https://doi.org/ 10.33472/AFJBS.6.5.2024. 6010-6034

African Journal of Biological Sciences

Antidiabetic And Antioxidant Activity of *Bergenia ligulata* (Paashanbheda)

1. Dr. Vastvikta Sahai, Assistant Professor, Krupanidhi College of Pharmacy, Bengaluru, INDIA, Email id: vasusahai26@gmail.com, Phone no.: 7506074793

2. Mrs. Yogeshwari Jambhulkar*, Assistant Professor, Krupanidhi College of Pharmacy, Bengaluru, INDIA. Email id: yognandini20@gmail.com ,Phone no.: 8530270377

3. Dr. Paramita Das, Professor & Head of Department (Pharmaceutical Chemistry), Krupanidhi College of Pharmacy, Bengaluru, INDIA. Email id: paramitadas04@gmail.com

4. Dr. Gururaj MP, Principal, Vivekananda Institute of Pharmaceutical Sciences, INDIA. Email id: gurureceptor@gmail.com

5. Dr. Padma P Prabhu, Professor, Srinivas College of Pharmacy, Mangalore, INDIA

Article History
Volume 6, Issue 5, 2024
Received: 25 May 2024
Accepted: 02 Jun 2024
doi: *10.33472/AFJBS.6.5.2024. 6010-6034*

Abstract

With an emphasis on *Bergenia ligulata's* hypoglycaemic, antioxidant, and anti-diabetic qualities, the study investigated the plant's medicinal potential in the treatment of diabetes. Rats were given Streptozotocin (STZ), which is produced by Streptomyces achromogenes, to induce diabetes by damaging β-cells and reducing insulin output. In rats, Bergenia ligulata extract significantly improved insulin levels and glucose tolerance through hypoglycaemic effects. It also protects against the frequent side effects of diabetes, such as protein breakdown and muscular atrophy.
The study examined *Bergenia ligulata's* possible therapeutic uses in the treatment of diabetes, with a focus on the plant's hypoglycaemic, antioxidant, and anti-diabetic properties. To cause diabetes in rats, Streptozotocin (STZ), was administered. This caused damage to β-cells and decreased insulin production. *Bergenia ligulata* extract produced hypoglycaemic effects that markedly increased insulin levels and glucose tolerance in rats. It also offered a defence against the common consequences of diabetes, such as muscle atrophy and protein breakdown.

Additionally, glibenclamide, a common anti-diabetic medication, along with *Bergenia ligulata* extract normalised cholesterol and triglyceride levels in diabetic rats, lowering cardiovascular risk factors. According to the findings, the hypoglycaemic and antioxidant qualities of *Bergenia ligulata* extract make it potentially as effective a natural therapy for diabetes as glibenclamide.

Keywords:
Antidiabetic activity, *Bergenia ligulata*, methanolic extract, hypoglycaemic, antioxidant

1. Introduction

Diabetes is an inability of the body to turn glucose (sugar) into energy. The main source of fuel for our bodies is glucose. (1) Food is transformed into lipids, proteins, and carbohydrates during digestion. Diabetes is a chronic disease that may be identified by high blood glucose levels. Diabetes is a condition in which the body either cannot create enough insulin or cannot utilize the insulin it produces as well as it should. (2) Diabetes has an impact on many bodily organs, including the heart, blood vessels, nerves, feet, eyes, teeth, gums, kidneys, sex organs, and kidneys. (3)

The World Health Organisation reports that 8.5% of persons who were 18 years of age or older had diabetes in 2016. 2019 had 1.5 million fatalities directly related to diabetes, with 48% of these deaths happening before the age of 70. Diabetes contributed to an additional 4,60,000 deaths from renal disease, and elevated blood glucose accounts for 20% of fatalities from cardiovascular disease. Diabetes caused an increase in age-standardized death rates of 3% between 2000 and 2019. Diabetes caused a 13% rise in mortality in lower-middle income nations. (4)

Traditional herbal medicines are plant-derived natural products that have been employed by our rural people for ages for the management of various diseases. These kinds of traditional plant-based medicines treat about 80% of the world's population, with emerging nations using them at a higher rate than others. Because they are less harmful, have fewer side effects, are more affordable, and are easier to obtain than synthetic medications, natural remedies have drawn attention from the medical community recently. (5) (6)

Bergenia ligulata Wall (Family Saxifragaceae) is regarded as one of the most prominent instances of contentious medications and one of the most prized, endangered climatic medicinal plants. It is locally known as '*Paashanbheda'* (designating 'to dissolve stones') in Indian systems of medicine as the rhizomes of *B.ligulata* have been used for centuries in herbal formulations for the dissolution of kidney and bladder stones. The perennial plant B. ligulata has a very sturdy base and short, thick, meaty stems that are procumbent. When flowers bloom, the leaves are oblong or spherical and are 5 to 15 centimeters in length (March to May). Autumn leaves have short, stiff hairs that grow to a length of around 30 cm and develop a brilliant scarlet color. As leaves mature, their hairy upper and bottom surfaces nearly completely disappear. The 3.2 cm in diameter, white, pink, or purple flowers create a cymose panicle with a flexible flowering stem that is 10- to 25 cm long, leafless, and adorned

with styles. The C-glycoside known as berginine, b-sitosterol, gallic acid, tannin, and amino acids isoleucine, leucine, methionine, phenylalanine, and threonine are found in the root and rhizome and are what give these plants their medicinal properties. Only bergenine has antihepatotoxic, antiulcer, anti-arrhythmic, and burn-wound healing properties. neuroprotective, antifungal, antidiabetic, antilithiatic, anti-inflammatory, anti-nociceptive, anti-HIV, and immunomodulatory properties. (7) (8)

Drugs are obtained from natural sources and have a lengthy history of traditional use. They are easily available and pure forms of medication. Bergenia ligulata has been found to have a wide range of uses. It will have the least or no side effects. Bergenia species also possess several other biological activities like diuretic, anti-diabetic, antitussive, insecticidal, anti-inflammatory, antipyretic, anti-bradykinin, antiviral, antibacterial, antimalarial, hepatoprotective, antiulcer, anticancer, antioxidant, anti-obesity, and adaptogenic. (9)

2. Methods

2.1 Plant Material

The highest total contents of bioactive compounds have been noted in B. ligulata compared to other medicinally important Bergenia species. The following are the bioactive constituents Bergenin, Arbutin, Gallic acid, Protocatechuic acid, Syringic acid, Catechin, Ferulic acid, Paashaanolactone. Bergenia ligulata root powder was obtained from a local market in Bengaluru. (9)

2.2. Animals

Albino rats of strain Wistar weighing in the range of 150–200 gm was bought and kept in animal house. They were adult rats, age ranging from 3-6 months. The animals were given an unlimited supply of water along with a conventional laboratory chow pellet meal that was purchased from Hindustan Lever Limited in Mumbai, India. All the studies were conducted by the Animal Ethical Committee guidelines.

2.2 Diabetes Induction

The rats were starved for the whole night prior to having diabetes induced in them. Streptozotocin (65 mg/kg) dissolved in 0.1 M citrate buffer was injected in rats for diabetes mellitus induction. Saline was administered intraperitoneally to normal control rats instead of STZ citrate buffer. Diabetes was induced but no therapy was given to the second group, which was the typical control. Diabetes was introduced into the final three groups.

