



## Phytochemical Screening and Blood Glucose Level Lowering Effect of *Bergenia ligulata* Root Extracts.

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### Abstract

The present study was carried out to evaluate the blood sugar level lowering effect of different extract of *Bergenia ligulata* root i.e. Pet. ether, Solvent ether, Chloroform, Acetone and Ethanol extracts in alloxan induced diabetic rats. The duration of study was 15 days. Blood sugar level lowering activity was evaluated by measuring the Blood sugar level and lipid profile (Total Cholesterol, Total Glyceride, High Density Lipoprotein, Low Density Lipoprotein, and Very Low Density Lipoprotein). The acetone extract has shown significant effect on blood sugar level at a dose of (500 mg/kg,b.w, p.o/day). The study results suggested the *Bergenia ligulata* roots possess significant activity. The different extract of the root of *Bergenia ligulata* were subjected to preliminary phytochemical screening and reveals the presence of Alkaloids, Glycosides, Tannins, Flavonoids and Sterols.

**Key words:** *Bergenia ligulata* root, Blood sugar, Alloxan and Glucometer.

### 1. Introduction

Plants which are the major source of drugs in Indian system of medicine have the advantage of little or no side effects. Most of the medicinal plants are scientifically validated for their therapeutic efficacy and safety. There are several species of the medicinal plant popularly used in the treatment of diabetic problems. <sup>(1)</sup> Still there is a great need to search more effective drugs that can be used as an antidiabetic. *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 roots of *Bergenia ligulata* are used as an antidiabetic, diuretic, astringent, cardiogenic, wound healer, expectorant, and anti-haemorrhoidal. The root is used as a tonic

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in fever, diarrhoea and also as an antiscorbutic. <sup>(2-3)</sup> This plant explore very little for pharmacological activity. Thus we have studied this plant for blood glucose level lowering effect as mentioned in literature.

## 2. Materials and Methods

The fresh plants of *Bergenia ligulata* were collected in January from hilly areas of Dehradun, Uttarakhand, authenticated by Dr. Santosh Kumar Agarwal, Reader and Head, P.G Department of Botany, D.B.S (PG) College, Dehradun. A voucher specimen of the plant has been made and deposited at departmental herbarium. The roots of *Bergenia ligulata* were dried in shade and subjected to reduction to a coarse powder using grinding mill. The coarse powder was extracted successively in soxhlet apparatus with different organic solvents in increasing order of polarity (i.e. petroleum ether, diethyl ether, chloroform, acetone and ethanol).

The crude extracts were evaporated to dryness in Rota evaporator under low temperature and reduced pressure. The practical yield of various dried extracts were as Petroleum ether extract – (0.636 % w/w), Diethyl ether extract – (2.026 % w/w), Chloroform extract – (0.214 % w/w), Acetone extract – (17.792 % w/w ) and Ethanol extract – (12.538 % w/w). The different extract of the root of *Bergenia ligulata* were subjected to preliminary phytochemical screening. The qualitative chemical test of all the extracts of the root were carried out by using standard procedure. <sup>(4-7)</sup> The results so obtained are mentioned in the table- 1.

### 2.1 Animals Selection

Forty eight Wistar albino rats of either sex weighing 120-150 gm were used for the study. The animals were maintained at standard environmental conditions and fed on standard pellets and water *ad libitum*. All the animal

**Table 1.** Phytochemical screening of different extracts of root of *Bergenia ligulata*.

S.No.	Extract	Phyto-constituents present
1	Petroleum Ether	Fats, Steroids, Terpenoids
2	Diethyl Ether	Steroids
3	Chloroform	Alkaloids, Tannins, Glycosides
4	Acetone	Flavonoids, Tannins, Glycosides, Saponnins
5	Ethanol	Alkaloids, Glycosides, Flavonoids, Carbohydrates

experiments were approved by the institutional animal ethics committee.

### 2.2 Acute toxicity study

Acute toxicity studies were carried out for all the extracts of roots of *Bergenia ligulata* on healthy Swiss albino mice of body weight 25-35 g by using Up and Down or Stair case method.<sup>8</sup> All the animals were housed in polypropylene cages at room temperature fed on standard pellet diet and water

*ad libitum*. The maximum non – lethal dose was found to be 5 g/kg- body weight. Hence 1/10<sup>th</sup> of the dose was taken as test dose (500 mg/kg- body weight).

