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Biochemical and functional properties of Mung bean and its utilization in development of high fiber diet

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Abstract

Our present work was aimed at studying the proximate analysis of Mung bean, fiber, profile and production of high fiber diet which make reduction of plasma glucose level in diabetic rats. Compositional evaluation of Mung bean and its byproducts was carried out by percentage analysis. The estimated values of mung bean 8.85, 22.9, 1.04, 3.8, 3.76 were observed for moisture, protein, fat, fiber and ash respectively. The percentage contents of lignin, cellulose, and total carbohydrates contents of mung bean calculated as 13.9, 6.1 and 60.0 respectively. Likewise, Mung bean hull contained 4.4% moisture, 15.2% protein, 3% ash, 0.28% fat, 28.3% fiber, 4.5% lignin, 37.2% cellulose and 47.8% carbohydrates. In third analysis it was noted that bleached Mung bean hull had 2.4% moister 3.4% ash, 0.4% fat, 36.6% fiber, 7.6% protein, 2.3% lignin, 58.8% cellulose and 49.5% carbohydrates. The results of minerals analysis of Mung bean showed that Na (1800mg/100g), K (1032mg/100g), Ca (198 mg/100g), Mg (177mg/100g) and Fe (9.82mg/100g) in that order. Rats were made diabetic with alloxan determine the high fiber diet effect on plasma glucose in diabetic rats. The research showed that the high fiber diet of Mung bean reduced the plasma glucose level in all experimental diabetic rats as compared to control.

Keywords: Biochemical, funcationa, properties, mung bean, fiber, diet.

Introduction

By growing scientific investigations it is suggested that plant-derived food stuff have various potential health benefits, which is resulting in greater pressure of their consumption at a rate of 5%-10% per year. Moreover, expanding worldwide health organizations consequently have great emphasis on the need of food ingredients with multiple functional properties to improve health status and prevent chronic diseases[1]. Mung bean (*Vignaradiata*) commonly known as green gram, is an important pulse crop of Pakistan. In most of Asian countries, it is a diet cereal based. Mung bean is a rich source of protein (23%) complex carbohydrates (dietary fiber) minerals, and vitamins are considerably important in Asian vegetarian diet[2] Beans contain a number of bioactive components that are known to reduce the risk of coronary heart disease, diabetes, and obesity, and can considerably lower blood cholesterol[3]. Mung beans or green bean is one of the most important early summer-growing legumes grown widely throughout the tropics and subtropics reigns[4] and can be cultivated by many different cropping systems. It constitutes important cereal-based diets too many people in Pakistan, India, Thailand, Indonesia, the Philippines, and China[5]. It consumed indifferent form and way for example, as a viand, boiled, or cooked with vegetables or meat, as well as dessert or incorporated in bread or cake. It can be used to make sprouts for egg rolls and vegetable dishes[6]. Mung beans are known for their good digestibility and low flatulence. They are rich in phosphorus and provitamin A and relatively free from anti nutritional factors. The high protein levels and high lysine/ low methionine amino acid profile of mungbean complement the high carbohydrate and low lysine/ high methionine content of cereals to form a much blanched amino acid diet.

Mung bean is also well known in China for its potential of detoxification and is used to relax mental nerves, improve body temperature regulating, and help to reduce swelling in the summer. It is observed through many researches that it is also necessary for improving digestion and regulating the excretion system and to moisturize the skin[7]. Not only in conventional dishes but the seeds and sprouts of mungbeans are also widely used as a fresh salad vegetable or common food in Pakistan, India, Bangladesh, South East Asia, and western countries[8].

In addition, Mung beans have been shown to exert antitumor effects through several different mechanisms. The recombinant plant nucleases R-TBN1 and R-HBN1, similar to nucleases derived from pine pollen and mung beans, were found to be effective against melanoma tumors and were about 10-times more potent than bovine seminal ribonuclease (RNase). Due to their relatively low cytotoxicity and high efficiency, these recombinant plant nucleases appear to be stable biochemical agents that can be targeted as potential antitumor cytostatics[9]. Overall, regular consumption of mung beans could regulate the flora of enterobacteria, decrease the absorption of toxic substances, reduce the risk of hypercholesterolemia and coronary heart disease, and prevent cancer[10].

