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Research Article

Elucidating Hyperglycemic and Hyperlipidemic Potential of Basil Seed Gum Aqueous Extract Powder in Diabetic Rat Model

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Abstract

Lifestyle diseases evolved during 20th century to promote epidemiological landscape with increasing industrial growth and climate change. Diabetes mellitus, obesity, hypertension, heart diseases, stroke, pulmonary diseases, and mental diseases along with alcohol abuse and smoking increase the risk of mortality and morbidity in population. So, the current study was conducted to evaluate aqueous extract powder of *Ocimum basilicum* potential against streptozotocin induced hyperglycemia with its potential to ameliorate biochemical parameters associated with liver and kidney functionality and body weight in albino rats. Four groups of albino rats were selected having six rats in each group (control group, diabetic group, 0.5% and 1% treatment group. Biochemical parameters which were evaluated were AST, ALP, ALT, total bilirubin, total protein, albumin, globulin, creatinine, and urea along with estimation of total cholesterol, triglycerides, HDL, and LDL. Aqueous extract powder significantly reduced blood glucose levels at both doses. Body weight was also improved significantly. Biochemical parameters for liver and kidney tests along with lipid profile was also improved showed ameliorated effect of extract powder. Mainly 1% basil seed gum showed prominent improvement in blood glucose parameters along with other biochemical parameters. This study concludes that both levels of BSG powder have notable possible effect to reduce hyperglycemia and hyperlipidemia associated with diabetes. Therefor it may be helpful in diabetes management.

Keywords: Hypoglycemic Potential; Basil Seeds Gum; Albino Rats; Hyperlipidemia; Aqueous Extract

Abbreviation

BSG: Basil Seed Gum; TG: Triglyceride; LDL Cholesterol: Low-density Lipoprotein; HDL-cholesterol: High-Density Lipoprotein; ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase

Introduction

All along history, products that are derived from animal and plant sources provide the vital and required ingredient to cure diseases. Importance of plant medicine has been raised up day by day. Active ingredients in those plants have been identified having therapeutic effect to correct aliments abnormalities [1]. World health organization has been reported that more than 80% of the population is dependent upon herbal medicine because of better outcome, availability and cheap other than chemicals. Most of the plants is used as adjuvant therapy because they are not only better for treatment of disease but also has ability to prevent the disease and maintenance of health against pathological abnormalities [2].

Ocimum basilicum, A vital economical medicinal plant is belonged to labiatae family widespread in mostly Asian and African tropic and subtropic countries. it is indigenous to central and southeastern Asia. It has growth up to 30cm long, has white, round, and rosy flower clusters every year [3]. Basil has been known for many pro-health activities such as anti-inflammatory, anti-allergic, anti-cancer, anti-diabetic, anti-hyperlipidemic, anti-microbial, anti-septic, anti-spasmodic, anti-fungal, anti-viral, immune-stimulating and antioxidant properties. These properties are related to its phytochemical content of polyphenols and aromatic compounds. Seeds have been used as additives in different types of drinks and food products. Recent research studies have shown antidiabetic and antihyperlipidemic effect [4].

Globally it is estimated that 422 million people are currently suffering from this diabetes. In Pakistan diabetes is a growing non-communicable disease. Government and non-governmental organizations have done a lot of work to minimize the outcomes [5]. According to the second national diabetes survey, percentage of people suffering from Diabetes is 26.3% out of whom, 19.2% are already diagnosed cases while 7.1% are new cases. Prevalence of diabetes in urban and rural areas of Pakistan is 28.3% and 25.3%, respectively [6].

According to WHO global report, diabetes mellitus is a chronic, serious kneeling disease that occurs due to malfunctioning of pancreas, deficiency of insulin in the blood and high levels of blood glucose. Uncontrolled condition of disease can lead to other diseases like CVD, kidney problems, damage of eyes and blood vessels. There are two types of diabetes according to condition and complications like type I (insulin dependent) and type II (non-insulin dependent) [7].

Dyslipidemia is one of the adverse complication of diabetes. Strong correlation has been proved by so many studies in which patients with coronary heart disease having higher level of serum cholesterol and triglycerides level along with diabetes type I and type II because of increase in fatty acid flux [8]. Evidence based studies has provide adverse effect of insulin resistance linked with dyslipidemia. According to Framingham Heart study, prevalence of high cholesterol among diabetic patients were 13% in males and 24% in females. Level of high plasma triglycerides were 19% males and 17% in females [9]. Another study proved the link of dyslipidemia among type II diabetic patients as results showed that 22.3% patients were diagnosed with hypercholesteremia and 61.9% were diagnosed with hypertriglyceridemia. Abnormal HDL levels in females were 59.3% and in males were 47.6% among HbA1c values more than 7.0% with significant higher values of cholesterol [10].