Amongst these, one group was treated with the standard anti-diabetic drug Glibenclamide, while the other 2 groups were treated with different concentrations of Bergenia extract i.e., 250mg/kg and 500 mg/kg, and the experiment protocol was performed for 28 days. The doses were selected by referring several research articles. (10)

2.3. Oral glucose tolerance test

Rats that had been famished for an entire night were used for the test. Thirty minutes after the standard anti-diabetic medication glibenclamide and the corresponding amounts of extracts were administered, all four groups of rats received 2 mg/kg glucose. After predetermined intervals of 0, 30, 60, 90, and 120 minutes, blood samples were obtained by tail vein/retro-orbital puncture. The amount of glucose in the blood was measured using the GOD-POD kit. (11)

2.4.1. Glycated hemoglobin

The usual methodology was followed in measuring the glycated haemoglobin (HbA1c) level. (Selvin et al., 2010). Four millilitres of blood were drawn for this purpose, and the plasma was separated in an EDTA-containing bulb. Six times, the packed cell was cleaned with regular saline. To make the hemolysate, fill the packed cell with 1/4 of distilled water and 1/4 of carbon tetrachloride, then centrifuge for 20 minutes at 3000 r.p.m. Using the cyanmethemoglobin technique, the hemolysate's haemoglobin concentration was determined. Normal saline was used to adjust the haemoglobin concentration to 10 mg/dl. Hemolysate was obtained at a concentration of 10 mg/dl haemoglobin, to which 1.0 ml of 0.3 (N) oxalic acid was added and well mixed. After the mixture was allowed to cool to room temperature and steeped for one hour in boiling water, one millilitre of 40% TCA was added. After mixing, the material was centrifuged at 3000 rpm. After collecting 2 ml of supernatant, 0.5 ml of 0.7% thiobarbituric acid was added, and the mixture was then stored at a temperature of 37 °C for 40 minutes. At 443 nm, the reading was made in comparison to a blank that contained 0.5 ml of thiobarbituric acid and 2 ml of distilled water. The expression for glycosylated haemoglobin was GHb%. (12)

2.6.2. Concentration of Hexokinase

Using an assay mixture, spectrophotometric analysis was used to assess the hexokinase activity in hepatic tissue. 45 mM HEPES buffer, 7.5 mM MgCl2, 11 mM thioglycerol, and 3.7 mM glucose made up the test mixture. The tissue was homogenised at a tissue concentration of 50 mg/ml in 0.1 M phosphate buffer saline (pH-7.4) at a temperature of ice

cold. 0.9 ml of the assay mixture and 0.22 M 0.03 ml of ATP were collected and well mixed in a spectrophotometer cuvette. The cuvette was then filled with 0.1 ml of tissue supernatant, and absorbance at 340 nm was recorded. Hexokinase was represented as one unit, or μg/mg of tissue. (12)

2.6.3. Glucose-6 phosphate level

Using a conventional technique, the hepatic glucose-6-phosphatase activity was determined. (de Koning and van Dam, 1992). At a tissue concentration of 50 mg/ml, tissue was homogenised in 0.1 M phosphate buffer saline (pH = 7.4) at ice-cold temperatures. 0.1 ml of 0.1 M glucose-6-phosphate solution and 0.3 ml of 0.5 M maleic acid buffer (pH-6.5) were placed in a calibrated centrifuge tube and heated for 15 minutes at 37 °C in a water bath. One millilitre of 10% trichloroacetic acid (TCA) was used to halt the reaction, and it was then chilled in ice and centrifuged at 3000 ×g for ten minutes. At 340 nm, the optical density was measured. The amount of inorganic phosphate released per milligrams of tissue was used to express the enzyme activity. (12)

Statistical analysis

Using one-way analysis of variance, all the results in this experimental investigation were reported as the mean ± SEM (ANOVA). San Diego, California's Graph Pad Prism Version 5.0 was used for all statistical analysis. Every value was regarded as highly significant, more significant, and substantial. Where 0.05, 0.01, and 0.001 were the P values. (12)

Results

Biochemical Estimations of *Bergenia ligulata* root ethanolic extract

Proximate Analysis of *Bergenia ligulata*

Ash Value: The total Ash value of *Bergenia ligulata* root was reported 11.3%, water soluble ash 0.44%, water insoluble ash was reported 15.02%, and acid insoluble ash 0.38%. Extractive Value: In alcohol the extractive value was 19.12% and in water the soluble extractive value was 14.9%. Moisture content: It was recorded to be 0.41%.

Table 1: Ash and extractive value

Parameters	***Bergenia ligulata***
Total ash value	11.3%
Water soluble ash	0.44%
Water insoluble ash	15.02%
Acid insoluble ash	0.38%
Alcohol soluble extractive value	19.12%
Water soluble extractive value	14.9%
Moisture content	0.41%

Preliminary phytochemical screening

Preliminary phytochemical screening of the ethanolic extract of *Bergenia ligulata* showed alkaloids, flavonoids, phenolic compounds, tannins, glycoside, carbohydrates, and saponins. It showed the presence of Tryptophan as it gave orange colour for Pauly's test.

Table 2: Preliminary phytochemical screening

Test	**Inference**
Flavonoids	+
Saponins	+
Steroids	+
Alkaloids	+
Glycosides	+
Amino Acids	-
Carbohydrates	+
Phenols	+
Tannins	+
Coumarins	-

Oral Glucose Tolerance test

Rats that had been starved all night were used for the test. Thirty minutes after the usual anti-diabetic medication glibenclamide and the corresponding amounts of extracts were

administered, all four groups of rats received 2 mg/kg glucose. Blood samples were obtained by retroorbital puncture or tail vein after predetermined intervals of 0, 30, 60, 90, & 120 minutes, respectively. The GOD-POD kit was utilised to measure blood glucose levels.

Table 3: Effect of *Bergenia ligulata* extract (BL) on oral glucose tolerance test

Groups	**Time (min)**				
	0	**30**	**60**	**90**	**120**
Glucose Control	69.8	117.9	115.2	109.1	99
Glucose with BL (250mg/kg)	72.09	116.2	108.8	99.7	86.3
Glucose with BL (500mg/kg)	71.22	119.1	110.3	85.1	79.2
Glucose + Gli (2.5 mg/kg)	71.87	116.9	88.9	80.1	65.3

Blood glucose level, HOMA-IR and HOMA-β

Blood glucose level

Fig. 1, table 4 shows the anti-diabetic impact of Bergenia ligulata root extract on blood glucose percentage in rats with diabetes caused by streptozotocin. When the rats were given the extract at two different dosages (250 and 500 mg/kg), there was a decreased proportion of blood glucose ($P < 0.001$). We could deduce from the results above that different doses of *Bergenia ligulata* root extract had effects that were dosage dependant. The greatest drop in blood glucose percentage was seen on the final day of the trial, at 34.23% and 54.39%, respectively. Comparing the common anti-diabetes medication glibenclamide to diabetic control groups revealed a 65.75% reduction in blood glucose percentage (Fig. 1).

Effect of HOMA-IR and HOMA-β

Figure 1 shows the effects of different dosages of Bergenia ligulata root extract on the evaluation of insulin resistance using the homeostatic model. Rats with STZ-induced diabetes have higher levels of insulin resistance than normal control rats, although the extract's effects were slightly less pronounced at 250 and 500 mg/kg. Rats treated with Glibenclamide showed an even lower reduction in outcome.

Figure 1 also displays findings from HOMA-β (Homeostatic model assessment for β cell function). Relative to normal control rats, diabetic rats originating from STZ showed a significant reduction in the functioning of β cells. Diabetic rats given Bergenia ligulata root extract showed elevation; rats given a dosage of 500 mg/kg, like glibenclamide, showed a significant increase.

