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Estimation of Biochemical Properties, Production of Vigna radiata High Fiber Diet, and its Effect on Hyperglycemic rats

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ABSTRACT: Our current analysis was designed to determine the relative composition of the fiber profile of mung beans (Vigna Rediata) and its production into high-fiber diets that can reduce plasma glucose levels in hyperglycemic mice. The evaluation of the compositions of mung beans and their byproducts was conducted using percentage analysis. For moisture, protein, fat, fiber, and ash, the projected readings for mung beans were 8.85 , 22.9, 1.04, 3.8, and 3.76 , respectively. In Mung bean whole grain, the percentage contents of lignin were 13.9, cellulose was 6.1 , and the total carbohydrate content was 60.0 . Mung bean hulls were also found to have 4.4%, 15.2%, 3%, 0.28%, 28.3%, 4.5%, 37.2%, and 47.8% of the following: protein, ash, fat, fiber, lignin, cellulose, and carbs. The third sample's investigation revealed that the moisture level, smoke, fat, fiber, and protein content of bleached mung bean hulls were 2.4%, 3.4%, 0.4%, 36.6%, and 7.6%, respectively. The bleached mung bean shell included 2.3% lignin, 58.8% cellulose, and 49.5% carbs. Rats were given an alloxan dose to make them hyperglycemic (diabetic); this was done to see how a high-fiber diet affected the rats' blood sugar levels. The results of the trial revealed that, in contrast to the control group, the high-fiber mung bean meal decreased the plasma glucose level in the diabetic rats who were part of the trial.

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I. INTRODUCTION

Numerous international health organizations have advocated a range of plant-based functional meals, which has led to a need for significant dietary pattern modifications to promote health and avoid chronic illnesses[1]. There is increasing demand to increase the intake of plant-based foods at a pace of $5\% - 10\%$ per year as a result of increased scientific findings suggesting they offer different potential health advantages. Additionally, the necessity of food components having different functional qualities to improve health status and avoid chronic illnesses is being increasingly highlighted by global health organizations[2]. Very closely behind cereals (Gramineae) as the most significant crop for human consumption are legumes (Fabaceae/Leguminosae)[3]. Mung bean (Vigna radiata) Also referred to as greenish gram, is a significant pulse crop in Pakistan. It is based on diet cereal in the majority of Asian countries. Mung beans are high in vitamins, minerals, and protein (23%) as well as complex carbs (dietary fiber) are considerably important numerous bioactive ingredients found in Asian vegetarian diets have been found to decrease blood cholesterol and significantly minimize the possibility of diabetes, obesity, and coronary heart disease[4]. Among the most significant legumes that is extensively grown in the tropics and subtropics is the green beans during the early summer rains and can be grown using a variety of cropping techniques. A large number of individuals in China, Pakistan, India, Thailand, Indonesia, and the nation of the Philippines, it is an essential part of their diets. It can be eaten in a variety of ways, such as a snack, boiled, cooked with meat or vegetables, dessert, or mixed into bread or cake. Sprouts for egg rolls and vegetable dishes can be made with it[5]. Mung beans are renowned for their reduced flatulence and excellent digestion. Provitamin A and phosphorus are abundant in them, and they don't contain many antinutritional elements. A meal rich in amino acids is formed when Cereals' low lysine/high methionine content and high protein profile mix with Vigna Rediata low methionine/high lysine amino acid profile[6]. In China, mung beans are

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also highly renowned for their ability to cleanse the body. They are also utilized to ease mental tension, enhance body temperature regulation, and lessen summertime swelling. Numerous studies have shown that it is also essential for enhancing digestion and controlling the excretion system; nevertheless, mung bean seeds and sprouts are also commonly consumed in Pakistan, India, Bangladesh, and other Southeast Asian and Western nations as a nutritious salad vegetable and to moisturize the skin dishes[7]. Moreover, it has been demonstrated that mung beans work against cancer via a variety of methods. R-TBN1 and R-HBN1 are recombinant plant nucleases, which were roughly ten times more active than bovine seminal ribonuclease (RNase), were efficient against melanoma tumors[8]. They resembled nucleases generated from pine pollen and mung beans. Recombinant plant nucleases are stable biochemical agents with prospective applications as anticancer cytostatic, owing to their high efficiency and comparatively minimal cytotoxicity[9].

