfur-containing amino acids have also been shown to have an increasing effect on HDL-C and decreasing effect on LDL-C levels in serum (Yao et al., 2014).

The serum glucose concentration (Figure 1b) was significantly ($p < .05$) lower in rats fed RMD, SMD, and BMD than those in the CD fed group at the end of the 5 weeks feeding period. The serum insulin concentration (Figure 1b) was significantly ($p < .05$) lower in rats fed RMD and BMD than the CD-fed rats. Lower serum glucose level in

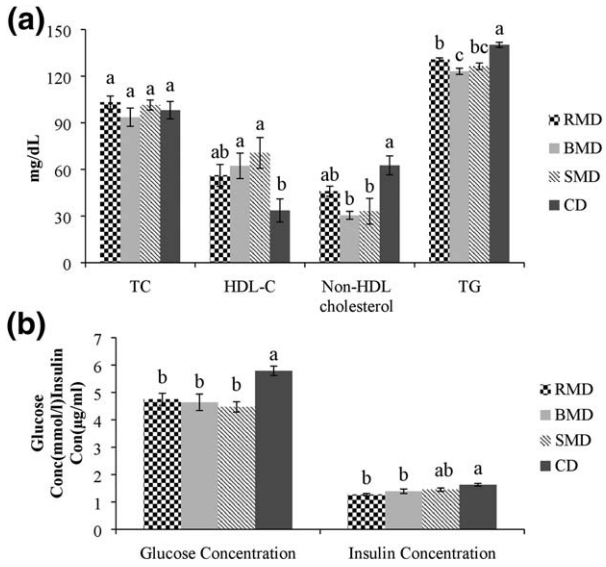

FIGURE 1 (a) Serum total cholesterol, high density lipoprotein cholesterol (HDL-C), Non-HDL-cholesterol (Non-HDL-C) and triglyceride (TG) concentration in rats fed with experimental diets for 5 weeks. Values are expressed as mean ± *SD*. Mean values within a group with different superscript letters are significantly different at $p < .05$. (b) Serum glucose and serum insulin concentration in rats fed with experimental diets for 5 weeks. Values are expressed as mean ± *SD*. Mean values within a group with different superscript letters are significantly different at $p < .05$

TABLE 3 Cecal weight, liver weight, and fecal matter weight of rats fed on experimental diets for 5 weeks

Diet group	Cecal weight (Wet g/100g b.w.)	Liver weight (Wet g/100g b.w.)	Fecal matter weight (g/rat/day)
RMD	0.70 ± 0.07^{a}	4.02 ± 0.13^{ab}	3.56 ± 0.04^{a}
BMD	0.64 ± 0.04^{ab}	4.18 ± 0.13^{ab}	3.25 ± 0.03^{b}
SMD	0.64 ± 0.05^{ab}	3.97 ± 0.11^{b}	2.63 ± 0.04^{c}
CD	0.56 ± 0.02^{b}	4.38 ± 0.10^{a}	2.07 ± 0.05^{d}

Values are expressed as means ± *SD*. Mean values within a column with different superscript letters are significantly different at $p < .05$.

mung bean incorporated diet-fed rats and lower serum insulin level in rats fed RMD and BMD diets support previous studies showing that mung bean nutrition had significant antidiabetic effect with potential for treating type 2 diabetes (Tachibana et al., 2013; Yao et al., 2014). It has been suggested that antidiabetic effect in mung beans may be due to its high fiber content and low glycemic index (Lerer-Metzger, Rizkalla, & Luo, 1996). Furthermore, the hypoglycemic effect observed in sprouted mung bean fed rats was supported by higher α-amylase inhibitory activity and α-glucosidase activity of sprouted mung beans.

3.5 | Cecal weight, liver weight, and fecal matter weight of rats fed on experimental diets for 5 weeks

The wet relative weights of the cecum (Table 3) were ($p < .05$) higher in RMD fed group than CD fed group, supported by higher total and insoluble dietary fiber content in raw mung beans than processed mung beans. The average liver weight was lower ($p < .05$) in SMD fed rats than the CD-fed rats while the average fecal matter weight of RMD, BMD, and SMD fed groups were significantly ($p < .05$) higher than CD fed group.

3.6 | Cecal bacterial population

The coliform bacterial population (Table 4) was lower ($p < .05$) in rats fed RMD, BMD, and SMD than CD fed rats. There were no differences in cecal *Lactobacillus* and *Bifidobacterium* population in rats fed experimental diets. The microbial population in the cecum provides evidence for cecal fermentation (Liyanage et al., 2007). Although there was no difference in *Bifidobacteria* and *Lactobacilli* populations among experimental groups, lower cecal *coliform* population in mung bean diet-fed rats compared to the control group indicated that mung bean diets may have improved the cecal microbial balance in rats as suggested previously (Akanbi & Agarry, 2014).

TABLE 4 Cecal microbial population in rats fed experimental diets for 5 weeks (log 10 cfu/g content)

Diet group	*Coliforms*	*Bifidobacterium*	*Lactobacillus*
RMD	3.17 ± 0.30^{a}	3.81 ± 0.47^{a}	5.73 ± 0.34^{a}
BMD	3.77 ± 0.12^{a}	3.91 ± 0.47^{a}	6.50 ± 0.34^{a}
SMD	3.51 ± 0.40^{a}	4.64 ± 0.47^{a}	5.93 ± 0.34^{a}
CD	4.16 ± 0.18^{b}	4.72 ± 0.47^{a}	6.16 ± 0.34^{a}

Values are expressed as means ± *SD*. Mean values within a column with different superscript letters are significantly different at $p < .05$.

4 | CONCLUSIONS

Results of this study showed that, processing reduced the total phenolic content, total flavonoid content, and antioxidant activity in mung beans while improving the soluble fiber content and in vivo hypocholesterolemic potential and maintaining the hypoglycemic potential. The results suggest that processed mung beans may be effective for treatment of hypercholesterolemia and hyperglycemia. It remains to be determined whether these effects are also valid in humans. Furthermore, toxicity evaluation of long-term feeding of high doses of processed mung beans would be necessary.

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CONFLICT OF INTEREST

The authors declare that this manuscript has no conflicts of interest.

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