The aim of the current study was to determine the nutritional composition of mung bean and its hypocholesterolaemic effect

on hypercholesterolaemic rats. As high fiber diet of Mung bean has potential to reduce cardiovascular disease risk more than other various sources of soluble fiber. Thus one of objective of this study is to ascertain this assumption by trials on chicks.

Materials and methods

The samples of Mung bean (seed) and Mung bean hull were procured from nearby market in Lahore for whole study. Mung bean and hull was sieved for cleaning. After drying the mungbean grains and its hull all extraneous matter was removed. Cleaned grains and hull was stored in zip lock bags for further analysis and processing. Mung bean and Mung bean hull used for proximate analysis.

Proximate Analysis: The moisture content of Mung bean sample was measured by the weight difference before and after oven drying at 120°C for 4h, and then extracting the fat with ether in a Soxhlet extractor for 4h[11]. Ash contents were assessed by using method of Kruawan K. et.al.[11] 3-5g of dried sample was in use in each determination. Crude protein content of Mung bean and hull was measured by the Kjeldahl method[11]. Fiber content was determined by digestion with acid and alkali using Fiber Tech System-M (Tecator).

Estimated of Lignin: Lignin content was determined by the standard method of ASTM 1961. According to this method refluxing of 1g of defatted sample with 70 mL 1.25% H_2SO_4 for 2 hours was done. Refluxed sample was then washed with hot water and then with chloroform. Washed sample was treated with 72% H_2SO_4 for four hours with constant stirring. Sample was filtered (add 10-12 mL distilled water) with Whatman filter paper No-1 and ignited it at 550°C for 4 hours.

Estimation of Cellulose: Cellulose estimated by using the method of refluxing.1g of defatted sample was refluxed with 15 mL of 80% acetic acid and 1.5 mL conc. HNO_3 acid for 2 hours. Then washing of refluxed sample was done. After thorough washing, sample was treated with 72% H_2SO_4 for 4 hours with constant stirring. In next step sample was filtered (add 10-12 mL distilled water) using Whatman filter paper No-1 then washed with distilled water until it become neutral and finally washing with alcohol to remove foreign contents and ignited it at 550°C for 6 hours[27].

Estimation of Carbohydrates: The Carbohydrates were determined by Subtraction method.

Fiber Extraction by bleaching: Mungbean hull were washed with distilled water to remove all the impurities, and hull treated with food grade hydrogen per oxide in an alkaline environment by using few drops of 40% NaOH (pH 10-12) for 1-2h at elevated temperature (50°C-100°C). Then the sample washed with distilled water thoroughly and after that neutralized with 0.1% HCl and again washed with distilled water to remove all the residue of acid. The bleached sample was dried with hot air and all the bleached sample was ground well[12].

Mineral Analysis: The dried powdered samples were first digested with nitric acid and perchloric acid and then the aliquots were used for the determination of sodium, potassium, calcium, magnesium and iron content. Sodium and potassium were determined by flame photometer[13]. Iron, calcium and magnesium determined by atomic absorption spectrophotometer[14]. Preparation of Acid Digest For the analysis of minerals, 1g powdered sample was taken in a 100ml digestion flask. 10ml of nitric acid (HNO_3) was added to it and the flask was placed in dark overnight. On next day, 5ml of the perchloric acid ($HClO_4$) was added to it. The mixture was then placed on a hot plate at 50°C for 15minutes and then the temperature was raised slowly up to 200°C. Heating was continued till the white dense fumes of perchloric acid were disappeared. After digestion, the contents were cooled and filtered through Wattman filter paper (#2). Then it was transferred to a 50ml volumetric flask and diluted with deionized water up to the mark.