II. MATERIAL AND METHODS

During the duration of the investigation, examinations of mung beans (seed) and hulls were obtained from a local Sargodha shop. To clean, the mung bean and hull were sieved. All unnecessary material was eliminated from the mung bean grains and their hull after they had dried. Zip lock bags were used to keep the cleaned grains and hull for additional examination and processing. Regarding the molecular study, mung beans and their hulls were utilized.

A. Proximate Analysis

A Soxhlet extractor was employed for the extraction of the fat along with ether for four hours after oven-drying the mung beans sample at 120°C. This allowed for the measurement of the moisture content of the sample[10]. The technique of[11] was used to evaluate the ash contents. Every analysis utilized three to five grams of dried sample. The Kjeldahl technique was used to determine the crude protein content of mung beans and their hulls. The Fiber Tech System-M (Tecator) was utilized to assess the fiber content during processing with both acid and alkali[10].

B. Estimated of Lignin

The standard method was used to figure out the lignin concentration. Using this approach, refluxing 1 g of defatted material with 70 mL of 1.25 percent H2 SO4 was carried out for two hours. (Rodrigues et al., 1999). Afterward utilizing warm water, the refluxed specimen was cleaned utilizing chloroform. A 72%H2SO4 treatment was applied to the washed sample and stirred continuously for four hours. The sample was burned for four hours at 550 degrees Celsius after being filtered (with 10-12 milliliters of distilled water added[12].

C. Estimation of Cellulose

Estimated cellulose by refluxing technique for two hours, 1.5 mL of concentrated HNO3 acid and 15 mL of 80% acetic acid were refluxed with 1 g of defatted material. After that, the refluxed specimen had been cleaned. After that, the refluxed specimen had been cleaned. Following a thorough cleaning, the specimen was immersed in 72% H 2 SO 4 for four hours while being continuously stirred. The specimen was subsequently washed along with alcohol to remove any remaining foreign contaminants, filtered (by adding $10-12$ mL of pure water) using Whatman filter paper No. 1, treated using water that had been distilled until it became neutral, and burnt for six hours at 550 oC27[13].

D. Estimation of Carbohydrates

The subtraction method was used to calculate the amount of carbohydrates.

E. Fiber Extraction by bleaching

After thoroughly cleaning the mung beans hulls with distilled water to get rid of all contaminants, the hulls underwent treatment with food-grade hydrogen peroxide under an alkaline atmosphere for one to two hours at a temperature between 50 and 100 degrees Celsius by adding a few drops of 40%NaOH(pH$10-12$). The specimen is subsequently completely cleaned with distilled water, neutralized with 0.1%HCl, and then rinsed once more with distilled water to get rid of any remaining acid. The sample that had been bleached was thoroughly crushed and dried using hot air[10].

F. Composition of Diet fed

To meet the animal's needs to feed protein during growth and development, a 1 kg mung bean fiber diet contains 18% casein. Additionally, the given formulation contains 7.5% maize oil to offer essential fatty acids. The starch from corn had been combined as a means of carbs. Subsequently, 3.5% of the mineral combination and 1% of vitamins were consumed. 45% of the fiber came from the bleaching hull of mung beans. Ad libitum access to water as well as food was granted[14].

TABLE I: 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

G. Functioning qualities of Mung bean high bran feed

The anti-hyperglycemic effect of a high-fiber formulation on hyperglycemic rats was assessed by using the alreadymade large bran feed to test the Mung bean's useful qualities.

H. The methods used in the experiment to induce hyperglycemia

i. Alloxan; **ii.** Enzymatic kits for biochemical analysis of blood serum to estimate cholesterol and glucose levels.

I. Choice of Animals

Rats' overall physiology is comparable to the one of human beings, and they are widely available, manageable, and reasonably priced. These factors led to the selection of albino rats as the experimental animals in the current study. Furthermore, the alterations in biochemistry and histopathology that rats undergo are quite comparable to those that people experience. Thirty healthy, gender-neutral albino rats weighing between 200 and 300 grams were used for the investigation. Rats were raised in cages with regulated humidity levels ($50-60\%$) and temperatures ($22 \pm 2°C$). Three equal groups of rats were created. Ten rats per group were given a thirty-day period to live the remaining group was given the medicine both separately and in combination, with one group serving as a control.

J. Alloxan

It has been suggested that Alloxan-induced hyperglycemia is a helpful experimental paradigm for assessing the efficacy of hyperglycemic medications. Diabetes was brought on by just one intraperitoneal injection of Alloxan at a dosage of 100mg/kg for body weight.

K. Enzymatic Kits

Using a spectrophotometer, albino rats' serum glucose and serum cholesterol levels were measured using commercial kits from the company Randox.

