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EVALUATION OF HYPOGLYCEMIC ACTIVITY OF PAKHANBED [BERGENIALIGULATA (WALL.) ENGL.] IN EXPERIMENTALLY INDUCED DIABETES IN ANIMAL MODEL

[1]Nasmeer MSM, [2]Najeeb Jahan,
[1]Senior Lecture, [2]Senior Lecture,
[1]Department of Ilmul Advia,
[1]Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka

Abstract: Diabetes Mellitus (DM) is the third leading cause of death after heart disease and cancer in many developed countries. Its prevalence is about 6% worldwide and the number of cases, presently estimated at more than 150 million, is predicted to be doubled by 2025. Therefore, the demand for safe and effective drugs for the treatment of DM is on the rise. Present study was carried out to evaluate hypoglycemic effect of rhizome of Pakhanbed, in experimental animals. The study was carried out in Wistar rats of 5 groups of 10 animals each. Group I was served as plain control. After overnight fasting groups II, III, IV and V were injected single dose of Streptozotocin (50mg/kg, i.p.). Group II was kept as negative control. Groups III, IV were given extract of Pakhanbed 120.75mg and 241.5mg/kg respectively. Blood samples were taken on 0th, 10th, 28th, and 56th days. The effect of test drug was observed on body weight, fasting blood glucose (FBG), postprandial blood glucose (PBG), urine output, urine sugar and compared with standard (group V), Glibenclamide (600µg/kg). Hydroalcoholic extract of test drug demonstrated significant increase in body weight of groups III, IV and V. On 28th day FBG, PBG were reduced significantly in group III ($p<0.05$) and group IV($p<0.1$), and on 56th day the reducing FBG and PBG were strongly significant in group IV ($p<0.01$). Percentage decreases in urine output and urine sugar were found significant. On the basis of above finding it can be concluded the test drug possesses significant hypoglycemic effect.

***Index Terms* - Diabetes mellitus, Pakhanbed, *Bergenia ligulata*, Streptozotocin, Ziabatus**

I. INTRODUCTION

Diabetes mellitus is the name given to a heterogeneous group of metabolic diseases that are characterized by chronic hyperglycemia and disturbances in carbohydrate, lipid and protein metabolism, resulting from defects in insulin secretion and/or insulin action. Diabetes is a worldwide health problem that affects increasingly people every year. It is the third leading cause of death (after heart disease and cancer) in many developed countries[1]. It affects more than 120 million people world-wide. Diabetes mellitus (DM) is a syndrome of chronic hyperglycaemia due to relative insulin deficiency, resistance, or both. It is also lifestyle-related disease known to trigger many complications, nephropathy, retinopathy, neuropathy, cardiovascular diseases and so on[2].New figures indicate that the number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030, if no urgent action is taken. This equates to approximately three new cases every ten seconds or almost ten million per year[3].

Recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases,scientific evidence in terms of modern medicine is lacking in most cases. However today it is necessary to provide scientific proof as whether to justify the use of plant or its active principles[4].

Many traditional plant treatments for diabetes mellitus are used throughout the world, and some of these plants have been scrutinized while a good number of them are yet to receive scientific scrutiny[5].

Bergenia ligulata a perennial herb with thick rootstock. Stem short, fleshy, flowers white, pink or purple occurs in temperate regions from Kashmir region to Bhutan. It is found in the Himalayas between the altitudes of 2,000 and 2,500 meters. The roots of *Bergenia ligulata* contain tannic acid, gallic acid, starch, mineral salt, albumin, glucose,mucilaginous matter, wax and aromatic substances. The root of *Bergenia ligulata* is used as an anti-diabetic drug, diuretic, astringent, cardiotonic, wound healer, expectorant, antipyretic, anti haemorrhoidal and antiscorbutic[6,7,8,9,10]. It was reported from acute toxicity study, that the maximum lethal dose was found to be 5g/kg b.w.[11].

