

Influence of blanched mung bean seeds on controlling blood glucose of diabetic male rats

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Abstract: Mung bean (*Phaseolus aureus* L.) is a leguminous SP. grown principally for its protein rich edible seeds. The seeds are prepared by cooking, fermenting, milling or sprouting. The present investigation aimed to study the effect of mung bean seeds, (raw), (raw and blanched) and (sprouted and blanched) on controlling blood glucose of diabetic animals. Chemical composition, minerals, phenolic compounds of the aforementioned materials were determined. To induce hyperglycemia, 50 rats (weighed 100 ± 5 g) were treated subcutaneous injection with alloxan (150 mg/kg body weight), then divided into ten groups (n= 5). The diabetic rats fed raw, raw and blanched and sprouted and blanched mung bean at the levels of 20, 30 and 40% of the diet, respectively, meanwhile the negative control (n= 5) and positive ones fed basal diet. At the end of the experiment (60 days), serum glucose, ALT, AST, ALP, LH, FSH and testosterone were determined. The data revealed that the sprouted and blanched mung bean seeds were the superior for controlling blood glucose (251, 246.7 and 249 vs. 118, 110 and 110 mg/dl under 20%, 30% and 40%, respectively) followed by blanched seeds (251.3, 240, and 242 vs. 124.7, 121.9 and 121.5 mg/dl under feeding of 20, 30 and 40%, respectively) and raw seeds which was slightly high. An improving in AST, ALT and ALP were found in parallel with that of blood glucose. Feeding mung bean resulted in significant decrease in FSH and LH and increase in testosterone compared with diabetic control.

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1. Introduction

Similar to most legumes, mung bean or green gram contains about 24% protein, 1% fat, 43% carbohydrates and 16% dietary fiber, in addition to considerable amounts of vitamins (A, B, C and E) and mineral (Dahiya *et al.* 2013). For food, seeds are prepared by cooking, fermenting, milling or sprouting (Khader and Raw, 1996). Dahiya *et al.* (2013) analyzed seven newly and three established varieties of mung bean for proximate composition, minerals, antinutrients and *in vitro* mineral accessibility. They contained 18 – 23g protein, 4 – 5.6g crude fiber and 2.5 – 4.1g ash/100g dry sample. Fe, Zn, Ca, Na and K ranged from 3.4 to 4.6, 1.2 – 2.3, 79 – 115, 8.1 – 13.5 and 362 – 415 mg/100g sample, respectively. They found that phytic acid and polyphenols were negatively correlated with *in vitro* minerals accessibility and nutrient digestibility. Protein and starch digestibility ranged from 53 to 67% and 20 – 29 mg maltose released/g dry weight. Mung bean (*Phaseolus aureus*, syn. *Vigna radiatus*) are thought to be beneficial as an anti-diabetic, low glycemic index food, rich in antioxidant and have lipid – lowering effects (Lee *et al.*, 2000). Bhatti *et al.* (2000) studied the effect of heat and cooking on the nutritional value of mung bean. They found that protein efficiency ratio (PER) was decreased by cooking but it improved the net protein utilization (NPU) and true digestibility (TD) of the mung bean diet. Mung bean substrate was enriched with phenolic antioxidants and levo-dihydroxy

phenylalanine (L-DOPA) through solid-state bioconversion (SSB) by *Rhizopus oligosporus*, with the goal to enhance health-linked functionality. The major implication is that SSB is a good strategy to improve the phenolic content of mung bean for enhance functionality with improving antioxidant activity that contributes to α -amylase inhibition relevant to potential diabetes management, (Randhir and Shetty,2007). Yao *et al.* (2011) reported that mung bean (*Vigna radiate* L) is rich in bioactive compounds including D-Chiro-inositol (DCL), vitexin and isovitexin which have beneficial effects on patients with diabetes. Stephan *et al.* (2003) evaluated the methanolic extract as estrogenic effect of mung bean sprout compound with other extracts of soybean, alfa alfa, green beans, kudzu root, red clover blossom and its sprouts. They found that the extracts exhibited preferential activity towards the estrogen receptor B (ERB) and these legumes are sources of phytoestrogen with high levels of estrogenic activity. The present investigation aimed to study the relationship between mung bean seed and controlling blood glucose of diabetes.