Table 4: Effect of *Bergenia ligulata* extract (BL) on blood glucose level, HOMA-Insulin resistance and HOMA- β

Groups	**Blood Glucose level (mg/dL)**	**HOMA-IR (µmol/min)**	**HOMA-β (µU/ml)**
Normal Control	95.5	1.46	127
Drug Control (STZ)	351.5	1.56	12.7
BL (250mg/kg)	211.3	1.14	21.8
BL (500mg/kg)	143.6	1.17	29.5
Gli (2.5 mg/kg)	106	1.18	31.3

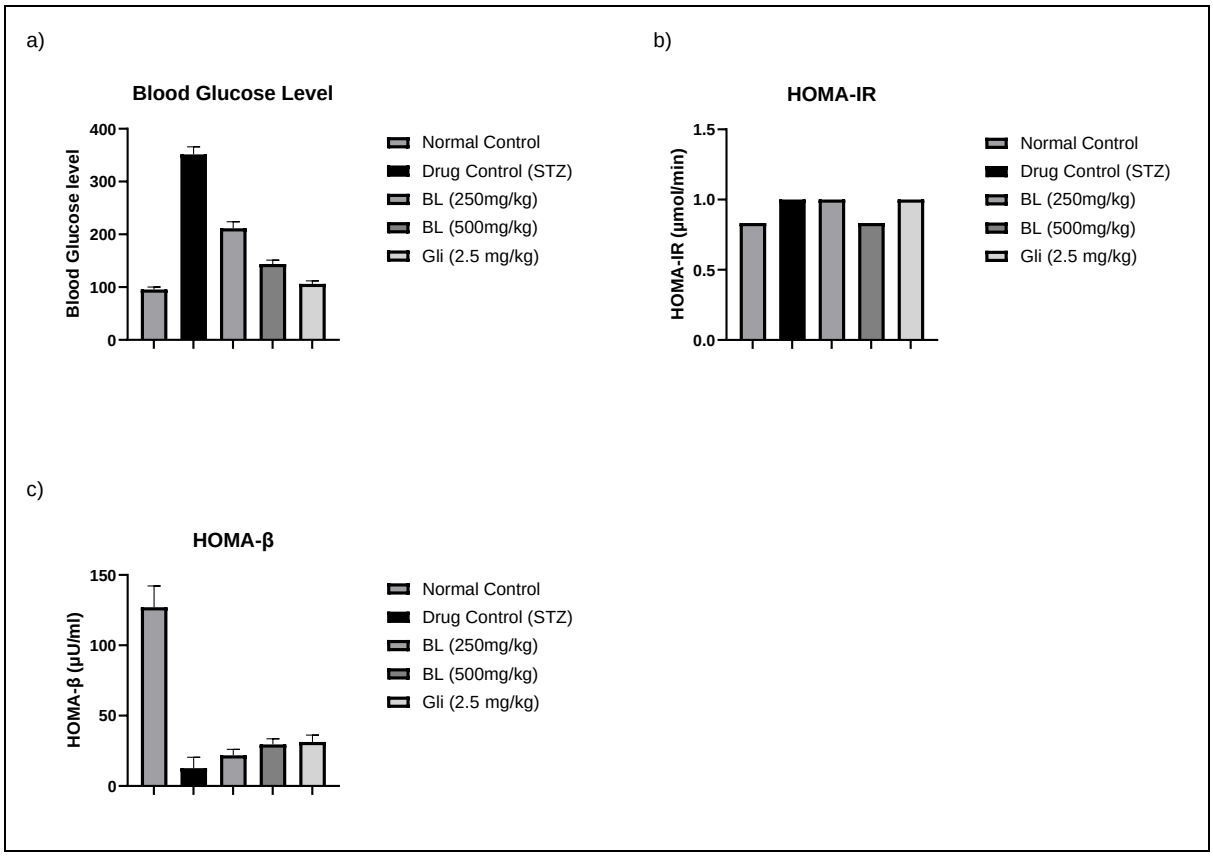

Fig. 1. Demonstrates how a Bergenia ligulata extract affects the various diabetic indices in rats with diabetes that have been produced by streptozotocin (STZ). As stated in the material and methods, a) blood glucose level, b) HOMA-IR, and c) HOMA-β technique. For determining statistical significance, Dennett's test was applied. (*P < 0.05, **P < 0.01 and ***P < 0.001).

Plasma Insulin level

The effect of distinct doses of *Bergenia ligulata* root extract on plasma insulin was tabulated and shown in Fig. 2. When compared to rats in the control group that were given a vehicle alone, diabetic rats initiated on streptozotocin (STZ) showed a significant decrease in plasma insulin levels. However, rats' plasma insulin level was noticeably raised (P < 0.001) when different dosages of Bergenia ligulata root extract were given to them orally. Comparing the usual medication glibenclamide with a 500 mg/kg dosage of Bergenia ligulata root extract, identical results were obtained in terms of raising plasma insulin levels.

Table 4: Effect of *Bergenia ligulata* extract (BL) on plasma insulin level

Groups	**Plasma Insulin level**

Normal Control	4.17
Drug control (STZ)	1.02
BL (250mg/kg)	1.67
BL (500mg/kg)	3.51
Gli (2.5 mg/kg)	3.55

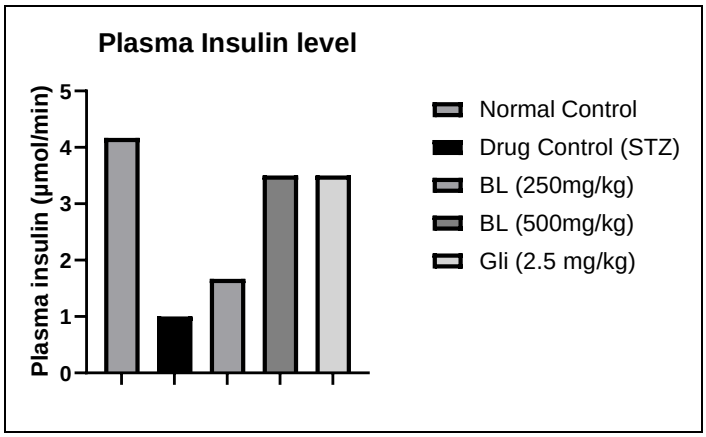

Fig. 2. Shows the effect of *Bergenia ligulata* on the plasma insulin level in the STZ induced diabetic rats. Dennett's test was used for evaluation of statistically significance (*P < 0.05, **P < 0.01 and ***P < 0.001).

Hepatic Parameters

Effect on Glycated haemoglobin

The measure of glycated haemoglobin was inflated in rats having diabetes which was induced by streptozotocin. Three doses of distinct concentrations of *Bergenia ligulata* root extract (250 and 500mg/kg) was administered along with glibenclamide (2.5 mg/kg) to group rats for the study. On day 28, the last day of the trial, it was noted that there had been a noticeable decrease (P < 0.001) in the amount of glycated haemoglobin compared to the diabetic control groups of rats. (Fig. 3).

Effect on hexokinase concentration

The measure of the hexokinase was noted having a downfall in streptozotocin originated diabetic rats. The rats were given varying quantities of Bergenia ligulata root extract (250, 500 mg/kg) and standard medicine glibenclamide (2.5 mg/kg). On the last day of the trial, there was a noteworthy increase (P < 0.001) in the quantity of hexokinase as compared to the diabetic rats. (Fig. 3). Hexokinase level at *Bergenia ligulata* root extract dose 250 mg/kg was

high when comparing with remaining groups that received various doses of *Bergenia ligulata* root extract and glibenclamide.

Effect on glucose-6-phosphate level

The glucose-6-phosphate measure was elevated in diabetic Wistar rats (diabetes caused by streptozotocin). Different rat groups were given glibenclamide (2.5 mg/kg), the typical anti-diabetic medication, and three different dosages of Bergenia ligulata root extract (250, 500 mg/kg). On the 28th day of the investigation, after this dosage, it was found that P was less than 0.001, indicating that the amount of glucose-6-phosphate had decreased in contrast to the diabetic control groups of rats. (Fig. 3). *Bergenia ligulata* root extract dose 500 mg/kg was most effective when compared 250mg/kg dose of *Bergenia ligulata* root extract.