The rhizome of the plant is widely used as a curative for diabetes mellitus in Indian system of medicine and also used as a folklore remedy to control the blood glucose level without any scientific evidence[12].Hence this study was undertaken to investigate the effect of hydro alcoholic extract of rhizome of *Bergenia ligulata* (Wall.) Engl. In Streptozotocin induced diabeticrats[13].

II. RESEARCH METHODOLOGY

2.1 Plant material

The rhizome of the plant “Pakhanbed” *Bergenia ligulata* was procured from Unani drug shop, City market, Bangalore, during the month of April 2011. The authentication of the drug was carried out by Dr. Siddamallayya N, Botanist of National Ayurveda Dietetics Research Institute (NADRI), Dept. of Ayush, Ministry of Health and Family Welfare, Govt. of India, Ashoka Pillar, Jayanagar, Bangalore-11. (Ref. No. Drug Authentication/SMPU/BNG/2010-11/45 dated 20.04.2011. Annexure: 1) The specimen of plant material was retained in the NADRI and a similar specimen of the plant material has been submitted in the herbarium of Dept. of Ilmul Advia, NIUM, Bangalore, for the record and future reference.

2.2 Preparation of hydro alcoholic extract:

The dried rhizome of the plant cleaned manually and coarse powder was prepared by electrical grinder. In 50% of hydro alcoholic solvent, about 200 gm of powdered rhizome of *Bergenia ligulata* was extracted by Soxhlet apparatus. The test drug and solvent was taken in the ratio of 1:6. The temperature was maintained at 80-90 ^{0}C for 6-7 hrs. The liquid extract was cooled and filtered by Wattmann filter paper40, and then placed on water bath at a temperature of 60-70 ^{0}C until the entire solvent evaporated and then extract was filtered and dried on water bath. The extract was weighted and the yield percentage was calculated with reference to the weight of the crude drug. The calculated yield percentage was 23.03 % w/w.

2.3 Phytochemical Screening

The hydroalcoholic extract was subjected to qualitative phytochemical investigation for the identification of the phytoconstituents viz. alkaloids, reducing sugars, flavonoids, glycosides, phenols, terpenes, sterols, and saponins[14,15,16].

2.4 Animals

The study was carried out on healthy albino rats of Wistar strain of either sex weighing about 150-200 gm, 2-3 month old. The animals were obtained from Biogen Laboratory Animal Facility (Reg No. 971/bc/06/CPCSEA), Bangalore. They ware acclimatized to the laboratory condition for 5 days before the experimental studies. The animals were housed in polypropylene cages and kept under standard environmental condition of 12 hrs light and dark cycle, temperature ($25^0 \pm 2^0$ C) and humidity (55 ± 15%). They were fed with standard food pellets and water *ad libitum*. Study was conducted after obtaining the ethical clearance by the Institutional Animal Ethics Committee (No: 953/C/CPCSEA/2006) of National Institute of Unani Medicine, Bangalore.

2.5 Induction of Diabetes Mellitus

Rats were fasted 12 hours, with free access to water. Diabetes was induced by single dose of intra peritoneal injection of streptozotocin (STZ, Sigma Aldrich, USA) at 50 mg/kg[17],in freshly prepared citrate buffer (pH 4.5) on 0thday. The blood glucose levels were measured on 0th, 10th, 28th and 56th days of experiment using a glucometer (SD Code Free, purchased from Standard Diagnostic, Korea).Animals with blood glucose above 200mg/dl were considered as diabetic and were taken for the experiment. The experiment was continued for 56 days.

2.6 Experimental Design

This test was carried out by the method of Rakietan N*et al*[13](1963). The animals were divided in to 5 groups of 10 animals in each as follows:

Group I was served as plain control and given distilled water after single dose of 0.1M Citrate buffer (pH 4.5). After overnight fasting group II, III, IV and V were injected single dose of Streptozotocin, 50 mg/kg in 0.1M Citrate buffer. Group II was kept as negative control after induction of diabetes. Group III (test group A), Group IV (test group B) were given hydro alcoholic extract of rhizome of *Bergenia ligulata* 120.75mg/kg, 241.5 mg/kg respectively. Group V was given Glibenclamide 600μg/kg b.w. and served as standard control.