### 2.3 Glucometer

Glucometer manufactured by Abbott Pvt. Limited was used to measure level of blood sugar. A small drop of blood was placed on a disposable test strip, which the meter reads and uses to calculate the blood glucose level.

The meter displays the level in mg/dl.

#### 2.4 Standard Drug

Gliclazide was obtained as in gratia sample from Ranbaxy laboratories, Ponta Sahib, Himanchal Pradesh. The drug was powdered in pestle mortar and triturated with tween-80 to make a fine suspension.

#### 2.5 Plant Extract

The suspension of the different extracts of roots of *Bergenia ligulata* in tween-80 were employed for assessment of present activity at dose (500 mg /kg b.w, p.o/day). The test dose was decided by using “up and down” method. <sup>(8)</sup>

#### 2.6 Induction of Diabetes

Alloxan monohydrate purchased from Central Drug House (CDH), New Delhi were used as diabetes inducing agent. Before induction of diabetes, the normal blood glucose level of all the animals were measured with the help of Glucometer and after that the animals were allowed to fast for 18 hours. Diabetes was induced by a single injection of alloxan monohydrate at a dose of 150 mg/kg b.w <sup>(9)</sup> intra peritoneally in freshly prepared 10 mmol/l sodium citrate; pH 4.5. <sup>(10)</sup>After 72 hours of the injecting of alloxan, blood glucose level was measured to confirm the induction of diabetes. The animal with sugar level 200 mg/dl were selected and considered as diabetic. All the animals were divided in seven groups containing six animals in each.

Group-I, animals and Group-II, Control animals were treated with tween-80 suspension only. Group-III, group diabetic animals were treated with suspension of standard drug Gliclazide in tween-80 at a dose of 10 mg/kg b.w. p.o/day.<sup>9</sup> Group-IV-VIII, animals were treated with Ethanolic, Acetone, Chloroform, Diethyl ether and Petroleum ether extract suspension of root of *Bergenia ligulata* in tween-80 at a dose of

500 mg/kg b.w. p.o/day respectively.

#### 2.7 Statistical analysis

The data obtained during the study was subjected to statistical analysis. All the results obtained are expressed as Mean  $\pm$  S.E.M. The data were analyzed using one way Analysis of variance (ONE WAY ANOVA) and the group means were compared by Dunnet test. Values were considered statistically significant when  $p < 0.01$ . Sigma stat was used for the analysis of data.

### 3. Result and Discussion

Alloxan produces selective necrosis of  $\beta$ -cells of islet of langerhans of pancreas and it leads to the deficiency of insulin. Insulin deficiency produces elevation of blood glucose level and also causes excessive catabolism of proteins and amino acids that are released, utilized for gluconeogenesis. It also stimulates lipolysis in the adipose tissue and gives rise to hyperlipidemia that can be used to evaluate the anti-diabetic activity by measuring the Lipid Profile.

The Present study was aimed to finding out whether the roots of *Bergenia ligulata* possesses blood sugar level lowering activity and which extract shows significant activity in alloxan induced diabetic animals. Different extracts of roots of *Bergenia ligulata* (viz: - Ethanol, Acetone, Chloroform, Diethyl ether, Petroleum ether) were administered orally at a dose of 500 mg/ kg b.w, p.o/day for 10 days. Blood sample were collected at the end of treatment period from individual animal from the retro orbital plexus under ether anesthesia and centrifuged at 3000 rpm for 10 minutes to separate the serum. The level of blood sugar was measured with the help of Abbott Glucometer. All the extract except Petroleum ether extract shows marked decrease in elevated blood glucose level. The percentage reduction in blood glucose level as compared to control group animals are shown in the table- 2. Serum

was used to measure the lipid profile (Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein HDL) by using Diagnostic Kits. Level of Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were determined by the method of Friedwald's formula <sup>(11)</sup> and shown in table-3. All the parameters showed an increase in the level of lipid profile as compared to alloxan treated diabetic animals (control group) except HDL. But the experimental animals treated with the

acetone and ethanolic extract of root of *Bergenia ligulata* showed a significant decrease in the level of TC, TG, LDL, VLDL & an increase in HDL as compared to control group.

Review of literature shows that some flavonoids and saponins isolated from medicinal plants significantly reduce blood sugar levels. Flavonoids stimulate the secretion of insulin in  $\beta$ -cells of islet of langerhans of pancreas. It is possible that the presence of glycosides,

**Table 2.** Effect of different extract of root of *Bergenia ligulata* on Blood glucose level in alloxan induced diabetic animals in (mg/dl).