Table 5: Effect of *Bergenia ligulata* extract (BL) on hepatic parameters

Groups	**Hexokinase (µg/mg of tissue)**	**Glucose-6-Phosphatase (µg/mg of tissue)**	**Fructose-1,6-Biphosphatase (µg/mg of tissue)**	**Glycated Haemoglobin (A1C) (%)**
Normal Control	155.3	7.8	36.5	4.6
Drug Control (STZ)	103	14.5	72.6	8.4
BL (250mg/kg)	132	13.6	48.5	7.4
BL (500mg/kg)	129.6	12.8	45.3	6.4
Gli (2.5 mg/kg)	158.1	11.5	48	6.2

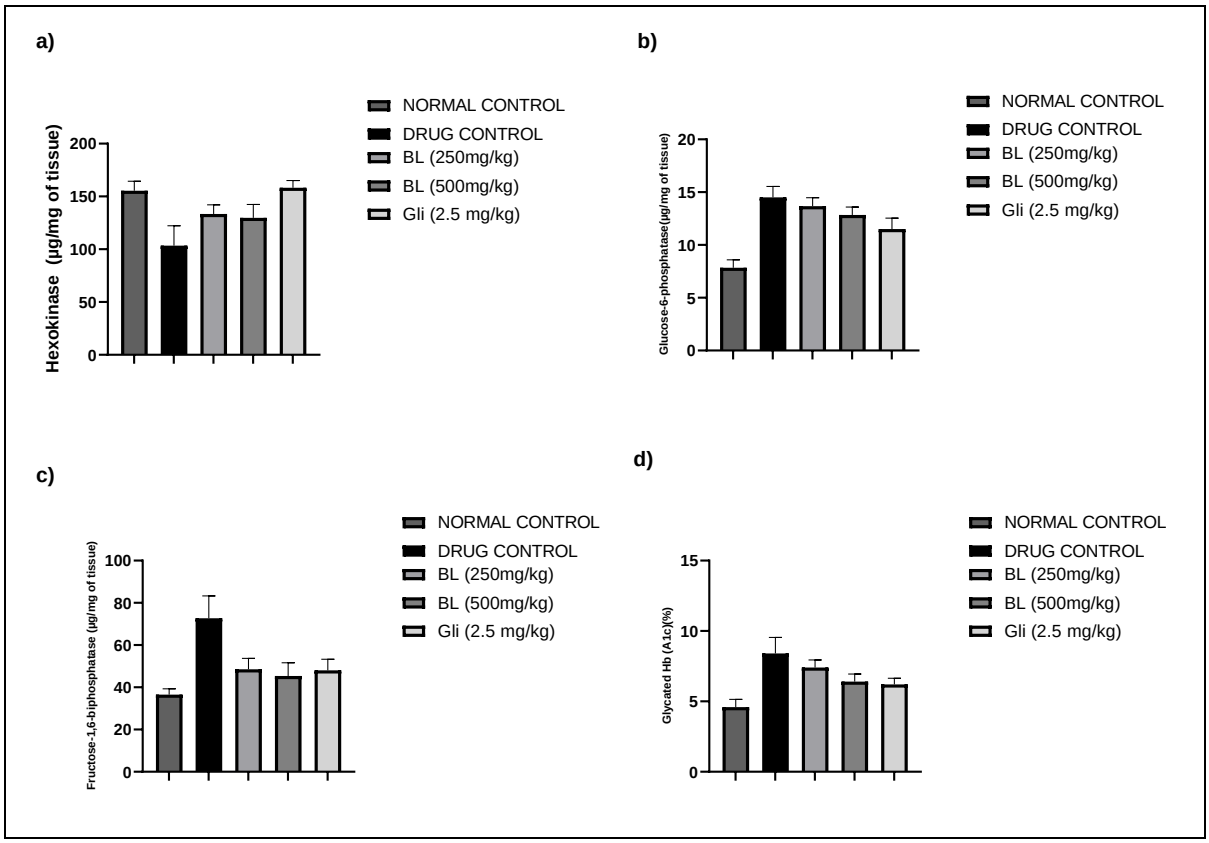

Fig. 3. Shows the effect *Bergenia ligulata* root extracts on the different hepatic enzymes in the streptozotocin (STZ) induced diabetic rats. **a:** hexokinase, **b:** glucose-6-phosphatase, **c:** frustose-1-6, biphosphatase and **d:** glycated haemoglobin described in method as described in the material and methods. For determining statistical significance, Dennett's test was applied. (*P < 0.05, **P < 0.01 and ***P < 0.001).

Lipid Parameters

Effect on cholesterol level

In the diabetic rats treated with streptozotocin, there was a discernible increase in cholesterol levels. The administration of two concentrations of root extract from Bergenia ligulata caused the level of total cholesterol to decrease. (Fig. 4). It was recorded that the level of serum cholesterol of diabetic rats that were untreated was quite elevated than that of diabetic rats (group I & II) receiving *Bergenia ligulata* root extract.

Effect on HDL cholesterol

It is likely that the diabetic rats treated with streptozotocin had a decrease in their HDL cholesterol levels. After giving varying doses of *Bergenia ligulata* root extract, observable elevation in HDL cholesterol level was noted in comparison to the diabetic rats (Fig. 4).

Effect on LDL cholesterol

LDL cholesterol was elevated in diabetic rats treated with streptozotocin. This rise is shown to decrease upon intake of dosages of Bergenia ligulata root extract.

Effect on level of VLDL cholesterol

Diabetic wistar rats treated with streptozotocin showed elevated levels of VLDL cholesterol (Fig. 4). Following dosages of the common medication glibenclamide and the extract from the roots of Bergenia ligulata indicated a decreased amount of VLDL cholesterol ($P < 0.001$).

Table 6: Effect of *Bergenia ligulata* extract (BL) on lipid parameters

Groups	**Total cholesterol(mg/dL)**	**HDL (mg/dL)**	**TG (mg/dL)**	**LDL (mg/dL)**	**VLDL (mg/dL)**
Normal Control	60.3	40.8	70.8	11.2	15.6
Drug control (STZ)	122.5	24.3	167.1	97	37.5
BL (250mg/kg)	115	29.5	153.8	78.5	25.3
BL (500mg/kg)	103.3	31.6	119.7	65.5	22.1
Gli (2.5 mg/kg)	101.5	33	117.3	63.6	22