2. Material and Methods

Materials:

Mung bean seeds (*P. aureus* L) were obtained from field crops research Institute, Agric. Res. Center, Giza, Egypt. All chemicals and authentic references, materials were Sigma products. Male albino rats (55)

of Sprague Dawley strain were obtained from the animal house, FTRI, ARC, Giza, Egypt.

Methods:

After cleaning, mung bean seeds were divided into three portions, the 1st was found as raw material, 2nd was blanched and the 3rd was sprouted and blanched. The three portions were subjected to the following analysis. Chemical composition, minerals using Perkin-Elmer atomic absorption spectroscopy, and total phenols according to the methods outlined in AOAC (2000).

Phenolic compounds were fractionated using HPLC according to the method described by Ben-Hammouda *et al.* (1995).

Biological investigation:

The experimental animals of Sprague Dawley strain (55 rats weighed 100 ±5g) were housed in well aerated cages under hygienic conditions and fed basal diet (Reeves *et al.*, 1993) for one week for adaptation, then divided into two main groups. The 1st one (5 rats) fed basal diet (-ve group), and the 2nd ones treated subcutaneous injection with alloxan (150 mg/kg body weight) to induce hyperglycemia, then divided into 10 subgroups as follows:

- 2nd fed basal diet (+ve control).
- 3rd fed 20% raw mung bean.
- 4th fed 30% raw mung bean.
- 5th fed 40% raw mung bean.
- 6th fed 20% blanched mung bean.
- 7th fed 30% blanched mung bean.
- 8th fed 40% blanched mung bean.
- 9th fed 20% sprouted and blanched mung bean.
- 10th fed 30% sprouted and blanched mung bean.
- 11th fed 40% sprouted and blanched mung bean.

At the end of the experimental period (60 days), the rats were fasted over night before sacrificing and blood was collected from orbital venous plexuses. The blood was centrifuged at 4000 rpm for 15 min to obtain serum. The following determinations were carried out. Serum glucose, was determined according to the method described by Trinder (1969). ALT and AST were determined as described by Thefweld (1974), while ALP was determined using the method of King and King (1954). Luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone hormone were measured by Radio Immune Assay(RIA) methods according to the methods of Glzieher and Dozier (1976). The obtained data were statistically analyzed using student's t-test (Snedecor and Cochran, 1982).

3. Results and Discussion

Chemical composition of raw and sprouted mung bean seeds either balanced or un- blanched were determined (Table 1). The data revealed that mung bean seeds characterized with high protein content (26.8%), carbohydrates (50.12%) and dietary fiber

(17.92%). Sprouting slightly increased protein, fat, fiber and ash and decreased markedly carbohydrates. Blanching of raw or sprouted mung bean seeds affected chemical composition by decreasing protein content and carbohydrates and increasing dietary fiber and ash content. The data cleared that blanching resulted in releasing of some protein (albumin) to the blanching water and also soluble sugars meanwhile, sprouting resulted in decreasing carbohydrates due to metabolic pathway during sprouting. Blanching of raw mung bean seeds or sprouted ones decreased all minerals (Table 2) due to its partial solubility in blanching water. On the other hand, all minerals increased due to sprouting compared with the raw ones which may be due to the decreasing of some components during sprouting. It could be pointed that Fe, P, K, Na and Zn were increased by about 1.28, 1.32, 1.32, 2.86 and 1.47 times due sprouting followed by blanching compared with raw and blanched ones, respectively. The other minerals slightly affected due to either sprouting or blanching. *P.aureus* seeds have shown to contain a considerable amount of iron especially after sprouting. It is well known that Fe play an essential role in iron deficiency anemia, the most common nutritional deficiency disorder in Egypt. (Kamal *et al.*, 2002). Table (3) shows the effect of sprouting and blanching in the total phenols and its fractions of mung bean seeds. The data revealed that total phenols decreased by either sprouting or blanching by about 8%, 9.87% and 18.22% for blanched raw, sprouted and sprouted followed by blanching, respectively compared with the raw seeds. The major phenolic compounds were caffeic, chlorogenic and ferulic which amounted in 7.43, 6.72 and 5.66 mg/100g seeds which amounted in 48.65% of the total phenols of raw seeds. Similar trend was found due to the blanching or sprouting and blanching. The decreasing of the phenolic compounds may be due to solubility or the metabolic process during sprouting. Table (4) showed a significant increase in serum glucose of the alloxanzied experimental animals after 4 days of injection. Feeding mung bean resulted in decreasing serum glucose after 14 days and continuous decreasing was observed till 60 days of the experiment. It is worth mentioning that the sprout and blanched mung bean resulted in almost the same glucose content as that of control (-ve) at the end of the experiment (110 vs 111 mg/dL) followed by raw and blanched mung bean (121 vs 111mg/dL). This means that the sprouted and blanched mung bean seeds improved the health status of diabetic animals. The hypoglycemic effect of mung bean can be explained through different mechanisms. Mung bean seeds contained complex carbohydrates, vegetable protein, dietary fiber and phytoestrogens particularly isoflavones Genistein and diadzein (Vijay *et al.* 2002). Moreover, isoflavones have antidiabetic properties

