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Bougainvillea spectabilis Exhibits Antihyperglycemic and Antioxidant Activities in Experimental Diabetes

Pratibha Chauhan, MSc[1], Sunil Mahajan, MPhil[1], Archana Kulshrestha, PhD[1], Sadhana Shrivastava, PhD[1], Bechan Sharma, PhD[2], H. M. Goswamy, BAMS[1], and G. B. K. S. Prasad, PhD[1]

Abstract

The study investigates the effects of aqueous extract of *Bougainvillea spectabilis* leaves on blood glucose, glycosylated hemoglobin, lipid profile, oxidative stress, and on DNA damage, if any, as well as on liver and kidney functions in streptozotocin-induced diabetes in Wistar rats. Daily administration of the aqueous extract of *B spectabilis* leaves for 28 days resulted in significant reduction in hyperglycemia and hyperlipidemia as evident from restoration of relevant biochemical markers following extract administration. The extract also exhibited significant antioxidant activity as evidenced from the enzymatic and nonenzymatic responses and DNA damage markers. The extract restored kidney and liver functions to normal and proved to be nontoxic. A marked improvement in the histological changes of tissues was also observed. The present study documented antihyperglycemic, antihyperlipidemic, and antioxidative potentials of the aqueous extract of *B spectabilis* leaves without any toxicity in streptozotocin-treated Wistar rats.

Keywords

blood glucose, diabetes mellitus, hyperglycemia, hyperlipidemia, oxidative stress

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Diabetes mellitus is a clinical syndrome affecting the human population on an epidemic level globally. Diabetes is a group of metabolic disorders characterized by hyperglycemia resulting from the deficiency of insulin secretion by the pancreas or the inefficacy of produced insulin.[1] Due to the absence of effective and affordable treatment for both types of diabetes, it exists as a serious threat to public health.[2] Obesity mainly due to changes in life style, such as increased energy intake, decreased physical activity, and sedentary work culture, leads to an epidemical increase in type 2 diabetes.[3] A series of *in vitro* experiments provided considerable knowledge into the physiological and biochemical aberrations of the diabetic state, which is often associated with changes in lipid, protein, and carbohydrate metabolisms.[4] Insulin resistance associated with decreased insulin secretion leads to oxidative stress with increased reactive oxygen species, which results in tissue damage through multiple mechanisms, including metabolic dysfunction and changes in the structure and properties of proteins, lipoproteins, and DNA.[5] *In vivo* studies showed the consequences of oxidative stress as it disrupts activity of receptors, enzymes, signal transduction pathways, and transport proteins. It also causes secondary damage to other biomolecules.[6] Significant structural changes are clearly oxidative in nature and are involved in the pathogenesis of vascular complications.[7] Because of the resultant macrovascular and microvascular injury typical to this disease, insulin and oral hypoglycemic agents like sulfonylureas and biguanides are in regular use but the long-term use of these therapeutics results in serious side effects.[8] Furthermore, these therapeutic agents are relatively expensive for developing countries.[9] Because of the adverse effects of conventional medication, the quest for the development of more effective natural antidiabetic agents has been explored. Recently, attention has been focused on one of the oldest remedies, that is, traditional herbal therapy well known to mankind.[10] Many traditional herbs, through their hypoglycemic and antioxidant actions, may also protect the organs involved in diabetes mellitus.

[1] Jiwaji University, Gwalior, India
[2] Allahabad University, Allahabad, India

Corresponding Author:
G. B. K. S. Prasad, PhD, School of Studies in Biochemistry, Jiwaji University, Gwalior 474011, India.
Email: gbksprasad@gmail.com

Bougainvillea spectabilis (Common name: Bougainvillea) is a thorny woody vine, grown in tropical and subtropical parts of India. Its leaves contain various active ingredients such as furanoids, saponins, flavonoids, quinones, phenols, sterols, triterpenoids, glycosides, tannins, and small amounts of sugars. The extract of its leaves contains pinitol, which serves as hypoglycemic agent and exhibits insulin like effect.[11,12] Glucosidase inhibitory activity of *B spectabilis* against murine pancreatic and intestinal glucosidase is suggested to be one of the important underlying mechanisms of antidiabetogenic activity.[13] The present study was undertaken to investigate the protective and therapeutic potential, if any, of *B spectabilis* against streptozotocin-induced diabetes in Wistar rats. The histological improvement in the diabetic rats due to treatment with the extract of *B spectabilis* was also observed.

Materials and Methods

Plant Material

Apical leaves of *B spectabilis* were collected from the healthy plants available locally at Gwalior, India. The plant was identified by Prof A. K. Jain, Institute of Ethnobiology, Jiwaji University, Gwalior, India. A specimen was deposited in the same Institute with Voucher Specimen No. IOE-511. The shade-dried leaves were coarsely powered and extracted with distilled water using a magnetic stirrer for 24 hours at ambient temperature (about 26°C). The resulting extract was filtered and the filtrate was evaporated under reduced pressure in a vacuum rotatory evaporator. The resulting powder was used for experimentation.