Blood (by pricking the tip of the tail) and urine were collected before and after meal for fasting blood sugar, postprandial blood sugar, and urine sugar and urine protein in each group of the animals on 0th day, 10th day, and after treatment on 28th and on 56th days, of the experiment.. At the end of the experiment i.e. on the 66th day all the animals were sacrificed under anesthesia by Thiopentone sodium 40 mg/kg, i.p.[18]Liver tissues were collected for the estimation of hepatic glycogen content.

2.7 Estimation of Hepatic Glycogen content

Glycogen content in the liver was measured according to the Wagtendonk*et al.*[19].A 200 mg of liver sample was weighed on a torsion balance and finely ground with 20 ml of 5% TCA in a mortar[20].The precipitate of proteins was filtered off and the clear filtrate submitted to analysis. 16.5 ml of Lugol's solution (iodine reagent), was prepared by dissolving 1 g of iodine and 2 g of KI in 20 ml of water, is added to 990 ml of an aqueous solution, containing 25% (w/v) of KCl.In a colorimeter tube (1.2 cm. diameter) 2 ml of the liver extract of 5 groups of rats were added to 3 ml of iodine reagent, separately. After mixing, the optical densities of all the liver extracts samples were read in a photometer at 650 nm against a blank (obtained by adding 2 ml of 5% TCA to 3 ml of reagent in the same way). The amount of glycogen in different extract sample of rats liver were read from a glucose calibration curve, which was prepared by using standard glucose[21]

2.7 Estimation of body weight and food consumption

Body weights of all rats in each group were taken on 0th, 10th, 28th and 56th days of experiment by using Gold Teck balance. A known weight of rat diet was given and consumption of food was measured by subtracting the remaining food weight. Consumed food weights was taken daily, and mean weight was taken on 10th, 28th and 56th days of experiment.

III. RESULTS AND DISCUSSION

3.1 Plant material

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Blood (by pricking the tip of the tail) and urine were collected before and after meal for fasting blood sugar, postprandial blood sugar, and urine sugar and urine protein in each group of the animals on 0th day, 10th day, and after treatment on 28th and on 56th days, of the experiment.. At the end of the experiment i.e. on the 66th day all the animals were sacrificed under anaesthesia by Thiopentone sodium 40 mg/kg, i.p.[18]Liver tissues were collected for the estimation of hepatic glycogen content.

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diameter) 2 ml of the liver extract of 5 groups of rats were added to 3 ml of iodine reagent, separately. After mixing, the optical densities of all the liver extracts samples were read in a photometer at 650 nm against a blank (obtained by adding 2 ml of 5% TCA to 3 ml of reagent in the same way). The amount of glycogen in different extract sample of rats liver were read from a glucose calibration curve, which was prepared by using standard glucose[21]

3.8 Estimation of body weight and food consumption

Body weights of all rats in each group were taken on 0th, 10th, 28th and 56th days of experiment by using Gold Teck balance. A known weight of rat diet was given and consumption of food was measured by subtracting the remaining food weight. Consumed food weights was taken daily, and mean weight was taken on 10th, 28th and 56th days of experiment.

3.9 Statistical Analysis

The results were tabulated and the statistical analysis were performed by Analysis of Variance (ANOVA) repeated measure has been used to find the significance of study parameters between three or more groups of rats , Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. Data were considered **"+"** Suggestive significance at P value: $0.05<P<0.10$, "*" moderately significant at P value: $0.01<P \leq 0.05$, "**" and Strongly significant at P value: $P \leq 0.01$.

IV. RESULTS AND DISCUSSION

4.1 Phytochemical screening

The phytochemical screening of hydroalcoholic extract revealed the presence of alkaloids, reducing sugars, flavonoids, glycosides, phenols, terpenes, sterols, and saponins.