such as inhibiting intestinal branch border uptake of glucose, having α -glucosidase inhibitor action and demonstrating tyrosine kinase inhibitory properties (Lee and Lee, 2001). Besides the above mentioned mechanisms, change in insulin receptor numbers and affinity, intracellular phosphorylation and alteration in glucose transport was affected due to isoflavones. In addition, the protective effects of isoflavones have been attributed mainly to their antioxidant properties (Vedavanam *et al.*, 1999). Also, isoflavones scavenge free radical, chelate redox active metal ions and increase metallo thionine expression; the enzyme which can protect cells from heavy metal toxicity and exerts antioxidant activity (Lee and Lee, 2001). Recently, Yao *et al.* (2011) reported that mung bean (*Vigna radiata*) is rich in bioactive compounds including D-Chiro-inositol(DCL), vitexin and isovitexin which have beneficial effects on patients with diabetes. AST, ALT and ALP showed a values of 79.7, 32.8 and 7.3 IU for the control(-ve) which increased markedly after alloxanization (134.4, 39.2 and 18.8 IU, respectively). Feeding mung bean seeds resulted in decreasing AST, ALT and ALP according to the processing method. It could be mentioned that sprout and blanched mung bean seeds resulted in best treatment which showed AST, ALT and ALP values almost the same of control(-ve) followed by blanched

raw and raw seeds at the end of the experiment. The data confirmed the serum glucose development due to feeding mung bean seeds. Table (5) showed the effect of feeding processed mung bean seeds in follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone hormone (TH) of the experimental animals. The data revealed an increase TH and decrease in LH and FSH compared with control (+ve) this can be explained due to phytoestrogen which are a diverse group of non - steroidal plant derived compounds that are structurally similar to estrogen steroids and have an affinity for estrogen receptor and acts as estrogen agonist and estrogen antagonist (Helna *et al.*, 2005). Due to their chemical similarity to estrogen, phytoestrogen compete with own estrogen for cellular receptor sites, if estrogen level is high, plant derived estrogen decrease over all estrogen activity, as estrogen fall, they can provide some of the same function as estrogen (Paley, 2005). Where phytoestrogen bind to estrogen receptor and exert both hormonal and anti-hormonal effects (Kurzer, 2003). The obtained data were supported by Huang *et al.* (2001) who reported that the regulation of LH and FSH in mammals is accomplished primarily not only through the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus but also due to feed back effects of steroids.

Table (1): Chemical composition of sprouted and blanched mung bean seeds compared with raw ones (on dry weight basis)

Chemical composition (%)	Raw		Sprouted	
	Unblanched	Blanched	Unblanched	Blanched
Crude protein	26.80	24.37	27.83	23.92
Total carbohydrate	50.12	46.68	35.65	34.82
Total lipids	1.26	1.15	2.61	2.55
Dietary fibre	17.92	23.17	28.72	31.36
Ash	3.90	4.63	5.21	7.35

Table (2): Minerals content of sprouted and blanched mung bean seeds compared with raw ones (on dry weight basis)

Element (mg/100g)	Raw		Sprouted	
	Unblanched	Blanched	Unblanched	Blanched
Ca	123.36	111.20	113.00	103.00
Fe	5.93	5.40	7.82	6.91
Mg	155.85	140.75	156.52	150.11
P	343.00	311.95	426.10	412.32
K	958.98	870.20	1165.20	1151.13
Na	14.84	13.53	43.48	38.70
Zn	2.51	2.15	3.48	3.17
Cu	0.67	0.58	0.81	0.66
Mn	1.14	0.98	1.12	0.97

Table (3):The phenolic compounds content of sprouted and blanched mung bean seeds