Due to increasing burden of diabetes day by day and lack of affordable treatment especially in developing countries such as South Asian countries such as Pakistan. Pakistan faces double burden of diseases such as increasing spectrum of communicable diseases along with high prevalence of non-communicable diseases that puts burden on government, health departments with affordability and availability. It is very difficult to find affordable treatment for diabetes mellitus and its complication dyslipidemia. To update ourselves and to fill the gap knowledge, we aimed to determine the basil seed gum powder efficacy against diabetes mellitus *in vivo*. We also evaluated powder effect against dyslipidemia. Different dosage level of BG powder on bodyweight of rats.

Material and Methods

Basil seeds were purchased from local market. Basil seeds were chosen based on maximum gum extraction yield and viscosity. Seeds were carefully cleaned from stones and dirt. Basil seeds were washed and kept in beaker. Beaker was filled with water according to seed ratio. 50:1 (w/w) water seed ratio was maintained throughout procedure. The temperature of water bath was set to 68.71°C. Beaker was kept in water bath for 20 minutes and stirred continuously [11].

Gum was extracted from sample by applying mechanical force. Mucilage was separated from seeds by using rubber spatula to seeds on mesh screen. Separated gum from seeds were shifted to 50 ml Eppendorf tube. Sample was centrifuged for 20 minutes at 6000 rpm. This method was adapted to separate gum from seeds [12]. This polysaccharide was dried in drying oven at 45°C until water was completely evaporated from the sample. After that, sample was ground in grinder to convert it into a fine powder. Dried basil seed gum powder was then stored in the form of packets.

Ethical statement

All the procedures done during the experiment were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Moreover, prior approval of this work was taken from Ethi-

cal Review Committee of the University of Veterinary Animal Sciences, Lahore, Pakistan.

Efficacy trials

Twenty-four 24 Sprague Dawley Albino rats (divided into 4 groups, 6 rats in each group) were bought and kept in stainless steel cages. Acclimatization of rats with the environment was done for a period of 2 weeks whereby they were given regular feed and water. The housing temperature for rats were maintained at 25 ± 2^0C with a relative humidity of 55 ± 5^0C. The animal house was well lit and airy.

Diabetes was induced to rats by using streptozotocin except negative control group. Fresh solution was prepared in lab by adding streptozotocin powder with 0.1M citrate buffer with pH 4.5. solution was filtered for attaining clean solution avert from any impurities. It was administered in a peritoneal cavity through injection at a dose of 55 mg/kg as a single dose to overnight fasting rats. diabetic group rats were monitored carefully to observe the symptoms of diabetes. When symptoms of Diabetes started appearing, presence of Diabetes was confirmed by taking blood sample from rats' tails. It was declared from the value of blood glucose which was greater than 250 mg/dl.

Basil seed gum powder to be delivered to rats was calculated using the formula:

The treatment plan for efficacy trials of rats is shown in table 1.

Group	Diet plan
G_0	Regular feed given to healthy rats
G_1	Regular feed given to diabetic rats
G_2	Basal feed with 0.5% basil seed gum powder given to diabetic rats
G_3	Basal seed with 1%basil seed gum powder given to diabetic rats

Table 1: Treatment plan for efficacy trial.

Weight of rats was measured on weekly basis and dose of basil seeds gum powder was adjusted accordingly.

Biochemical analysis

Blood of rats was withdrawn at baseline (0 day) and at termination of study (28th day) from tails of rats in yellow cap vacutainers which were coated with thrombin a blood clotting activator. Samples were delivered to AM Pets Care Lab located at Shahab Park, Lahore.

Blood glucose levels

Blood glucose levels of all group of rats were measured at 1st day of trial and was checked after end of trial. Glucose levels of rats was checked through simple glucometer. Drop of blood was taken from tail was put on glucose strips. After a few seconds, glucometer showed blood glucose value which was noted down immediately.

Serum LFTs and RFTs

This test was performed to check the efficacy of basil seed gum. Sample were centrifuged to collect the serum for further usage. For liver function tests and kidney functions tests, blood serum samples were delivered to AM Pets Care Laboratory located in Lahore.