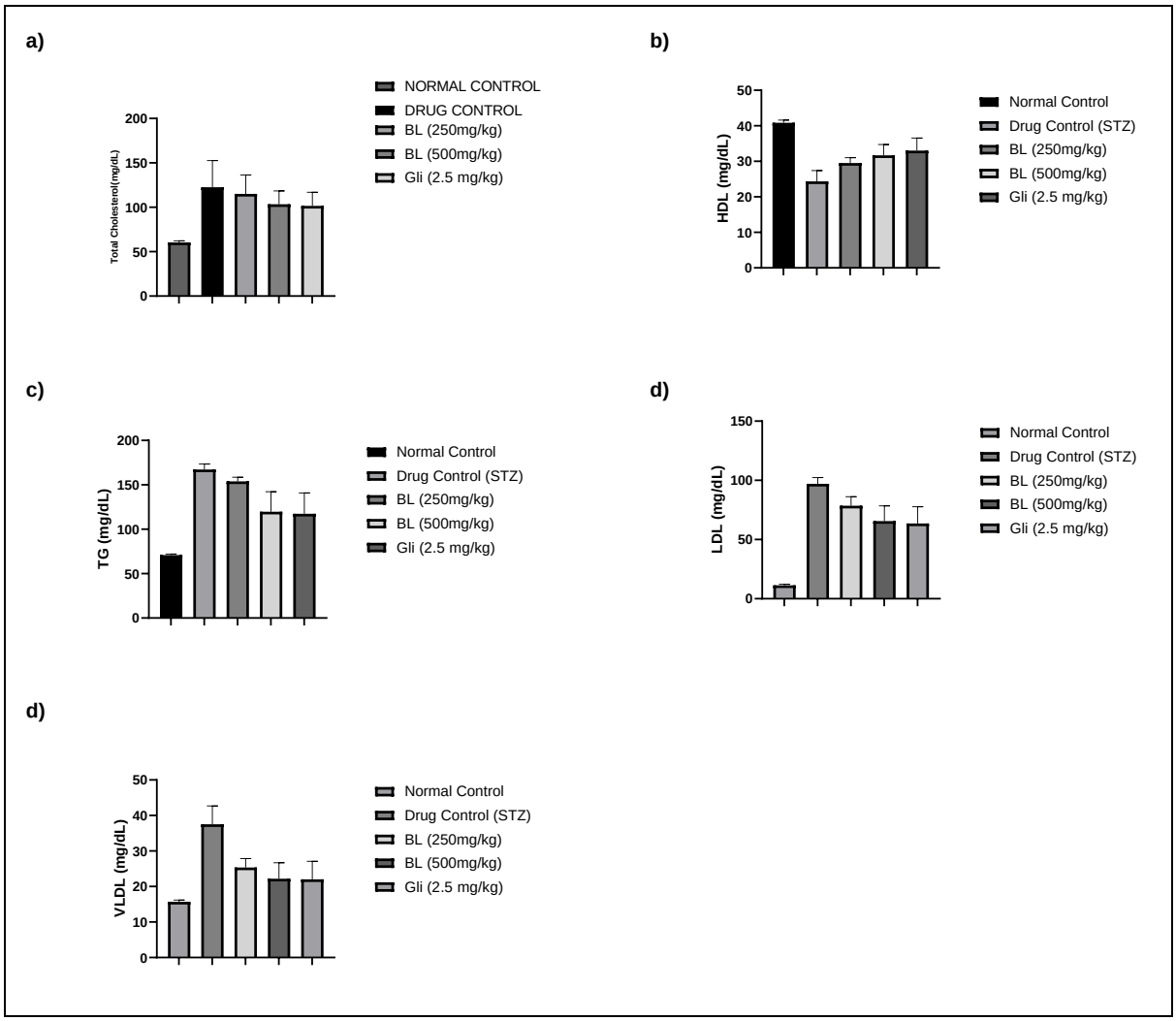

Fig. 4. Illustrates how the lipid parameters in rats given streptozotocin (STZ) to produce diabetes are affected by Bergenia ligulata. The following are the values given in the material and methods: a: TC, b: HDL, c: TG, d: LDL, and e: VLDL. The statistical significance was assessed using Dennett's test (*P < 0.05, **P < 0.01 and ***P < 0.001).

Hepatic Parameters

The levels of ALP, AST, and ALT are displayed on graphs linked to liver parameters in Figure 5. Different levels of ALP (IU/L) in different groups are displayed in Graph A. To achieve optimal outcomes, it is evident from the data that rats receiving treatment with Bergenia ligulata root extract and glibenclamide had lower ALP levels than untreated STZ-originating diabetic rats. A 500 mg/kg dosage of the extract had results comparable to those of glibenclamide. Rats' AST concentrations are shown in Graph B. Anytime there is injury to the heart or liver, AST is released into the blood. Diabetic STZ rats had higher levels of AST than rats given Normal Control. After using an extract from the roots of Bergenia ligulata, the

level decreased. A dosage of the extract of 250 and 500 mg/kg had similar outcomes to Glibenclamide. The ALT level, which should be lower under normal circumstances but is seen to be higher in diabetic rats, is shown in Graph C. The level seemed to decrease when dosages of Bergenia ligulata root extract were given.

Table 7: Effect of *Bergenia ligulata* extract (BL) on lipid parameters

Groups	**ALT (IU/L)**	**ALP (IU/L)**	**AST (IU/L)**
Normal Control	76.5	126.2	126.3
Drug control (STZ)	194	222.3	223
BL (250mg/kg)	181.5	177	175.1
BL (500mg/kg)	153	170.8	171.6
Gli (2.5 mg/kg)	134	171.3	170

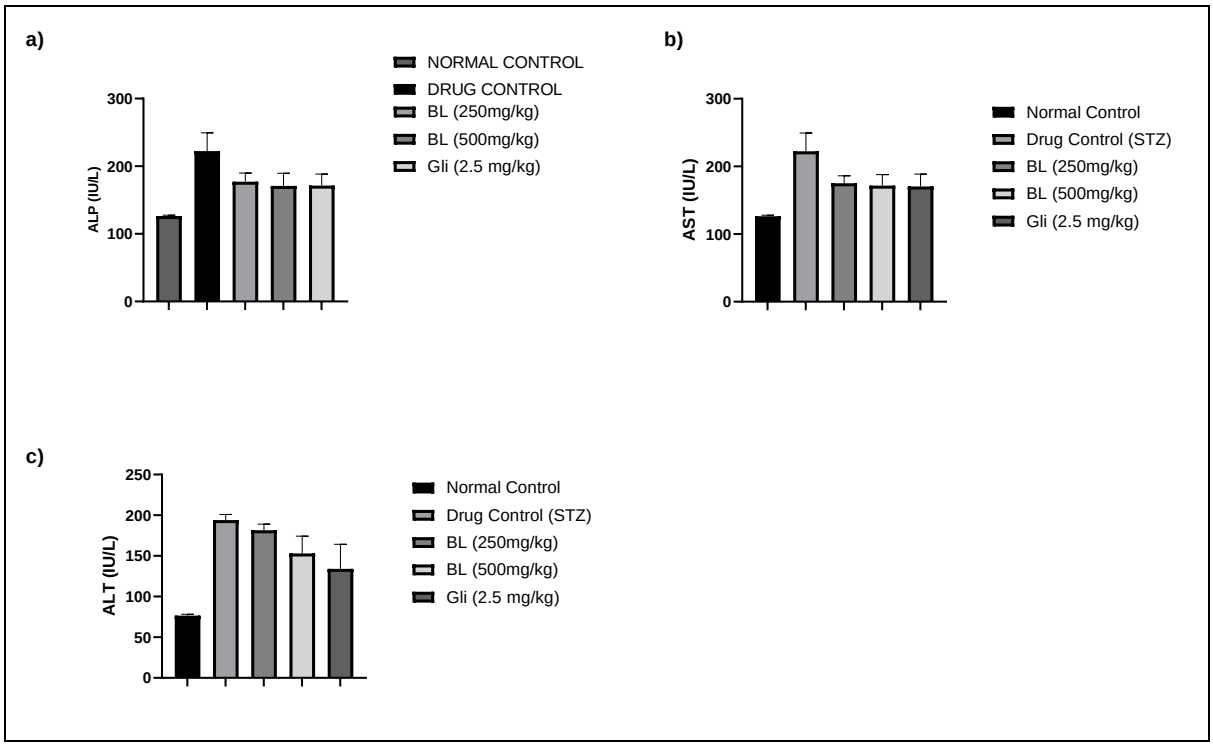

Fig. 5. Exhibits how Bergenia ligulata affects the liver parameters in rats with diabetes caused by streptozotocin (STZ). The content and techniques describe the following

methodologies: a) ALP, b) AST, and c) ALT. For determining statistical significance, Dennett's test was applied. (*P < 0.05, **P < 0.01 and ***P < 0.001).

Antioxidant Parameters

A curve of the plasma malonaldehyde levels is shown in Figure 6. MDA concentration rises in rats with diabetes. Rat groups administered dosages of glibenclamide and Bergenia ligulata root extract showed a decrease in MDA levels. In rats treated with 500 mg/kg of extract and in rats treated with glibenclamide as a typical medication, this level nearly returned to normal.

Table 8: Effect of *Bergenia ligulata* extract (BL) on antioxidant parameters

Groups	**MDA(µ/g)**	**CAT (nmol/ml)**	**SOD (µ/mg)**
Normal Control	0.4	52.1	17.2
Drug control (STZ)	2.52	21.3	13.4
BL (250mg/kg)	1.27	25.3	16.1
BL (500mg/kg)	1.18	28.5	16.4
Gli (2.5 mg/kg)	1.14	38	17.2