L. Experimental Design

There were five groupings created from the animals. Eight rats made up each group. 100mg/kg body weight of Alloxan administered intraperitoneally in one shot using a 0.1 mol/L buffered citrate preparation, was used to cause diabetes. Rats that had blood glucose levels higher than 150mg/dl were classified as diabetic and used in subsequent studies. By assessing the fasting blood glucose levels after ten days, diabetes in the rats treated with alloxan was proven.

M. Blood collection and analysis

Through the coccygeal artery of rats with albino coloring, one milliliter of blood was taken out. Afterward, the serum was separated by centrifuging blood at 3000 rpm for 10 minutes. Spectrophotometers were utilized to measure the serum glucose levels in albino rats using commercial kits from the company Randox.

N. Estimation of Serum Glucose

Principle:

In the presence of glucose oxidase, glucose is ascertained by enzymatic oxidation. With the help of peroxidase, the hydrogen peroxide that is produced combines 4 -aminophenazone as well as phenol to produce a reddish-violet quinoneimine dye suitable to be utilised to serve as an indicator. Reaction

$$\text{Glucose } + \text{O2} + \text{H2O glucose oxidase} \longrightarrow \text{Gluconic acid } + \text{H2O2}$$
$$\text{2H2O2} + \text{4-aminophenazone } + \text{ phenol peroxidase} \longrightarrow \text{Quinoneimine } + \text{4H2O}$$

Sample: Serum:

If the serum is made within 39 minutes of the sample being taken, glucose will remain stable for 24 hours at $+2$ to $+8°C$. Reagent: i. Enzyme Reagent, ii. Stan-

TABLE II: Animal conditions and Treatment.

Groups	Animal conditons	Treatment
1	Control group (Normal)	Chick starter diet
2	Control group+ high fiber diet	High fiber mug beans diet (45% fiber) 15 g/ day / rat

dard. Acceptable ranges: $75-115$mg/dl for blood and plasma (fasting).

Calculations:

A test divided by an ordinary yield the glucose concentration (mg/dl), which is then multiplied by 10016 to determine the serum glucose content.

O. Statistical Assessment

Values of P for analysis of variance (ANOVA) less than ($p<0.05$) were deemed statistically significant, and if higher than ($p>0.05$), they were deemed significant17. All statistical analyses were conducted using this methodology[15].

III. RESULT AND DISCUSSION

A. Proximate analysis

The biochemical makeup of the bleach hull and whole grain mung bean was examined in a study. The elements of chemical assessment include moisture, ash, fat and fibre, protein, carbs, and minerals (all percentages are given). Additionally, to ascertain the cellulose and lignin composition of the entire grain and hull of mung beans. For both normally and diabetic rats, a high-fiber diet made of mung bean grain and hull were prepared. One of five diets was given to the rats at will, and each measurement was made three times[10].

By using the standard deviation of three replications, the mean values were determined. According to Table 3, the average value of moisture ash and fat levels in three mung bean experiment samples. The results indicated that the mung bean whole grain (8.85%) had a higher moisture content than both the treated (2.4%) and untreated (2.4%) hulls. A comparable trend was seen when looking at the ash amounts. Whole grains had a higher ash concentration (3.76%) than both bleached (3.4%) and untreated (3.0%) hulls. The fat levels of the entire bean were 1.04%, the bleached hull was 0.4%, and the hull had 0.28% fat.

The deviation from the mean was used for determining the averages. Table 7 displays data evaluation of changes in blood glucose levels during a 30-day eating cycle of a high-fiber mung bean meal. The initial class in the present investigation consisted of control and chick starters, and on the first day, their normal glucose level was 116.20$\pm$ 5.26mg/dl. There was a substantial difference ($p<0.05$). subsequently the 15^{th} and 30^{th} days, the glucose levels were 114.20 ± 1.48 and 114.20 ± 1.64mg/dl, respectively. The glucose level in the second group (normal+high fiber diet) was 114.00 ± 4.00mg/dl on day one, however, it dropped to 113.20 ± 2.16 and 100.80 ± 1.92mg/dl on days 15 and 30 , accordingly. A steady and statistically noteworthy decrease In glucose was observed ($p<0.05$). On the other hand, the glucose level in the third category (diabetic+high fiber diet) was 115.60 ± 5.85mg/dl on day one and 152.60 ± 12.66 mg/dl on day fifteen. Moreover, the third group's glucose level dropped to 117.00 ± 3.00mg/dl following 30 days. The glucose level dropped consistently and proved of statistical importance ($p<0.05$).