Drug Doses

The extract reconstituted in 0.5% dimethyl sulfoxide (100 mg/kg body weight) and glibenclamide in normal saline (600 µg/kg body weight) were used in the study. Animals in the control group received normal saline orally.

Chemicals

Streptozotocin used for the induction of diabetes in animals was purchased from Sigma-Aldrich Co (St Louis, MO). Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, urea, uric acid, and creatinine in blood plasma were assayed using standard kits from Coral Clinical Systems (Goa, India). All other chemicals for estimation of reduced glutathione (GSH), superoxide dismutase (SOD), catalase, and thiobarbituric acid reactive substances (TBARS) were procured from HiMedia (Mumbai, India). One touch glucometer (ACCU-CHEK Sensor) of Roche Diagnostics (Berlin, Germany) was used for regular monitoring of blood glucose in experimental animals.

Experimental Animals

Male Wistar rats of body weight 180 to 200 g, procured from the Defense Research and Development Establishment, Gwalior, India were used for the study. All the animals were kept in department's animal house under standard laboratory conditions (25 to 30°C with 45% to 55% relative humidity for 12 hours each of light and dark cycle). The animals were kept on a normal pellet diet and water *ad libitum.* The rats used in the present study were maintained in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals regulations and guidelines for the care and use of laboratory animals.

Induction of Diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5. Hyperglycemia or increased blood glucose was confirmed by checking the blood glucose by an ACCU-CHEK sensor glucometer at 48 hours after streptozotocin injection. Rats showing hyperglycemia with blood glucose >200 mg/dL 48 hours after streptozotocin injection were considered diabetic and were used in the study.

Study Design and Treatment Schedule

In the experiment, a total of 24 rats were used. The rats were divided into 4 groups of 6 each. The experimental period was 28 days beginning after the induction of streptozotocin diabetes.

Group 1: Normal untreated rats
Group 2: Diabetic control rats (streptozotocin group)
Group 3: Diabetic rats administered daily with aqueous extract of *B spectabilis* (100 mg/kg body weight) for 28 days
Group 4: Diabetic rats given glibenclamide (600 µg/kg body weight daily)

All the treatments were given once a day orally at 12.00 noon for 28 days. Throughout the study, standard food and water were made available to the animals for 24 hours. Blood glucose and body weight of rats in every group were recorded at weekly intervals. Experimental animals were cautiously observed every day for general physical conditions and behavior. Any morphological/physiological changes in the skin, eyes, respiratory pattern, and behavior pattern were noted. The onset of toxicity and signs of toxicity were also noted with water and food intake. The study protocol was approved by the Institutional animal ethics committee.

Sample Collection

At the end of the experiment, blood samples were collected and all rats were sacrificed. Sera was separated and stored at −20°C till use, for estimation of biochemical parameters. The abdomen and thorax were opened. The kidney, liver, and pancreas were removed and washed thrice with ice-cold saline and blotted dry individually on filter paper. These organs were used for the preparation of their homogenates for estimation of activities of tissue glutathione peroxidase (GPx), glutathine S transferase (GST), and glutathione reductase (GR).

Preparation of Tissue Homogenate

The tissues were weighed and 10% (w/v) tissue homogenate was prepared by homogenizing the tissues in 0.1 M phosphate buffer (pH 7.5) separately. After centrifugation (10000 rpm at 4°C for 10 minutes), the supernatant was collected and used for the estimation of various biochemical parameters.

Biochemical Parameters

Estimation of Blood Glucose, Glycated Hemoglobin, and Weight of Animals. Fasting blood glucose estimation was done weekly on tail prick blood by using the ACCU-CHEK sensor glucometer. It uses glucose oxidase–specific strips and works on the principle of reflectance photometry. HbA1c was measured after hemolysis of the anticoagulated whole blood specimen. HbA1c was determined by the ion exchange resin method.[14] The weight of animals was recorded at weekly intervals during the course of experimentation.

Lipid Profile. The lipid profile parameters such as total cholesterol,[15] serum triglycerides,[16] serum HDL-cholesterol,[17] LDL, and very low density lipoprotein (VLDL) were estimated. All the estimations were carried out on fasting serum samples using commercial kits (Crest Biosystems India Pvt Ltd, Goa, India).

Kidney Function Tests. Kidney function markers such as serum creatinine,[18] serum urea,[19] and serum uric acid[20] were estimated by using kits manufactured by Crest Biosystems.

Liver Function Tests. Liver function markers such as bilirubin,[21] AST, and ALT[22] were estimated by using kits manufactured by Crest Biosystems.