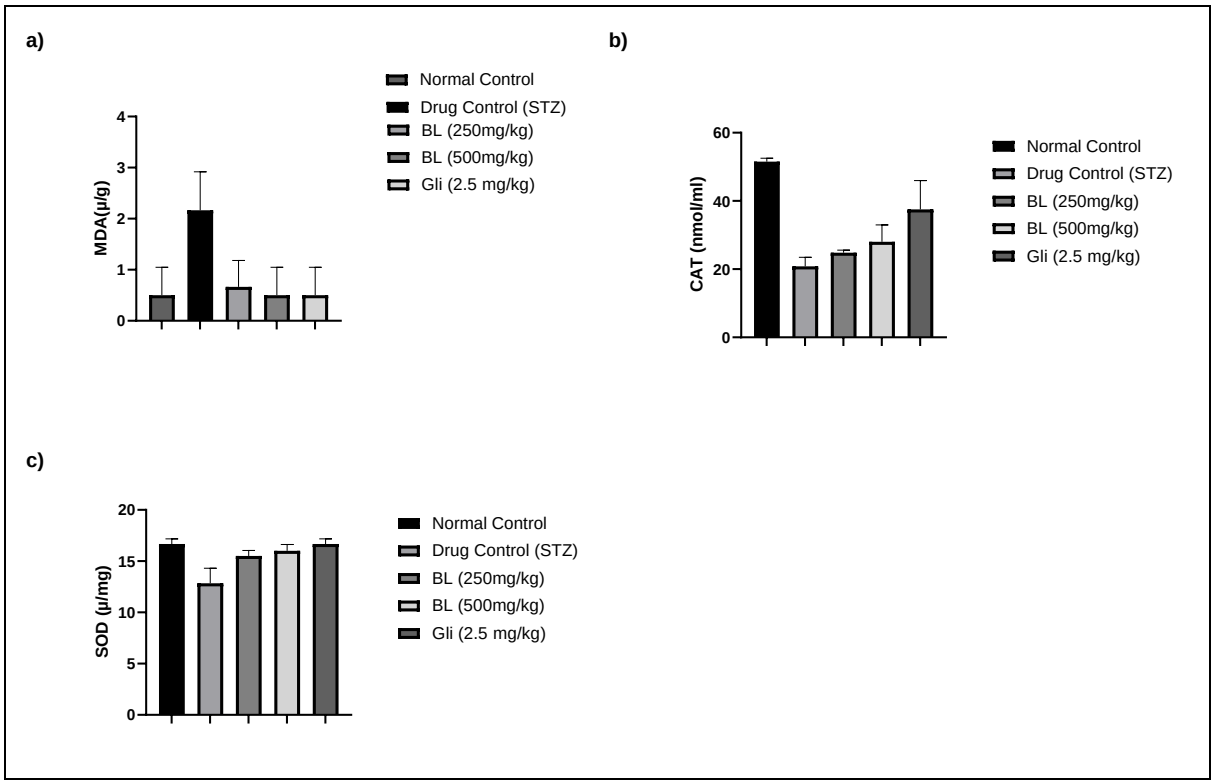

Fig. 6. Shows the effect of *Bergenia ligulata* on the antioxidant parameters in the streptozotocin (STZ) induced diabetic rats. **a:** MDA, **b:** CAT and **c:** SOD method as described in the material and methods. The statistical significance was assessed using Dennett's test (*P < 0.05, **P < 0.01 and ***P < 0.001).

Renal Parameters

Blood urea nitrogen (BUN), total protein content, and creatinine level are examples of renal markers. The graph illustrating the creatinine level indicates that the diabetic rats produced by STZ had a higher level than the group of normal control rats. Rats given glibenclamide and those treated with Bergenia ligulata root extract both show decreased concentration. When rats given extract from the roots of Bergenia ligulata were given STZ, their blood levels of BUN, which were previously high, somewhat decreased. (Fig. 7). Under diabetic circumstances, total protein concentration falls; this was seen to be higher in diabetic rats administered dosages of Bergenia ligulata root extract. Total protein rose along with the dosage of Bergenia ligulata root extract.

Table 9: Effect of *Bergenia ligulata* extract (BL) on renal parameters

Groups	**Creatinine (mg/dl)**	**BUN (mg/dl)**	**Total Protein (g/dl)**
Normal Control	0.93	47.3	8.6
Drug Control (STZ)	1.43	88.4	3.2
BL (250mg/kg)	1.08	68.9	4.9
BL (500mg/kg)	1.1	59.1	6.7
Gli (2.5 mg/kg)	1.01	51.9	7.1

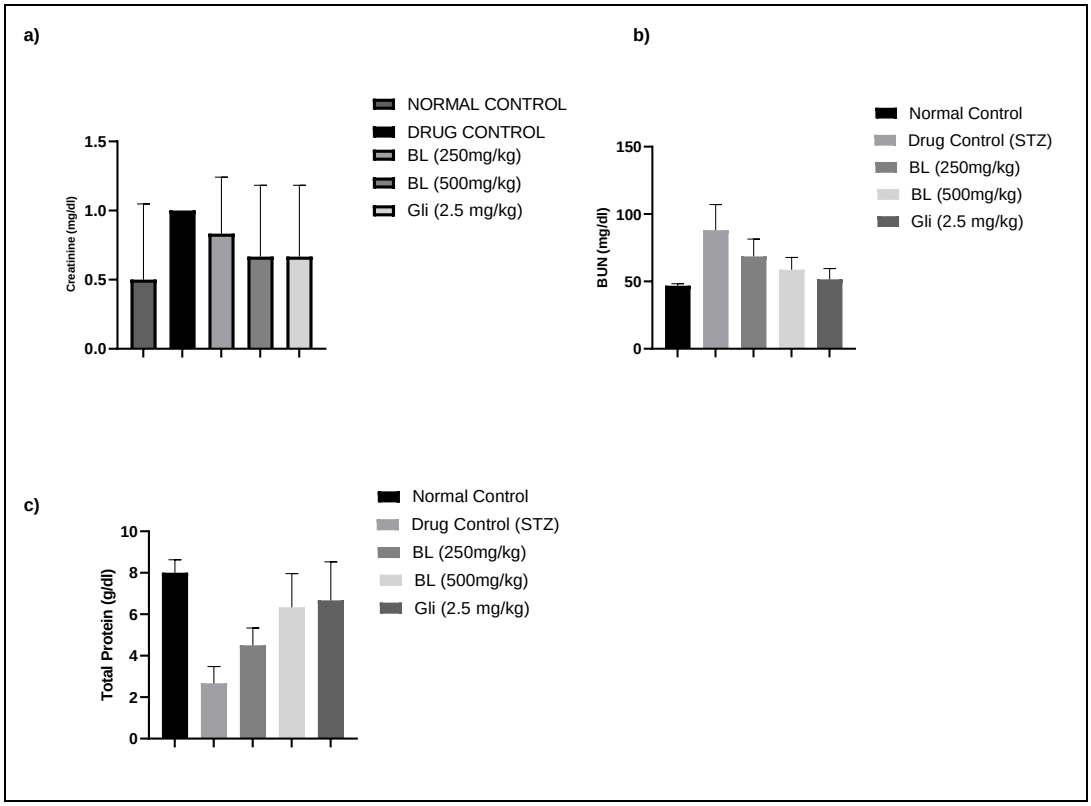

Fig. 7. Depicts how Bergenia ligulata affected the renal parameters in rats given streptozotocin (STZ) to produce diabetes. as stated in the material and methods: a) creatinine, b) BUN, and c) total protein technique. The statistical significance was assessed using Dennett's test (*P < 0.05, **P < 0.01 and ***P < 0.001).

SGLT and GLUT2 parameters

Protein-facilitated glucose transfer across cell membranes is made possible by the transmembrane carrier protein glucose transporter 2. The mRNA relative expression (fold) is used to study this; it has been shown that while the level increased in diabetic rats that were started on STZ, it decreased when the animals were given different dosages of Bergenia ligulata root extract or glibenclamide. A dosage of 500 mg/kg had positive outcomes. The kidney's GLUT 2 level is shown in Fig. 8 graph b. When rats receiving Bergenia ligulata root extract or glibenclamide medication were administered, the increased level in diabetic rats with STZ origins was lowered. The impact of a root extract from Bergenia ligulata on the expression of SGLT1 and GLUT2 in the jejunum was shown in Figure 8. SGLT1 and GLUT2 expression was upregulated in the STZ control group, whereas SGLT1 and GLUT2 expression was downregulated in the dose-dependent treatment with Bergenia ligulata root extract.