* Significant as $p<0.05$. **Insignificant as $p>0.05$. Major consequences on glucose levels were seen throughout the first day of the research in all groups. Additionally, eating a high-fiber diet had a substantial impact on glucose levels at days 15 and 30 when contrasted with the control group.

IV. DISCUSSION

Mung bean is the primary crop used to supply human consumption of protein and energy for feed and food. Both the protein content and the amount of fiber in mung beans are higher. Therefore, the purpose of this investigation is to determine the biochemical makeup of different mung bean samples and use their bleached hulls to construct a high-fiber diet. Bleached hulls are used in cuisine as a source of fiber. A proximate evaluation was conducted on mung beans to determine a number of characteristics, containing lignin, cellulose, fiber, proteins, ash, fat, and carbohydrates. Investigation that is close to crucial for evaluating seed quality and frequently serves as the foundation for calculating nutritional value. Table III through 6 present a proximate analysis of the mung bean sample, which includes the hull, whole grain, and bleached hull varieties, and the hypoglycemic effect of the mung bean high fiber diet. These results are linked with previous results for differ-

TABLE III: Proximate composition and bioactive compound of Mung bean samples.

Sr. No	Proximate and biochemical components Mean ± S.D.	Mung bean samples		
		Whole grain	Hull	Bleached Hull
1	Moisture	8.85 ± 0.02	4.4 ± 0.29	2.4 ± 0
2	Ash	3.76 ± 0.05	2.9 ± 0.1	2.4 ± 0.17
3	Fat	1.04 ± 0.03	0.28 ± 0.01	0.4 ± 0.01
4	Crude Fiber	3.8 ± 0	28.3 ± 0.5	36.6 ± 0.58
5	Protein	22.9 ± 0.21	16.2 ± 0.32	7.6 ± 0.15
6	Lignin	13.9 ± 0.34	4.5 ± 0.32	2.3 ± 0.1
7	Cellulose	6.1 ± 0.17	37.2 ± 0.26	58.8 ± 0.76
8	Carbohydrates %	60 %	47.82 %	45.59 %

TABLE IV: Sugar descriptive data (milligrams/d1).

Sr. No	Groups	Glucose level at 1^{st} day before alloxan Mean ± S.D	Glucose level at 15^{th} after alloxan Mean ± S.D	Glucose level at 30 Mean ± S.D
1	Control	115.99 ± 5.26	114.10 ± 1.48	114.10 ± 1.64
2	Normal + High fiber diet	114.00 ± 4.00	113.20 ± 2.16	100.80 ± 1.64
3	Diabetic + High fiber diet	115.60 ± 5.85	152.58 ± 12.66	117.00 ± 3.00

TABLE V: Analysis of Variance of Glucose.

Dependent variables	Sum of squares	df	Mean squares	F
Glucose level at 1^{st} day	4699.600	2	2349.800	90.377^{**}
Glucose level at 15^{th} day	14786.533	2	7393.267	132.654^{*}
Glucose level at 30^{th} day	10097.733	2	504.867	98.351^{*}

ent varieties of mung bean[16]. When compared to hull, whole grain had a higher moisture content, which was followed by bleached hull. Shuchen and his associate reported comparable outcomes using entire Mung beans. The three samples' ash contents' percentage values also revealed a slight variation. But the highest level of ash concentration was seen in whole mung beans. Husk and the bleached hull of Mung beans. Previous researchers have also carried out work along these lines[17]. Legumes are generally low in fat, with most species containing less than 5% lipids. Soybeans are an exception, with a much higher fat content of 17.7% to 21%, making them a valuable source of plant-based oils. In our investigation in mung bean also have low lipid content compared to other legumes. Mung beans, peas, lentils, and kidney beans have very low lipid levels, typically below 2.5%[18].