Groups	Blood glucose level ( 0th day)	Blood glucose level ( 3rd day)	Blood glucose level ( 10th day)	Reduction (%)
Normal Group	91.0 $\pm$ 4.033	94.167 $\pm$ 2.971	92.667 $\pm$ 3.621	1.59 %
Control Group	99.5 $\pm$ 4.311	312.167 $\pm$ 21.311	347.33 $\pm$ 31.711	*
Standard Group	97.0 $\pm$ 3.715	353.667 $\pm$ 21.980	124.33 $\pm$ 13.391***	64.85 %
Ethanol Extract	96.83 $\pm$ 2.442	323.333 $\pm$ 7.719	179.333 $\pm$ 4.794**	44.54 %
Acetone Extract	87.5 $\pm$ 3.510	320.833 $\pm$ 9.973	116.5 $\pm$ 2.232***	63.69 %
Chloroform Extract	99.0 $\pm$ 3.357	332.833 $\pm$ 32.479	279.5 $\pm$ 30.865*	16.02 %
Diethyl Ether Extract	99.5 $\pm$ 2.895	345.167 $\pm$ 21.820	288.5 $\pm$ 11.464*	16.42 %
Petroleum Ether Extract	99.33 $\pm$ 3.159	315.667 $\pm$ 14.921	328.5 $\pm$ 14.227	*

\* No reduction in blood glucose level. Data are expressed as mean  $\pm$  SEM one way Anova method (n=6). The significance on comparison with control group. \* mark, \*P< 0.05, \*\*P< 0.01, \*\*\*P< 0.001.

**Table 3.** Effect of different extract of root of *Bergenia ligulata* on lipid profile in alloxan induced diabetic animal (in mg/dl).

Groups	TC	TG	HDL	LDL	VLDL
Normal Group	153.32 $\pm$ 6.2	86.83 $\pm$ 5.5	45.4 $\pm$ 1.5	90.48 $\pm$ 4.9	20.78 $\pm$ 1.9
Control Group	293.11 $\pm$ 16.6	223.25 $\pm$ 17.2	46.0 $\pm$ 1.1	225.12 $\pm$ 14.2	56.23 $\pm$ 8.0
Standard Group	146.83 $\pm$ 6.1***	108.00 $\pm$ 6.1***	51.50 $\pm$ 1.9***	73.73 $\pm$ 6.7***	9.28 $\pm$ 2.1***
Ethanol Extract	174.83 $\pm$ 1.9***	131.66 $\pm$ 7.9***	45.66 $\pm$ 1.5***	102.83 $\pm$ 5.9***	23.98 $\pm$ 3.5***
Acetone Extract	158.50 $\pm$ 5.9***	115.50 $\pm$ 6.1***	50.83 $\pm$ 1.9***	94.56 $\pm$ 4.8***	20.22 $\pm$ 1.2***
Chloroform Extract	243. $\pm$ 2.6*	190.58 $\pm$ 8.5*	35.86 $\pm$ 2.9*	198.56 $\pm$ 5.6	48.68 $\pm$ 6.5*
Diethyl Ether Extract	258.12 $\pm$ 4.8	185.22 $\pm$ 6.8*	38.23 $\pm$ 2.9*	210.5 $\pm$ 9.2	53.28 $\pm$ 5.5
Petroleum Ether Extract	289.16 $\pm$ 15.6	193.66 $\pm$ 6.0*	35.23 $\pm$ 2.1*	215.16 $\pm$ 14.0	55.2 $\pm$ 8.2

(TC-Total Cholesterol, TG-Total Glyceride, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein, VLDL-Very Low Density Lipoprotein). Data are expressed as mean  $\pm$  SEM one way Anova method (n=6). The significance on comparison with control group. \* mark, \*P< 0.05, \*\*P< 0.01, \*\*\*P< 0.001.

flavonoids and tannins in acetone extract are responsible for their activity. <sup>(12-13)</sup> Further studies are necessary to elucidate in detail the mechanism of the action of drug. Based on the results of this study we conclude that Acetone extract of roots of *Bergenia ligulata*

which shows maximum decrease in the elevated blood glucose level and significant effect on the lipid profile can be used to control the blood sugar level. However study is in progress to isolate the compound responsible for the activity.

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