Composition of Diet fed: 1kg Mung bean fiber diet contained 18% casein which is necessary tocomplete the protein requirement in growth and development of animal, 7.5% corn oil was incorporated in provided formulation to get necessary fatty acids. For source of carbohydrates corn starch was mixed. Next 1% vitamins and 3.5% mineral mixture was in diet respectively. 45% Mung bean bleached hull was used as a fiber source. Diet and water were provided ad libitum[15].

Table-1: Composition of high fiber diet.

Ingredients	Amount (%)
Mung bean fiber	45
Casein	18
Corn oil	7.5
Corn starch	25
Mineral mixture	3.5
Vitamins	1

Functional properties of Mung bean high bran feed: Prepared high bran feed was thenutilized for the fortitude of purposeful characteristics of Mung bean by the evaluation of antihyperglycemic influence of high fiber formulation on hyperglycemic rats.

Experimental Design and Induction of Hyperglycemia: i. Alloxan, ii. Enzymatic kits for biochemical analysis of blood serum for estimation of glucose and cholesterol level.

Choice of Animals: In present study albino rats were selected as experimental animals because of several reasons such as biochemical and histopathological changes produced in rats are

relatively similar as observed in humans, general physiology of rats is similar to humans, rats are easily available, easy to handle and economical. The study was conducted on thirty healthy albino rats of either sex weighing between 200 to 300gm. Rats were reared in cages, under controlled conditions of temperature (22±2°C) and humidity (50-60%). Rats were divided into three groups equally. Each group was consisting of 10 rats and these rats were left 30 days one group treated as a control while the other remaining group received the drug singly and in combination.

Alloxan: Alloxan included hyperglycemia has been described as a useful experimental model to evaluate the activity of hyperglycemic agents. Diabetes was induced by a single intraperitoneal injection of Alloxan at a dose of 100mg/Kg body weight.

Enzymatic Kits: Commercial kits of the company Randox were used to determine serum glucose and serum cholesterol levels in albino rats by spectrophotometer.

Experimental Design: The animals were divided into five groups. Each group consisted of eight rats. Diabetes was induced by a single intraperitoneal injection of Alloxan prepared in 0.1mol/L citrate buffer at a dose of 100mg/kg body weight. The rats with blood glucose level above 150mg/dl were considered as diabetic and were used in the further experiments. Diabetes was confirmed in the Alloxan treated rats by measuring the fasting blood glucose after 10 days.

Table-2: Animal conditions and Treatment.

Groups	Animal conditions	Treatment
1	Normal (Control)	Chick starter diet
2	Normal+high fiber diet	High fiber Mung bean (45% fiber)
3	Hyperglycemic+high fiber diet (diabetes)	High fiber Mung bean (45% fiber)

Blood collection and analysis: From coccygeal vein of albino rats 1ml blood was collected. Then by centrifugation of Blood at 3000rpm for 10minutes, serum was parted. Commercial kits of the company Randox were used to determine serum glucose levels in albino rats by spectrophotometer.

Estimation of Serum Glucose: Principle: Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as an indicator.

Reaction

Glucose+ O_2 +H_2O →Gluconic acid + H_2O_2

$2H_2O_2$+4-aminophenazone+ phenol →<u>peroxidase</u> Quinoneimine +$4H_2O$

Sample: Serum: Glucose is stable for 24 hours at + 2 to + 8°C if the serum was prepared with in 39 min. after collection.

Reagent: i. Enzyme Reagent, ii. Standard.

Procedure: 12 test tubes were taken; two tubes out of 10 were labeled as blank and standard. Remaining 10 test tubes were labeled as 1, 2, 3……10 for sample of rat serum discretely from each diabetic group. By micro pipette 1000µl reagent was added in all the tubes. 10 µl of standard solution from the kit was added to the tube labeled as standard and 10 µl of serum sample was taken in tubes labeled as 1, 2, 3……10. Incubation of all the tubes at 37°C for 10 minutes after thorogh mixing of whole tube contents was done. After incubation, the absorbance of standard (AbsStd) and the sample (AbsS) was measured against the blank (AbsB) at wavelength of 546nm. This process was used for the rest of samples.