4.2 Body weight and Food intake

The 50% hydroalcoholic extract of rhizome of Pakhanbed demonstrated significant increase in body weight of rats of groups III, IV and V by 7.35% ($p<0.01$), 31.0% ($p<0.1$), 11.8 ($p<0.01$), respectively. During the study it was observed that there was no significant difference in food consumption of animals of all the groups. However, it was found that there was significant increase in food consumption of animals of all the groups.

4.3 Fasting Blood Glucose (FBG)

It is evident from the present investigation that STZ administration at the dose of 50 mg/kg body weight to the animals of group I, II, III, IV and V causes significant diabetogenic response from 0th day of STZ administration to 10th day. After 28 days of treatment the FBG levels were found to be decrease in groups III, IV and standard group. Interestingly, the increase in glucose levels in negative control group (group II) was found to be highly significant ($p<0.001$) when compared to the other groups. When comparisons were made between group II and drug treated (group III, IV) animals, blood glucose levels were found to be declined sharply from 10th day to 56th day. After 56 days of treatment the decrease in fasting glucose levels were found to be moderately significant ($p<0.05$) in group IV and there was no significant effect observed in group V, on plasma glucose levels. The percentage decline in blood glucose levels of test group B was found to be strongly significant ($p\leq0.01$) than test group A ($p<0.05$).

4.4 Postprandial Blood Glucose (PBG)

On 0th day just after induction of diabetes mellitus, and on 10th day PPG levels were found to be significantly ($P< 0.001$) raised in all the STZ treated groups when compared with plain control. After 28 days when groups III and IV compared with negative control group (group II) reduction in PPG levels was found to be strongly significant at $P=0.001$ and $P=0.01$, respectively. A moderately significant decreases in PPG level of group III ($P<0.05$) and group IV ($P<0.05$) was seen when compared with these groups with standard group (group V). On 56 days the decreases in PPG levels were found to be moderately significant ($P<0.05$)

in group III and in group IV, and it was found to be strongly significant (P<0.01) when compared to standard control. When the test groups were compared with each other, the decreases in PPG level was found to be strongly significant (P<0.01) in test group B, these results shows that the drug has dose dependent effect.

4.5 Urine output and Urine Sugar

Percentage decreases in urine output and urine sugar were found significant on 28th day. The percentage reduction of urine output was found to be 30 % and 63% after 28 days in groups III and IV respectively, when compared to the urine output of all the STZ treated groups on 10th day. There was decreased in the number of animals (urine sugar) from 7 to 3 after 28 days treatment and was found to be further decrease to 2 after 56 days in group IV.

4.6 Hepatic glycogen content

Hepatic glycogen levels found to be resettled towards plain control in group III

Table 01

Effect of test drug on Fasting Blood Sugarin experimentally induced Diabetes Mellitus

Group	FBS(mg/dl)			
	0th day	10th day	28th day	56th day
Group I	79.30±8.58	74.70±8.42	78.20±9.07	91.50±10.46
Group II	77.50±14.05	142.25±87.39	239.38±125.15 d*	329.25±185.50 c* d**
Group III	85.00±9.29	269.57±149.19	297.86±159.89	184.00±90.27 d**
Group IV	75.50±16.09	157.57±115.45	202.25±70.52	135.67±55.73 d**
Group V	73.63±5.29	81.00±38.33	181.00±189.81	189.67±182.03

n=10 in each groups. Test used:ANOVA tests with Chi-square/ Fisher Exact test, where,

+P:0.05<P<0.10 (Suggestive significance), *P: 0.01<P ≤ 0.05 (Moderately significant)**P: P≤0.01(Strongly significant)and a, b, c, d and e = comparison with plain control(group I), negative control (group II), test group A (group III), test group B (group IV), standard group (group V), respectively.