Hyperlipidemic assays

high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and very low-density lipoprotein (VLDL), were estimated by using standard enzymatic methods following commercially available kits (Randox) according to manufacturer advice respectively.

Statistical analysis

Obtained data was entered in Statistical Package for the Social Sciences (SPSS) version 23.0. One-way Annova was employed during the experiment and differences were significance at P-value < 0.05. All results were expressed as Mean ± Standard Deviation.

Results

Effect of basil seed gum powder weight of rats

Figure 1 showed the status of weight of rats every week of trial. This graph showed overall the effect of disease and treatment on different rat groups. As in this trial not mortality occurred the weight of each rat in each group was noted every week. Group G_1 was positive control having not any disease and treatment that perfectly elaborate mean value of weight that seems to be same in this trial. G_2 is negative control group means diabetic group without treatment that represents drastic change of weight status every week. treatment groups weight status is improving every week as mean value of weight is increasing in trial as highest weight is recorded 240g in last week of trial of 1% BSG group.

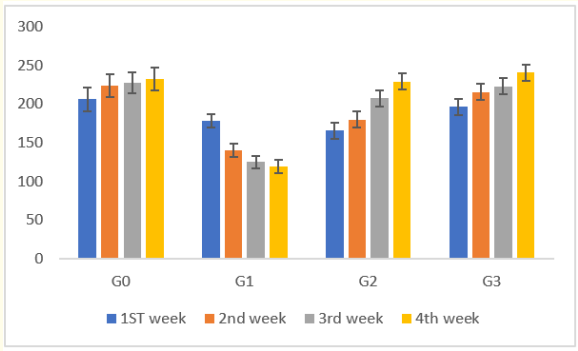

Figure 1: Graphical presentation of Rats weight status over weekly in mean ± S.D. Where, G0 = Control group G1= Diabetic group without treatment G2= Diabetic group with 0.5% basil seed gum powder diet G3= Diabetic group 1% basil seed gum powder diet.

Figure 2: Graphical presentation of mean ± S.D of blood glucose level of rats Where, G0 = Control group G1= Diabetic group without treatment G2= Diabetic group with 0.5% basil seed gum powder diet G3= Diabetic group 1% basil seed gum powder diet.

Effect of basil seed gum powder on blood glucose levels of rats

Mean squares of blood glucose level of rats along with different treatments and trial period have been mentioned in figure 2. Trial duration was found to have significant effect on blood glucose levels of rats (P-value < 0.05). As per the results, rats in control group had higher levels of blood glucose levels (88.83mg/dl) at termination of trials, as compared to the start of trials (82.66 mg/dL). Diabetic group G_1 had highest blood glucose levels among other groups as no treatment was given to this group. It is evident that blood glucose levels for this group at day 0 were 139.3 mg/dL and 154.50 mg/dL at the termination. Among treatment groups, blood glucose level was under control after fortification of feed with basil seed gum powder. In 0.5% basil seed gum powder treatment blood glucose levels at day 1 of trial, blood glucose levels were 143 mg/dL while at the termination, levels were found to be 75.66 mg/dL. On the other hand, in 1% basil seed gum powder treatment, blood glucose levels were 156.83 mg/dL at start of trials, while 80.33 mg/dL at termination.

Effect of basil seed gum powder on other biochemical parameters of rats

Table 2 shows mean squares regarding various biochemical parameters of rats and their association with duration of trials. As is evident from the table, mean squares for Bilirubin total, ALT, AST, ALP, Total Protein, Globulin, Urea and Creatinine for treatment groups were significantly different with regards to duration of trials (P-value < 0.05). However, this effect was not significant for treatment groups for the parameter of Albumin (P-value >0.05).

Tests	Weeks	G_0	G_1	G_2	G_3
Bilirubin Total (mg/dL)	1ST week	0.12 ± 0.02	0.18 ± 0.02	0.21 ± 0.03	0.25 ± 0.05
	4th week	0.17 ± 0.06	0.18 ± 0.01	0.07 ± 0.01*	0.11 ± 0.05*
ALT (U/L)	1ST week	25.33 ± 2.33	52.20 ± 2.94	69.33 ± 3.38	47.83 ± 16.98
	4th week	34.33 ± 10.34	55.00 ± 2.82	27.33 ± 2.94*	30.66 ± 6.47*
AST (U/L)	1ST week	182.23 ± 39.69	197.00 ± 22.75	258.33 ± 16.15	217.33 ± 15.15
	4th week	162.50 ± 6.89	167.77 ± 12.02	80.16 ± 7.08*	193.33 ± 10.85*
ALP (U/L)	1ST week	138.83 ± 6.43	279.00 ± 18.83	180.33 ± 19.86	276.60 ± 32.67
	4th week	134.33 ± 3.82	175.46 ± 14.93	130.00 ± 8.94*	158.33 ± 35.92*
Total Protein (g/dL)	1ST week	6.38 ± 0.2	7.20 ± 0.55	7.53 ± 0.16	7.57 ± 0.25
	4th week	6.10 ± 0.37	6.00 ± 0.34	6.38 ± 0.57*	7.20 ± 0.60*
Albumin (g/dL)	1ST week	2.76 ± 0.34	2.95 ± 0.21	3.30 ± 0.58	3.56 ± 0.79