Normal values: Serum, plasma (fasting): 75-115mg/dl.

Calculations: The concentration of glucose in serum was calculated by the following formula: Glucose concentration (mg/dl)= (A test/A standard) x 100[16].

Statistical Analysis: All Satistical Analysis was done with analysis of variance (ANOVA) p values less then ($p<0.05$) was considered of statistical significance and if more than ($p>0.05$) than it was in significant[17].

Results and discussion

Proximate analysis: A study was conducted to investigate the biochemical composition of Mung bean whole grain hull and bleach hull. The components of chemical analysis are moisture, ash, fat and fiber, protein, carbohydrate and minerals (All determinations are in %). As well as, to find out the fibre profile (Lignin and cellulose) in Mung bean whole grain and hull. High fiber diet was prepared from Mung bean grain and hull for normal and diabetic rats. Rats were fed ad libitum one of five diets, all the determinations was carried in triplicate (AOAC, 2012).

Table-3: Moisture, Ash and Fat contents in Mung bean (whole grain, hull and bleached hull)

Mung bean samples	Moisture contents Mean ± S.D	Ash contents Mean ± S.D	Fat contents Mean ± S.D
Whole grain	8.85 ± 0.02	3.76 ± 0.05	1.04 ±0.03
Hull	4.4 ± 0.29	3.0 ± 0.1	0.28 ± 0.01
Bleached Hull	2.4 ± 0	3.4 ± 0.17	0.4 ± 0.01

The mean values were calculated by applying standard deviation of three replications. Table-3 depicted that the mean value of

moisture, Ash and Fat contents in three trial samples of Mung bean. It was noted that the whole grain of Mung bean had highest value of moisture (8.85%) than the treated (2.4%) and untreated hull (2.4%).Similar pattern was indicated in case of ash contents, in whole grain ash was higher (3.76%) as compared to bleached hull (3.4%) followed by untreated hull (3.0%). Fat contents in whole bean 1.04% bleached hull 0.4% and in hull was 0.28% respectively.

Table-4: Fiber, Protein contents and carbohydrates in Mung bean (whole grain, hull and bleached hull).

Mung bean samples	Fiber contents Mean ± S.D	Protein contents Mean ± S.D	Carbohydrates %
Whole grain	3,8 ± 0	22.9 ± 0.21	60.00
Hull	28.3 ± 0.5	16.2 ± 0.32	47.82
Bleached Hull	36.6 ± 0.58	7.6 ± 0.15	49.59

The mean values were calculated by applying standard deviation of three replications.

Compositional data for whole mungbean seeds hull and bleached hull fiber, protein and carbohydrate are summarized in Table-4. It was observed that fiber contents in the whole grain Mung bean had maximum value as compared to bleached hull. As well as, hull of Mung bean before bleaching showed the lower value of fiber. Analysis of the whole mung bean revealed a good protein content of the seeds i.e. 22.9% and in hull it was 16.2%. On the other hand protein contents in bleached hull was significantly ($p>0.05$) lower than the whole mungbean (7.6%).

It was noted that whole grain of mung bean had highest percentage of carbohydrate contents i.e. 60.0%.

While the hull of Mung bean before bleaching had lower percentage of carbohydrate (47.82%). Similarly, higher value of carbohydrates was reported in case of bleached hull (49.59%) then the hull (47.82%).

Table-5: Lignin and cellulose contents in Mung bean (Whole grain, hull and bleached hull).

Mung bean samples	Cellulose contents Mean ± S.D	Lignin contents Mean ± S.D
Whole grain	6.1 ± 0.17	13.9± 0.34
Hull	37.2 ± 0.26	4.5± 0.32
Bleached Hull	58.8 ± 0.76	2.3± 0.1

The mean values were calculated by applying standard deviation of three replications.