Phenolic compounds (mg/100g seeds)	Raw		Sprouted	
	Unblanched	Blanched	Unblanched	Blanched
Gallic acid	3.18	2.91	2.97	2.73
Proto catechuic acid	4.53	4.17	4.34	3.90
Chlorogenic acid	6.72	6.10	6.28	5.60
Caffeic acid	7.43	6.91	7.12	6.34
P-Coumaric acid	2.99	2.74	2.88	2.62
Ferulic acid	5.66	5.11	5.21	4.85
Coumarin	4.58	4.13	4.38	4.00
Cinnamic acid	2.63	2.47	2.50	2.28
Total phenols	40.72	37.45	36.70	33.30

Table (4): Effect of feeding sprouting and blanching of mung bean seeds on blood glucose and liver function of diabetic animals

Treatments	Serum glucose (mg/dl)			Liver function at the end of the experiment (60 days)		
	After alloxanization (4 days)	After 30 days	At the end of experiment (60 days)	AST (IU)	ALT (IU)	ALP (IU)
Control(-)G1	111.67±4.1	109.67±4.5	111.0±4.1	79.7±0.5	32.8±1.1	7.3±0.2
Control(+)G2	255.81±9.6*	248.33 ±7.6*	241.7±6.07*	134.4±2.3*	39.2±1.3*	18.8±1.1*
Fed raw mung bean						
20% G3	301.5±7.41*	177.3 ±6.5#	143.7±9.1#	115.1±2.0*	25.9±0.6*	16.1±0.7*
30%G4	270.3±5.9*	161.7±2.7#	139.81±7.8#	100.1±1.8*	31.14±1.4*	14.1±0.7*
40% G5	252.7±8.6*	139.38±1.4#	121.76±8.8#	96.0±2.0*	31.6±1.4*	10.3±0.1*
Fed blanched raw mung bean						
20% G6	251.3±2.1*	133.7±3.7#	124.7±8.5#	94.2±1.9*	31.5±1.2*	18.7±1.1*
30%G7	240.0±8.3*	147.2±4.2#	121.9±1.5#	93.6±3.1*	29.8±0.8*	14.8±1.3*
40% G8	242.1±9.6*	145.3±4.8#	121.5±4.6#	89.5±2.5*	28.1±0.6*	13.2±0.9*
Fed blanched sprouted mung bean						
20% G9	251.0±7.6*	140.1±1.8#	118.7±4.5#	85.9±1.4*	29.3±0.8*	7.8±0.1
30%G10	246.7±5.4*	131.6±2.0#	110.1±5.0#	80.5±1.2*	27.9±0.7*	7.5±0.2
40% G11	249.1±6.5*	128.6±1.7#	110.1±6.2#	78.8±0.8	25.9±0.6*	7.2±0.1

* Significant compared with negative control. # Significant compared with positive control.

Table (5): Effect of feeding sprouted and blanched mung bean seeds on serum Follicle stimulating (FSH), Luteinizing (LH) and Testosterone (TH) hormones of diabetic animals

Treatment	Follicle stimulating hormone (FSH) (mIU/ml)	Luteinizing hormone (LH) (mIU/ml)	Testosterone hormone (TH) (ng/ml)
Control(-ve) G1	0.26±0.4	2.33±0.35	1.14±0.15
Control(+ve) G2	1.43±0.26*	3.50±0.37*	0.56±0.08*
Fed raw mung bean			
20% G3	0.41±0.14#	1.03±0.1*#	1.53±0.02#
30%G4	1.16±0.08*	0.96±0.03#	1.98±0.15#
40% G5	1.11±0.14*	1.82±0.16#	3.11±0.07#
Fed blanched raw mung bean			
20% G6	0.56±0.12#	0.86±0.03*#	1.35±0.08#
30%G7	0.58±0.02#	1.88±0.06#	1.78±0.01#
40% G8	0.92±0.13*	1.95±0.03#	2.48±0.12#
Fed blanched sprouted mung bean			
20% G9	0.77±0.03#	0.95±0.02*#	1.34±0.07#
30%G10	0.82±0.01#	1.45±0.06#	1.88±0.02#
40% G11	1.35±0.01#	2.05±0.03#	2.80±0.06#

* Significant compared with negative control. # Significant compared with positive control.

From the above mentioned data, it could be concluded that sprouted and blanched mung bean seeds resulted in improvement the health status of the diabetic animals.

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