	4th week	2.86 ± 0.33	3.41 ± 0.54	3.72 ± 0.26	3.67 ± 0.90
Globulin (g/dL)	1ST week	1.73 ± 0.51	4.55 ± 0.91	4.88 ± 0.15	4.59 ± 0.27
	4th week	1.38 ± 0.11	4.28 ± 1.19	2.26 ± 0.13*	2.6 ± 1.22*
Urea (mg/dL)	1ST week	18.72 ± 0.74	30.66 ± 1.20	26.66 ± 8.40	25.5 ± 2.34
	4th week	22.5 ± 2.07	30 ± 2.60	26.08 ± 1.42*	18.85 ± 1.46*
Creatinine (mg/dL)	1ST week	0.39 ± 0.06	0.57 ± 0.08	0.54 ± 0.04	0.46 ± 0.08
	4th week	0.35 ± 0.10	0.61 ± 0.24	0.35 ± 0.10*	0.5 ± 0.06*

Table 2: Effect of basil seed gum powder on other biochemical parameters.

Where, * = P-value < 0.05, G_0 = Control group, G_1= Diabetic group without treatment, G_2= Diabetic group with 0.5% basil seed gum powder diet, G_3= Diabetic group 1% basil seed gum powder diet, all values are expressed as Means ± SD.

Serum lipid profile

Lipid profile was analyzed by estimating total cholesterol, triglycerides, High lipid density lipoprotein (HDL) and low-density lipoprotein (LDL).

In this study when rats were injected with streptozotocin for high blood glucose level. Eventually hyperglycemic effect disturbed lipid profile of rats administering towards hyperlipidemia. As table 3.4 showed total cholesterol, Triglycerides, HDL, and LDL vales of different groups. By applying annova it is noted that control group has normal ranges of lipid profile as compared to diabetic group, in which all parameters' ranges are abnormal except HDLC. We all know that high density lipoprotein (HDL) is considered good cholesterol, so this range was disturbed and estimated low in range due to progression of disease. When these diabetic groups were treated with 0.5% and 1% of basil seed gum their lipid profile parameters were improved eventually. Cholesterol level was improved along with triglycerides, HDL, and LDL. As it is noted from table that diabetic group treated with 1% BSG showed highly significant results as lipid profile parameters were improved high as compared to 0.5% treatment group.

Groups	Weeks	T.C (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal Ranges		**80-114**	**43.78-84.86**	**33.4-50**	**30.18-72.86**
G_0	1st week	91.5 ± 1.71	45.6 ± 1.52	37.4 ± 1.61	30.8 ± 1.62
	4th week	95.4 ± 1.35	59 ± 1.14	45.2 ± 1.12	55.4 ± 1.15
G_1	1st week	125.8 ± 1.62	95.4 ± 1.66	29.8 ± 1.53	86 ± 1.75
	4th week	126.2 ± 1.05	98.8 ± 1.01	32 ± 1.12	89.4 ± 1.14
G_2	1st week	129.2 ± 1.02	99.6 ± 1.04	28.9 ± 1.02	85.6 ± 1.0
	4th week	101.8 ± 1.12*	72.8 ± 1.11*	37.8 ± 1.11*	69.3 ± 1.15*
G_3	1st week	130.5 ± 1.03	98.5 ± 1.01	27.5 ± 0.98	85.9 ± 0.87
	4th week	87.2 ± 0.85**	78.3 ± 0.82**	40.7 ± 0.56**	70.7 ± 0.82**

Table 3: Mean ± S.D of lipid profile parameters of different rats' groups.