Table 02

Effect of test drug on Postprandial Blood Sugar in experimentally induced Diabetes Mellitus

Group	Blood Sugar (mg/dl)			
	0th day	10th day	28th day	56th day
Group I	83.30±11.01	95.20±16.76	115.60±7.45	115.5±11.0
Group II	243.63±114.57	400.50±116.79	466.50±141.3	330.8±187.8
Group III	170.25±59.55	510.43±76.94	424.3±187.1 e+ d*	399.0±166.61 d**
Group IV	233.00±40.89	498.43±143.71	389.8±201.5 e*	334.0±143.8
Group V	229.25±54.91	313.86±153.68	349.7±160.7	393.7±195.9 c*d**

n=10 in each groups. Test used:ANOVA tests with Chi-square/ Fisher Exact test, where,

+P:0.05<P<0.10 (Suggestive significance), *P: 0.01<P ≤ 0.05 (Moderately significant)**P: P≤0.01(Strongly significant)and a, b, c, d and e = comparison with plain control(group I), negative control (group II), test group A (group III), test group B (group IV), standard group (group V), respectively.

Fig. 1: Effect of test drug on Effect of test drug onBody weightin experimentally induced Diabetes Mellitus

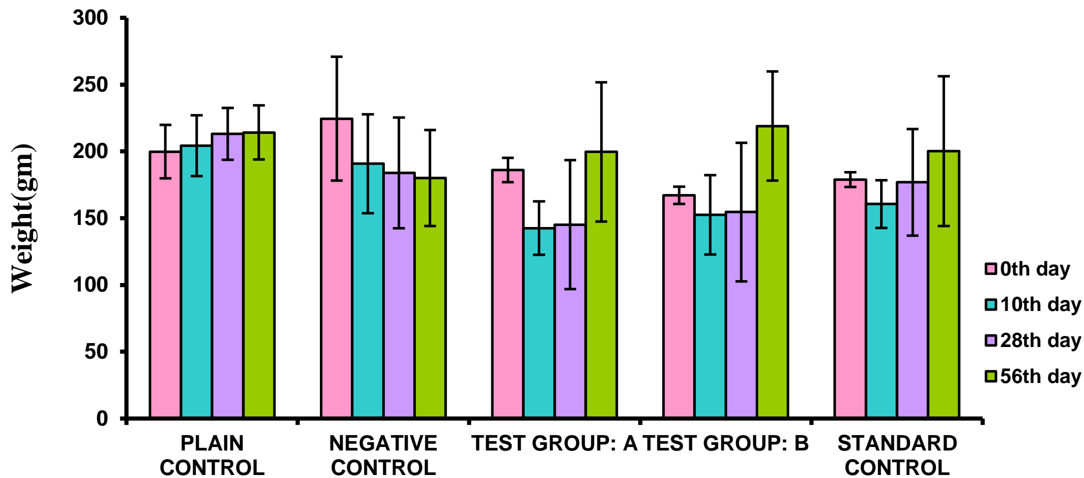
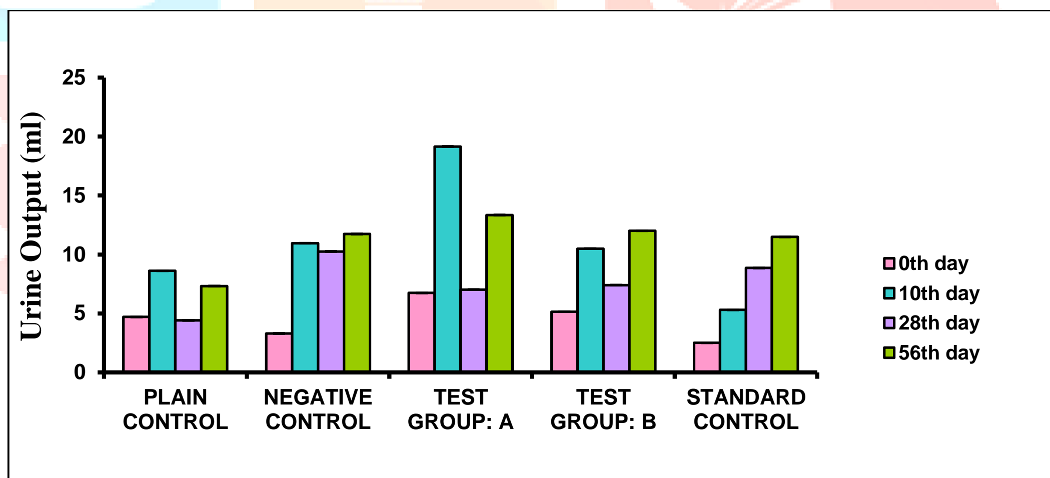