Table 10: Effect of *Bergenia ligulata* extract (BL) on SGLT1 and GLUT2 parameters

Groups	**mRNA relative expression of SGLT1 (Fold)**	**mRNA relative expression of GLUT2 (Fold)**
Normal Control	1.1	1.9
Drug Control (STZ)	6.5	5.9
BL (250mg/kg)	6.48	4.8
BL (500mg/kg)	4.03	4.0
Gli (2.5 mg/kg)	3.31	3.3

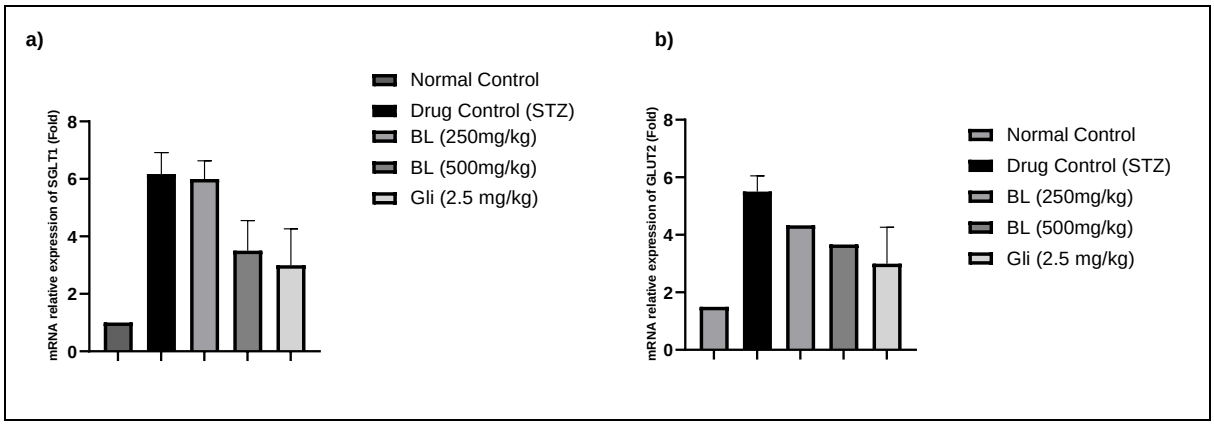

Fig. 8. Demonstrates the effect of *Bergenia ligulata* on the SGLT1 and GLUT2 expression in the jejunum in the streptozotocin (STZ) induced diabetic rats. **a:** SGLT1 and **b:** GLUT2 method as described in the material and methods. The statistical significance (*P < 0.05, **P < 0.01 and ***P < 0.001) was assessed using Dennett's test.

SGLT2 and GLUT2 expression in the renal

It was observed that there was a similar trend in the expression of SGLT1 and GLUT2 in the renal tissues. SGLT1 and GLUT2 expression levels were greater in the rats in the STZ-induced group; however, treatment with Bergenia ligulata root extract dose-dependently brought expression levels down to almost normal levels. Results were nearly the same for the rats in the Glibenclamide-treated group. (Fig. 9).

Table 11: Effect of *Bergenia ligulata* extract (BL) on SGLT2 and GLUT2 parameters

Groups	**mRNA relative expression of SGLT2 (Fold)**	**mRNA relative expression of GLUT2 (Fold)**
Normal Control	1.15	1.3
Drug control (STZ)	5.4	7.53
BL (250mg/kg)	3.53	5.35
BL (500mg/kg)	3.02	4.45
Gli (2.5 mg/kg)	2.78	4.47

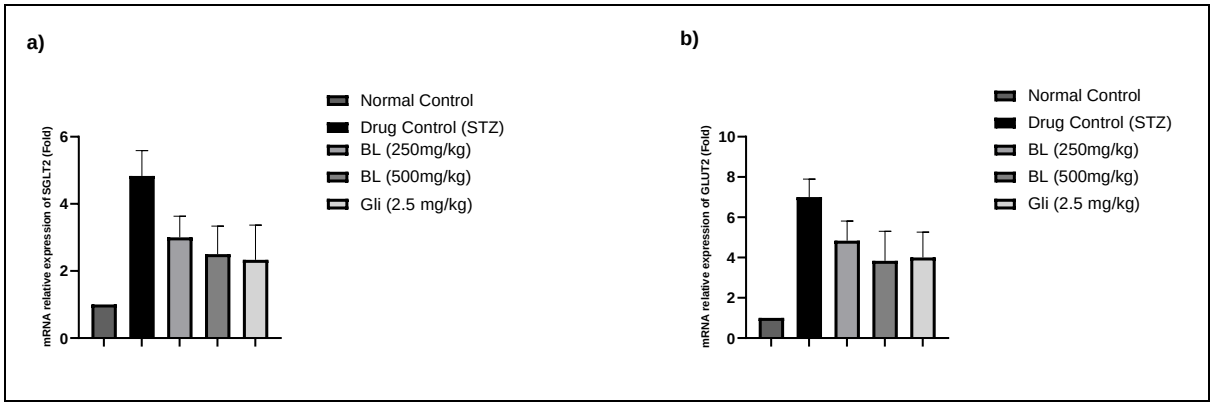

Fig. 9. Indicates how Bergenia ligulata affected the expression of SGLT1 and GLUT2 in the kidneys of rats given streptozotocin (STZ) to produce diabetes. The material and methods state a) SGLT2 and b) the GLUT2 approach. Dennett's test was used for evaluation of statistical significance (*P < 0.05, **P < 0.01 and ***P < 0.001).

Discussion

As per the results obtained, we can say that *Bergenia ligulata* possess hypoglycemic, antioxidant, and anti-diabetic activity by discussing about them here. Streptozotocin is a nitrosourea having immunosuppressive and anti-tumour properties acquired from the soil microbe Streptomyces achromogenes. Streptozotocin works via piercing β-cells via glucose transporter and induces ripping of DNA strands in β-cells leading to a downfall in endogenous secretion of insulin, this is due to the nitrourea part of Streptozotocin. This ripping of a strand of DNA provides a route to alterations in sugar and glucose levels in the blood. Alterations commence only after the administration of STZ. Two hours after inducing Streptozotocin (STZ), hyperglycemia occurs with a collateral thrust in insulin level. After around 6 hours, hyperglycemia is observed by the elevated insulin in the blood. Later, acute hyperglycemia evolves with reduced insulin in the blood. Throughout this trial, the anti-diabetic impact of *Bergenia ligulata* was described in detail. The ability of BL extract to lower blood glucose levels in OGTT (oral glucose tolerance test) rats showed that these rats could use glucose and that higher glucose tolerance in groups treated with varying amounts of BL extract resulted from improved glucose transport and consumption as well as insulin emission from β-cells. (13) (14)

In STZ induced diabetic rat groups, diminished body weight was recorded which occurred due to muscle deterioration and organizational proteins. Rat groups with diabetes were given

BL extract and glibenclamide dosages, and the rats' body weight significantly improved as compared to the rats in the diabetic control group. Bergenia ligulata extract plus glibenclamide shown protective efficacy against wasting (also known as a reversal of gluconeogenesis) at all doses. Rats with diabetes that were produced with streptozotocin (STZ) had lower plasma insulin levels. The different dosages of Bergenia ligulata extract administered to rat groups resulted in elevated levels of plasma insulin. This is likely due to the active ingredient found in the plant extract, which may act to either stimulate insulin secretion or shield fully functional β-cells from further degradation, allowing them to continue producing insulin as usual. For 28 days, using Bergenia ligulata extract orally resulted in a decrease in blood glucose and a rise in plasma insulin levels. The rat/animal group that received treatment with Bergenia ligulata extract may have seen an increase in insulin release from the β-cells in the islets of Langerhans. When comparing the hypoglycemic activity of glibenclamide and Bergenia ligulata extract, we may infer that their mechanisms of action may be comparable. According to certain studies, plants with high flavonoid content frequently exhibit hypoglycemic and anti-diabetic properties. Our research indicates that the root extract of Bergenia ligulata is rich in flavonoids and phenolic compounds.