Where, * = P-value ≤ 0.05, **= highly significant P- value ≤ 0.05, all values are expressed as Means ± SD, G0 = Control group, G1= Diabetic group without treatment, G2= Diabetic group with 0.5% basil seed gum powder diet, G3= Diabetic group 1% basil seed gum powder diet T.C= total cholesterol, TGL= triglycerides, HDL= high density lipoprotein, LDL= low density lipoprotein.

Discussion

Globally, prevalence of Diabetes Mellitus has been on a rise especially in developing and underdeveloped countries. Rapid urbanization, economic development and changing lifestyle are the major reasons behind this increase in prevalence of Diabetes across the globe. In Pakistan, approximately 27.4 million people are diabetic out of 207.77 million population according to the latest National Diabetes Survey of Pakistan. In Pakistan, the identified causes behind increase in Diabetes include environmental changes, emotional instability, caloric rich diet, excessive usage of internet and TV, excessive urbanization, and increased obesity in youth. Lifestyle modification (LSM) is still considered to be the most effective intervention when it comes to primary prevention of Diabetes [12].

In the current study, effect of basil seeds gum powder on hyperglycemia in rats was elucidated. It was seen that basil seeds gum powder could significantly decrease the blood glucose levels in hyperglycemic rats which showed that basil seeds had hypoglycemic potential. The results of our study are in line with those of researchers who also carried out trials on basil seeds. This hypoglycemic effect of BSG powder could be due to high dietary fiber content. when basil seeds are socked in water the gum is formed. This gum helped in decline of absorption of glucose from gut [13]. Other possible explanation to this hypoglycemic effect is increase insulin sensitivity. it activate glucose transporter type-4 (GLUT-4) which translocation in non-insulin dependent pathway which explains synergistic effect between active ingredients of gum and insulin [14]. Our study results are also in close relevance to the results of research conducted by Amit. In comparison to previous research the difference was only dosage level. As in current study the dosage of BSG is in percentage. So, it is investigated that more prominent effect of hypoglycemia is noted in 1% treatment group [15].

It is concluded from meta-analysis study that liver disease and diabetes are associated to each other. As liver play major role in controlling body metabolic processes such as gluco-regulation, carbohydrate homeostasis and insulin deterioration [16]. Due to diabetes all those mechanisms and increase insulin sensitivity that leads to abnormality of liver functionality enzymes such as AST, AST, ALP total protein and total bilirubin Patients with type II diabetes are more prone to liver pathophysiology [17]. Chronic kidney functions are also prolonging side effect of diabetes [18]. So, to evaluate its effect on liver and kidney functionality in current study, certain biochemical parameters were also considered such as total bilirubin, ALT, AST, and ALP, total protein, albumin, globulin, creatinine, and urea. Results showed that basil seed gum has significant effect on these biochemical parameters more efficiently in 1% treatment group of rats.

Basil seed gum tendency to have efficacy for hyperlipidemic effect by means of specific phytoconstituents [19]. This current study clearly showed the hyperlipidemic parameters in normal range. The reduction of plasma cholesterol occurs due to lower range of LDL. As LDL is considered bad cholesterol which means lipoprotein takes cholesterol from the liver and deposits in different organs and endothelial cell line of capillaries that eventually worse the pathophysiology for cardiovascular diseases [20]. So, these organic and aqueous fraction of *Ocimum basilicum* has tendency to limited LDL by improving hepatic enzymes functionality that eventually metabolize cholesterol present in liver [21]. On other hand these organic and aqueous fractions have protective effect for HDL which is known as good cholesterol. It helps in mobilization of free fatty acids and cholesterol metabolization and secretion in the form of bile acids [22]. So, in this current study streptozotocin induced diabetes showed drastic abnormality of total cholesterol, LDL, HDL and triglycerides as compared to control group. Diabetes with abnormal lipid profile leads to hypertriglyceridemia that eventually cause pancreatitis that leads to disease severity [23]. However, in our study by treating those diabetic groups with BSG powder have synergistic effect on lipid profile. Within diabetic treatment groups 1% BSG showed more prominent results as compared to 0.5% treatment group.

Conclusion

Therefor outcome of the present study established that oral intake of aqueous extract of *Ocimum basilicum* powder on streptozotocin induced diabetic rats overturned glucose level and complication associated with diabetes such as liver and kidney dysfunctionality. BSG powder may be considered appropriate contender as an alternate therapy option for disease. Beside this different phytoconstituents of seed should be considered responsible for hypoglycemic and hypolipidemic effect.

Conflict of Interest

There is not any conflict of interest.

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