Fig. 2: Effect of test drug on Urine outputin experimentally induced Diabetes Mellitus



DISCUSSION

Diabetes is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein characterized by increased fasting glucose and postprandial blood sugar level[22,23].The diabetes mellitus is classified into two major subtypes, IDDM (Insulin dependent diabetes mellitus) and NIDDM (Non insulin dependent diabetes mellitus)[24,25]. IDDM results from a cellular mediated auto immune destruction of the β-cells of the pancreas[24,25].However, NIDDM results from the development of insulin resistance and the affected individuals usually have insulin deficiency[26]. All IDDM patients need immediate insulin therapy. Initial therapy in NIDDM patients should be by dietary means alone, for 2-3 months but most patients will need oral antidiabetic drugs in addition[27].

Modern medicines like biguanides, sulphonylureas and thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses and fail to give a long term glycemic control[28].

Even the treatment in the Indian ancient pharmacopoeia mentioned specific treatment for the two types including dietary modifications, medicinal plant remedies and minerals. Moreover, researches conducted over last decades has shown plant and plant based therapies have a potential to control and treat diabetes[29].

For testing antidiabetic potential of plants, STZ induced hyperglycemia in rodents is considered to be a good preliminary screening model[30]and it is widely used.

Due to destruction of pancreatic β-cells, secretion of insulin decreases. The lack of insulin or deficiency gives rise to hyperglycemic condition i.e. Diabetes Mellitus, in which large amount of sugar present in the blood and urine. Because insulin is responsible to increase uptake of glucose from blood by activating glucokinase and glucose phosphatase enzyme and there by increases glucose transport as well as glucose utilization especially by muscles and adipose tissues; it promotes glycogen synthesis; it inhibits gluconeogenesis, and it prevents the free glucose to diffuse back into blood by inactivating glucose phosphatase enzyme. The net effect of insulin is to lowers blood glucose level i.e. hypoglycemic effect. Moreover, hypoglycemia is also produced by the drugs which delay the digestion and absorption of intestinal carbohydrate[1,27]

Therefore, the present study was designed by including a number of test and parameter that commensurate with different pathophysiological aspect and mechanism associated with DM, so that the test drug can be studied comprehensively for hypoglycemic effect. The parameters like body weight and food intake and the tests included to study were FBG, PPG level, urine sugar, and glycogen percentage in liver tissue and also an in-vitro test, α-amylase inhibitory assay was undertaken to underline the possible mechanism of action of the hypoglycemic effect of test drug.

In Diabetes deficiency of insulin affect carbohydrate, fat and protein metabolism, abnormalities of carbohydrate metabolism is associated with blood sugar. Abnormal fat metabolism causes conditions like acidosis and arteriosclerosis and abnormality of protein metabolism causes depletion of the body's protein. Failure to use glucose for energy, leads to increased utilization and decreased storage of proteins as well as fat. Therefore, a person with severe untreated diabetes mellitus suffers rapid weight loss and *asthenia* (lack of energy) despite eating large amount of food (*polyphagia*). Without treatment, these metabolic abnormalities can cause severe washing of the body tissues and death within a few weeks[31] (Guyton AC and Hall JE2000)

After Streptozotocin administration there was marked reduction in body weight of rats of all the groups except in group I (plain control). In the present study the significant increase of body weight in group III and Group IV towards plain control (group I) clearly demonstrates hypoglycemic effect of test drug.