The current study indicated that three distinct mung bean samples had the lowest level of crude fat. When compared to the whole grain of mung beans, as well as the proportion of fat and bleached husk is comparatively low. It has long been known that mung beans are an excellent source of fiber and protein[19]. The entire mung beans, specimens of mung bean hull as well as cleaned mung bean hull were examined chemically. The outcomes showed that the bleached mung bean hull had a noticeably higher fiber content than the other two samples. However, while the amount of fiber grew throughout the bleaching process, the amounts of protein, fat, moisture, and lignin dropped. Samples of mung beans roughly approximate the fiber contents recommended by[20] Additionally, elevated fiber bleached mungbean hulls are a rich source of fiber for the digestive system. Many Legumes have a high fiber content, like Fiber content in these legumes varies, ranging from 7% to 20%. Soybeans contain the highest fiber content (20%), followed by broad beans ($7.5\% - 13.1\%$), lentils (12%), and adzuki beans (127%). Mung beans, pigeon peas, and kidney beans have relatively moderate fiber content ($7\% - 10\%$)[18] which makes them low on the glycemic index and slow to digest. This promotes a healthy glucose metabolism and stable blood glucose levels. By reducing the glycemic value of meals, increasing your bean intake can help lessen the impact of high-GI foods[20] Since mungbean is a member of the leguminoseae family, which has a high protein content overall, it falls under this family. Legume seeds are generally rich in protein, but the amount varies significantly between species. Soybeans, Broad beans, lentils, and kidney beans also have relatively high protein levels, ranging from 35.1% to 42%, 26.1% to 38%, 23% to 32%, and 20.9% to 27.8%, respectively[18]. The entire grain has a greater protein content than the hull. However, compared to whole grain and hull, the bleached hulls have the lowest protein content (7.6%). Hulled and whole grain samples differed considerably ($P \leqslant 0.05$) in their protein contents. Hulled and whole grain samples differed considerably ($P \leqslant 0.05$) in their protein contents[21]. Most legumes in the table have carbohydrate content ranging from 37% to 62.9%. Adzuki beans have the highest carbohydrate content (62.9%), while kidney beans, peas, broad beans, lentils, and chickpeas contain around 40% to 46%. Soybeans, due to their higher protein and fat content, have the lowest carbohydrate percentage (7.7%). came in second, according to the computed value of carbohydrates in our investigation[18]. Furthermore, a substantial difference was noticed between the cellulose content of the bleached hull and the entire Mung bean grain in terms of age. In contrast, whole-grain mung bean and hull had a higher lignin concentration than bleached hull[22]. Additionally, it was noted that while mung beans are typically utilized as a source of protein, they can also help with micronutrient deficiencies. The legume is the fruit or seed of plants of the legume family, which are used for food, including lentils, common beans, broad beans, dry peas, chickpeas, cowpeas, and mung beans. Legumes are an excellent source of protein ($20-45\%$ protein), high amounts of the amino acids such as lysine and leucine, and lower amounts of methionine and tryptophan, complex carbohydrates, dietary fiber, unsaturated fats, vitamins, and essential minerals for the human diet. Providing in diet for the control of blood sugar[23].

chickpea-based diet helps avoid diabetes and obesity. Adiponectin is a hormone that helps to prevent type 2 diabetes and atherosclerosis. A randomized cross-over clinical study involving 32 diabetes patients fed a chickpea diet discovered that all patients had greater levels of adiponectin[24].

Determining the extent of the glucose level reduction by prepared formulation ingestion was the last step. The current study's findings showed that mung bean-based prepared meals can lower the amount of glucose in hyperglycemic rats. Several blood tests were performed to evaluate the impact of the glucose-lowering formulation. Significant decrease in plasma following 30 days, the diabetic high-fiber diet group's blood glucose level was shown to be higher than that of the control groups. It was discovered throughout this investigation that giving normal rats $14-16$ grams of high-fiber mung beans (normal+ high- fiber diet) can help them by lowering their plasma glucose levels.

Comparably, the outcomes of the three diabetic rat groupings demonstrated that a high-fiber diet had a favorable impact on blood glucose sensitivity as well as that dietary fiber is useful in managing and therapy diabetes. In contrast to the control group, significant variations in plasma glucose levels were noted throughout the trial over 30 days. My research needs to conduct a trial to evaluate the long-term health benefits of consuming legume fiber in both healthy individuals and those with existing health conditions. Over a period of 60 days or longer, plasma glucose levels will be closely monitored, with significant differences expected between the intervention group and the control group.

V. ETHICAL POLICY

All animal experiments conducted in this study complied with ethical standards and were approved by The University of Lahore Animal Ethics Committee. The study adhered to the guidelines established by the internationally recognized principles such as those outlined by the National Institutes of Health (NIH) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and reduce the number of animals used."

VI. CONCLUSION

The results of this study indicate that consuming a high dose of fiber has a final impact on blood sugar levels. The findings suggest that a high-fiber diet supplemented with mung beans may reduce blood glucose levels, reducing the risk of diabetes and improving insulin production in hyperglycemic circumstances.

DECLARATION OF COMPETING INTEREST

The authors have no conflicts to disclose.

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