Table-5 indicated that mean value of lignin contents in three samples of Mung bean. It was observed that maximum value was found in whole grain (13.9%) of Mung bean and smallest amount in bleached hull (2.3%) and the lignin value in hull was 4.5% vice versa. In case of bleached hull remarkable highest value of cellulose contents was seen in bleached hull as compared to hull and whole grain Mung bean.

Minerals analysis: The result of mineral analysis of Mung bean is presented in Table-6. Mungbean contained appreciable quantities of minerals. It was found that Mung bean had relatively higher concentration of Na (18mg 100g-1), K (1032mg 100g-1), Ca (198mg 100g-1), Mg (177mg 100g) and Fe (9.82 mg 100g1).

Table-6: Minerals compositions in Mung bean.

Sodium mg/100g	Potassium mg/100g	Calcium mg/100g	Magnesium mg/100g	Iron(Fe) mg/100g
18.00	1032.00	198.00	177.00	9.82

Table-7: Descriptive statistics of Glucose (mg/dl).

Groups	Glucose level at 1st day Mean ± SD (before alloxan)	Glucose level at 15th day Mean ± SD (after alloxan)	Glucose level at 30th day Mean ± SD
Control	116.20 ± 5.26	114.20 ± 1.48	114.20 ± 1.64
Normal+ High fiber diet	114.00 ± 4.00	113.20 ± 2.16	100.80 ± 1.92
Diabetic+ High fiber diet	115.60 ± 5.85	152.60 ± 12.66	117.00 ± 3.00

The mean values were calculated by applying standard deviation.

Data assessment to change in serum glucose level after feeding of high fiber diet of Mung bean for feeding period of 30 days are showing in Table-7. In this study the 1st group was control + chick starter, normal level of glucose at 1st day was 116.20 ± 5.26mg/dl. This difference was significant as $p<0.05$. While glucose level after 15th and 30th days was 114.20±1.48 and 114.20±1.64mg/dl. In case of second group (normal+high fiber diet) glucose level at 1st day was 114.00±4.00mg/dl whereas at 15th and 30th days glucose level was decreased to 113.20±2.16 and 100.80±1.92mg/dl respectively. Reduction of glucose was consistent and statistically significant as $p<0.05$. In contrast to that, in 3rd group diabetic+high fiber diet glucose level at 1st day was 115.60±5.85mg/dl, and 15th days it was 152.60±12.66 mg/dl. As well as decreased in glucose level after 30th days in 3rd group was 117.00±3.00mg/dl. The decreased in glucose was consistent and statistically significant as $p<0.05$.

Table-8: Analysis of Variance of Glucose.

	Dependent Variable	Sum of Squares	Df	Mean square	F
Source	Glucose level at 1st day	4699.600	2	2349.800	90.733*
	Glucose level at 15th days	14786.533	2	7393.267	132.654*
	Glucose level at 30th days	1009.733	2	504.867	98.351*

*Significant as $p<0.05$.

During this study significant effect on glucose level was noted in all groups at 1st day. As well as, significant effect observed after feeding high fiber diet on glucose level at 15th and 30th days as compared to control.

Discussion: Mungbean serve as the main crops for providing energy in feed and food, and the major supplies of protein consumed by human. Mung bean has higher concentration of protein and it hull have higher quantity of fiber. Consequently our present study was undertaken to find out the biochemical components of different samples of Mung bean and prepare the high fiber diet from bleached hull. Bleached hull used as fiber source in food. Proximate analysis of Mung bean was done and find out of various parameters such as moisture, ash, fat, fiber, carbohydrates protein, cellulose and lignin. Proximate analysis is important in determining quality of seed and often the basis for establishing the nutritional value. Proximate analysis of Mung bean sample (Mung bean whole grain, Mung bean hull and Mung bean bleached hull) and hypochloesterolemic effect of Mung bean high fiber diet are given in Table-3 to 6, which, correlated the earlier findings for Mungbean varieties by Phadgung P.T.[18].