Stabilization of blood glucose is important for diabetic patients, because it prevents hyperglycemia and the complications associated with diabetes. In the present study glucose levels in all the groups on 0th day were found to be clinically significant as there were in normal range (FBG ≤ 126 mg/dl) indicates that the higher dose possess a striking hypoglycemic effect and the effect of test drug may be correlated with that of insulin, as insulin is an anabolic hormone, which stimulates the entry of amino acids into the cells, enhance protein synthesis and reduces protein degradations[31]

The finding suggested that the test drug possesses hypoglycemic effect in dose dependent manner as the higher was found to produce greater effect than the lower dose. And the result proves that the test drug may possess the possible insulinomimic mechanism of action[32], it helps in activation of glucose and even the mode of action of the active compound of this plant material is mediated through enhancing secretion of insulin from the β-cells of langerhans insulin secretagogues or through extra pancreatic mechanism , and by activation of glucose uptake by the cells, or increase glycogenesis or by inhibiting gluconeogenesis in the liver or inhibiting the absorption of glucose from the intestine[33].

Some important ingredients eg. (+)-Afzelechin, (+)-3-Acetyl–5,7,4'-trimethoxyafzelechin, (+)-afzelechintetraacetate, (+)-5,7,4' trimethoxyafzelechin, (+)-tetramethoxyafzelechin have been reported to inhibit α-glucosidase enzyme which inhibits the absorption of glucose from the intestine and reduces blood glucose level[34]. Various pharmacological approaches are used to improve diabetes via different modes of action such as stimulation of insulin release, increase the number of glucose transporters, inhibition of gluconeogenesis and reduction of absorption of glucose from the intestine. One of the most beneficial therapies for type II diabetes is said to be the control of postprandial hyperglycemia after a meal[31].

The observations and results of the test drug on postprandial glucose (PPG) level in the present study have demonstrated the reduction of PPG level in dose dependent manner. This finding reveals, that the active component of the test drug possess α-glucosidase inhibitory activity. As it has been reported that the components of test drug like (+)-Afzelechin, (+)-3-Acetyl–5,7,4'-trimethoxyafzelechin, (+)-afzelechintetraacetate, (+)-5,7,4' trimethoxyafzelechin, (+)-tetramethoxyafzelechin inhibit α-glucosidase enzyme in the intestine and decrease the rate of hydrolytic cleavage of oligosaccharides[35], process of carbohydrate digestion spread to the lower part of small intestine and delays the overall absorption rate of glucose into the blood which decreases the postprandial rise in blood glucose and hepatic triglyceride synthesis. Further, this strategy helps in avoiding the onset of late diabetic complication[36.]

Liver, the major metabolic organ, has pivotal role in maintaining blood glucose homeostasis. Insulin regulates the metabolism of carbohydrates in the liver. The reduced glycogen content observed in diabetic rats may be associated with increase in glycogen phosphorylase activity. Disturbed activities of carbohydrate metabolizing enzymes in diabetic rats indicate that the carbohydrate metabolic pathways (glycolysis, glycogenolysis, glycogenesis and gluconeogenesis) were severely affected, which was probably due to insulin deficiency[37]

It has been reported that certain plant drugs, and isolated constituents of various plants[35]possess hypoglycemic activity. Earlier investigations of chemical constituents and their pharmacology revealed that flavonoids, glycosides, sterols, β-sitosterol[38,39], possess hypoglycemic activity. The phytochemicals mentioned above, have been reported to be found abundantly in test drug. So, the hypoglycemic effect of test drug may be due to the presence of these phytochemicals.

It has been reported from previous studies that Catechinand Bergenine the chemical constituents of rhizome of *Bergenia ligulata* possess anti-oxidant property[40], and therefore, it can be used in that pathogenesis of diabetes which involve the destruction of islets of pancreas due to the accumulation of free radicals[1].

Bergenin also plays a role in the mobilization and breakdown of fat and is becoming a popular component of thermogenic fat-burning dietary supplements, it enhance the activity of lipolytic adrenergic hormones like norepinephrine and stimulate lipolysis. It also opposes the lipogenic activities of insulin. The mechanism appears to be through the enhancement of norepinephrine to phospholipids of adipose cells. Bergenin displays moderate activity against an enzyme called protein tyrosine phospatase 1B (PTP1B). This enzyme negatively regulates insulin and leptin signalling and some of the positive effects of insulin and leptin (blood sugar and appetite regulation) may be enhanced by its inhibition[41]

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