STZ-originated diabetic rats elevate the level of lipid peroxidation, as non-primary evidence of originating free radicals. The amount of lipids increases in STZ-originated diabetic rats, resulting in diabetes and increased production of free radicals. Raising free radical concentrations has a crucial role in producing reactive oxygen species (ROS) and hyperglycemia. Unchecked generation of free radicals' damages tissues by attacking membranes through unsaturated fatty acid peroxidation. ROS can raise lipid peroxidation, modify the antioxidant defense system, and affect glucose metabolism in biological systems. In the end, lipid peroxidation causes severe harm to membranes and malfunctions. Reduced levels of endogenous antioxidant enzymes and susceptibility to the harmful effects of free radicals are observed in pancreatic β cells. SOD, GPx, and CAT levels spiked in STZ-originated diabetes, but MDA levels were decreased. The elevated origination of H_2O_2 in the diabetic pancreas and the increased quantity of SOD resulting from enhanced superoxide production, which has been linked to cell dysfunction, boosted the measure of CAT. Increasing SOD concentration without also increasing GPx causes cells' peroxide levels to rise, which can lead to overuse of peroxide. In contrast to diabetic control, different dosages of Bergenia ligulata extract to rat groups improve the assessment of endogenous antioxidants i.e. SOD, CAT, and GPx and stop the membrane from further deteriorating by lowering lipid

peroxidation. When it comes to intracellular glucose storage, glycogen is crucial. Since insulin initiates glycogen synthesis and damages glycogen phosphorylase, it stimulates intracellular glycogen deposition in several tissues through unmediated expression. In Streptozotocin (STZ)-induced diabetic rats, there was a decrease in the amount of glycogen stored in the liver, resulting in an insulin shortage. Various dosages of Bergenia ligulata extract were given to diabetic rats originating from streptozotocin (STZ)-induced hepatic glycogen recall, which increased insulin output. Because glucose is produced unrestrictedly in the blood and combines with blood haemoglobin to form glycated haemoglobin, diabetic rats treated with streptozotocin (STZ) had better levels of glycated haemoglobin (A1c). (15) Rats with diabetes and increased the levels of triglycerides and total cholesterol. Elevated triglyceride levels, or hypertriglyceridemia, and increased cholesterol, or hypercholesteremia, are the primary causes of the worsening of cardiovascular disease and atherosclerosis, a secondary consequence of diabetes. Different dosages of Bergenia ligulata leaf extract and glibenclamide were administered to diabetic rats that were STZ-originated—according to reports, glibenclamide and Bergenia ligulata extract increased total cholesterol and triglyceride levels back to normal. This may have been caused by an increase in insulin secretion that was initiated and maintained, which inhibited hormone-sensitive lipase, increased glucose utilization, and reduced the mobility of free fatty acids from fat deposits. STZ-originated Diabetes groups showed cardiovascular risk factors by having higher levels of LDL, which raises the coronary risk factor, and lower levels of HDL. Increased levels of TC and TG are linked to higher levels of LDL, VLDL, and lower levels of HDL in diabetic conditions. Diabetes causes cholesterol (LDL and VLDL) to lodge in peripheral tissue, and elevated LDL and VLDL levels frequently cause atheroma, or the buildup of plaque on the arterial walls, like atherosclerosis. Different dosages of Bergenia ligulata leaves extract were used to treat the STZ-originated diabetic groups. This resulted in significant reductions in total cholesterol, triglyceride LDL, VLDL, and MDA levels. According to the conclusions, different dosages of Bergenia ligulata extract considerably reduced abnormalities in blood lipid levels. (16)

5. Conclusion

In summary, the results of our study clearly showed that the methanolic extract of Bergenia ligulata sprouts has anti-diabetic properties since it lowers blood glucose levels and, in hyperglycemic rats, SGLT1 and GLUT2 levels. This extract's antioxidant activity was brought about by the reduction of lipid peroxidation and the elevation of SOD, GPx, and CAT

levels. The anti-hyperglycemic properties of the methanolic extract of Bergenia ligulata were inferred.

Reference:

1. Kumar, R., Saha, P., Kumar, Y., Sahana, S., Dubey, A., & Prakash, O. (2020). A Review on Diabetes Mellitus: Type1 & Type2. *World Journal of Pharmacy and Pharmaceutical Sciences*, *9*(10), 838-850.
2. Mekala, K. C., & Bertoni, A. G. (2020). Epidemiology of diabetes mellitus. In *Transplantation, bioengineering, and regeneration of the endocrine pancreas* (pp. 49-58). Academic Press.
3. Alam, S., Hasan, M. K., Neaz, S., Hussain, N., Hossain, M. F., & Rahman, T. (2021). Diabetes Mellitus: insights from epidemiology, biochemistry, risk factors, diagnosis, complications and comprehensive management. *Diabetology*, *2*(2), 36-50.
4. Arokiasamy, P., Salvi, S., & Selvamani, Y. (2021). Global burden of diabetes mellitus. In *Handbook of global health* (pp. 1-44). Cham: Springer International Publishing.
5. Samtiya, M., Aluko, R. E., Dhewa, T., & Moreno-Rojas, J. M. (2021). Potential health benefits of plant food-derived bioactive components: An overview. *Foods*, *10*(4), 839.
6. Jugran, A. K., Rawat, S., Devkota, H. P., Bhatt, I. D., & Rawal, R. S. (2021). Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. *Phytotherapy Research*, *35*(1), 223-245.
7. Kumar, P., Wal, P., Rai, A. K., Singh, Y., & Singh, S. P. (2022). Phytochemical Screening And In Vitro Antimicrobial And Antifungal Activity Of Bergenia Ligulata (Wall.) Leaves Extracts.
8. Fuloria, N., Goswami, R., Ambwani, S., Tandon, R., & Ambwani, T. K. Exploring in vitro efficacy of roots of Bergenia ligulata for urolithiasis management.
9. Singh, A., Tandon, S., Nandi, S. P., Kaur, T., & Tandon, C. (2021). Downregulation of inflammatory mediators by ethanolic extract of Bergenia ligulata (Wall.) in oxalate injured renal epithelial cells. *Journal of Ethnopharmacology*, *275*, 114104.
10. Furman, B. L. (2021). Streptozotocin-induced diabetic models in mice and rats. *Current Protocols*, *1*(4), e78.
11. Benedé-Ubieto, R., Estévez-Vázquez, O., Ramadori, P., Cubero, F. J., & Nevzorova, Y. A. (2020). Guidelines and considerations for metabolic tolerance tests in mice. *Diabetes, Metabolic Syndrome and Obesity*, 439-450.

12. Ahmed, D., Kumar, V., Verma, A., Gupta, P. S., Kumar, H., Dhingra, V., ... & Sharma, M. (2014). Antidiabetic, renal/hepatic/pancreas/cardiac protective and antioxidant potential of methanol/dichloromethane extract of Albizzia Lebbeck Benth. stem bark (ALEx) on streptozotocin induced diabetic rats. *BMC complementary and alternative medicine*, *14*, 1-17.
13. Rolta, R., Goyal, M., Sharma, S., Bharaj, D., Salaria, D., Upadhyay, N. K., ... & Sourirajan, A. (2022). Bioassay Guided Fractionation of Phytocompounds from Bergenia ligulata: A synergistic approach to treat drug resistant bacterial and fungal pathogens. *Pharmacological Research-Modern Chinese Medicine*, *3*, 100076.
14. Singh, A., Tandon, S., Nandi, S. P., Kaur, T., & Tandon, C. (2021). Downregulation of inflammatory mediators by ethanolic extract of Bergenia ligulata (Wall.) in oxalate injured renal epithelial cells. *Journal of Ethnopharmacology*, *275*, 114104.
15. Singh, A., Tandon, S., Nandi, S. P., Kaur, T., & Tandon, C. (2021). Downregulation of inflammatory mediators by ethanolic extract of Bergenia ligulata (Wall.) in oxalate injured renal epithelial cells. *Journal of Ethnopharmacology*, *275*, 114104.
16. Singh, A., Tandon, S., Nandi, S. P., Kaur, T., & Tandon, C. (2021). Downregulation of inflammatory mediators by ethanolic extract of Bergenia ligulata (Wall.) in oxalate injured renal epithelial cells. *Journal of Ethnopharmacology*, *275*, 114104.