The value of moisture content in whole grain was higher as compared to hull followed by bleached hull. The similar type of results of whole Mung bean was reported by Shuchen and his collaborator in 2009. In the same way percentage value of ash contents of three samples showed a minute difference. However whole Mung bean contained the highest amount of ash content than hull and Mung bean bleached hull. The work on similar lines has also been conducted earlier researchers[19]. In present investigation it was noted that smallest amount of crude fat found in three different samples of Mung bean. The percentage value of fat is relatively low in Mung bean hull and bleached hull as compared to whole grain of Mungbean. Mung bean has long been recognized as a good source of protein and fiber[20]. Its excess quantity of fiber lower the extra glycemic index than whole Mung bean and hull which may make suitable for people with blood sugar problems[19]. Chemical analysis of Mung bean samples (Whole Mung bean, Mung bean hull and bleached Mung bean hull) indicated that the bleached Mung bean hull have a remarkable increased amount of fiber content as compared to other two samples. However, during the process of bleaching the quantity of fiber increased but the values of moisture, fat, protein and lignin contents decreased. The fiber contents of Mung bean samples are approximately approached to the values suggested by Adel A. et.al.[21]. Moreover, high fibre content makes bleached hull of mungbean a good digestive food. Due to their high fiber content, legumes are digested very slowly, thus low on the glycemic index, and help maintain stable blood glucose level and healthier glucose metabolism. Eating more beans helps to reduce the effect of high glycemic index foods by lowering the glycemic value of meals[22]. As Mungbean belongs to the family of leguminoseae the member of this family having a high amount of protein and protein is a major content of Mungbean. The protein content of whole grain was higher than the hull. However, the bleached hull contains lowest amount of protein (7.6%) than that of whole grain and hull. There were significant differences ($P<0.05$) among the protein content of the whole grain and hull samples. Similar percentage values of protein were estimated by Saleem B. et al.[23]. In our study calculated value of carbohydrate was shown that whole grain Mung bean having a heist value than bleached hull followed by hull[24]. In addition to it was observed that the % age cellulose content of bleached hull was significantly higher than the cellulose content of whole Mung bean grain. On the contrary, that the lignin content of bleached hull was lower than the whole grain mung bean and hull[25]. The data on mineral analysis of mung bean revealed that the investigated varieties appear to be a rich source of sodium, calcium, potassium, magnesium and ferric (iron). Mung bean can effectively contribute towards the daily recommended dietary allowances[26] for all groups. It was also observed that generally mung bean is used for protein source but it can fulfill the micro nutrients deficiency as well.

The final part was determination of degree of reducing glucose level by the prepared formulation intake. Results of present study revealed that prepared feed from mung beans has ability to reduce glucose concentration in hyper glycemic rats. To determine the effect of glucose lowering formulation various blood tests were carried out. Significant reduction in plasma glucose level was detected in diabetic High fiber diet group after 30th days as compared to control groups. During this study it was noticed that intake of 14-16grams of high fiber diet of Mung bean (normal+high fiber diet) can be beneficial to normal rats because, depressing plasma glucose level. Similarly the consequences of the diabetic rats groups 3 indicated that a positive effect of high fiber diet in the reaction of glucose level, results showed that the dietary fiber are effective in the treatment and management of diabetes. Domino effect of present study were in accordance those reported by Phadung in 1983. Our experiment must be undertaken to substantiate the long term effects of legume fiber ingestion on health in both healthy individuals and subjects suffering from disorders. During the study different changes were observed in plasma glucose level throughout the 30th days as compared to control group.

Conclusion

It is concluded from the current study that consumption of high fiber intake have ultimate effect on blood glucose level. This conclusion recommends that high fiber diet using mung bean as supplement can reduce risk of diabetic disease by reducing glucose level and enhancing insulin potential in hyperglycemic conditions.

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