Oxidative Stress Markers in Blood

Oxidative stress markers such as GSH,[23] SOD,[24] TBARS,[25] and catalase[26] were estimated using standard procedures. GSH was estimated in whole blood (50 µL blood in 950 µL distilled water); TBARS, SOD, and catalase were estimated in hemolysate. DNA damage was evaluated by Comet assay[27] using lymphocytes.

Oxidative Stress Markers in Liver and Kidney Tissues

The activity of tissue GPx was measured using a coupled enzyme assay system.[28] The decrease in absorbance was monitored at 25°C at 340 nm. One unit of enzyme activity was defined as µmol of GSH consumed/min/mg protein.

The activity of tissue GR was measured in hepatic and renal tissue extracts.[29] The decrease in absorbance was monitored at 25°C at 340 nm. One unit of enzyme activity was defined as µmol of NADPH oxidized/min/mg protein.

Tissue GST enzyme activity was measured spectrophotometrically.[30] One unit of GST activity was defined as the production of mmol of CDNB-GSH conjugate/min under the assay conditions.

Histopathology

The tissues from liver, kidney, and pancreas were fixed immediately after removal in 10% formalin for routine histopathological examination. Histopathological evaluations were performed by a pathologist. Photomicrographs of the microscopic sections were taken with the help of a light microscope.

Statistical Analysis

The data were analyzed by one-way analysis of variance followed by Tukey's test to determine the level of significance (Sigma stat 3.5). A value of $P < .05$ was considered significant, and results are expressed as mean ± standard error of the mean.

Table 1. Effect of *Bougainvillea* Aqueous Extract on Body Weight, FBG, and HbA1c Levels*.

Groups	Body Weight (g)	FBG (mg/dL)	HbA1c (%)
NC	242.0 ± 3	81.50 ± 2.50	5.05 ± 0.15
DC	231.75 ± 10.09[a]	435.44 ± 11.16[a]	7.65 ± 0.55[a]
Bougainvillea	242.25 ± 3.86[b]	225.83 ± 29.40[ab]	7.25 ± 0.15[a]
Glibenclamide	249.75 ± 6.39[b]	151.17 ± 3.27[b]	7.35 ± 0.15[a]

Abbreviations: FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; NC, normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group.

Results

Effect of Bougainvillea spectabilis on Body Weight

All animals in the diabetic group showed a progressive fall in their body weight, which was constantly examined till the end of the study period (Table 1). Weight reduction induced by diabetes resulted in notable increase following *B spectabilis* treatment, which is comparable to that of glibenclamide (Table 1).

Effect of Bougainvillea spectabilis on Hyperglycemia

Table 1 shows the levels of fasting blood glucose (FBG) in normal and diabetic rats at the time of diabetes induction as well as after 7, 14, and 28 days of treatment. Streptozotocin induction resulted in a significant increase in blood glucose level as compared to a normal control group. The administration of aqueous extract of *B spectabilis* to diabetic rats resulted in a significant decrease in the level of FBG. This FBG lowering trend due to the treatment of aqueous extract of *B spectabilis* was comparable to a certain extent with that of glibenclamide. The diabetic rats administered with *B spectabilis* extract showed marked reduction in glycosylated hemoglobin levels as well (Table 1).

Effect of Bougainvillea spectabilis on Lipidemia

Table 2 shows the effect of *B spectabilis* on serum lipid profile. Diabetic rats showed significantly increased levels of total cholesterol and triglycerides and decreased levels of HDL cholesterol when compared with the normal rats. In rats treated with the aqueous extract of *B spectabilis* and glibenclamide, there were significant decreases in the contents of total cholesterol, triglycerides, and VLDL-cholesterol but no significant change in the levels of LDL and HDL-cholesterol was observed.

Effect of Bougainvillea spectabilis on Kidney and Liver Functions

Effect of aqueous extract of *B spectabilis* on kidney function was monitored by estimating urea, creatinine, and uric acid

Table 2. Effect of *Bougainvillea* Aqueous Extract on Lipid Profile*.

Groups	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
NC	80.65 ± 1.35	62.34 ± 0.56	32.13 ± 2.73	14.1 ± 2.4	16.13 ± 0.27
DC	132 ± 1.73[a]	88.8 ± 1.8[a]	19.50 ± 1.19[a]	42.86 ± 3.13[a]	26.4 ± 0.3[a]
Bougainvillea	89.2 ± 2.0[abc]	79.51 ± 1.51[ab]	30.64 ± 6.50	31.0 ± 5.2[a]	19.08 ± 0.15[abc]
Glibenclamide	95.42 ± 0.76[ab]	80.4 ± 1.1[ab]	33.17 ± 1.11[b]	28.2 ± 1.84[b]	17.8 ± 0.4[ab]

Abbreviations: TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; NC, normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group; and [c]in comparison to the glibenclamide treated group.

levels in serum. The data presented in Table 3 show significant reductions in uric acid and creatinine but there was no change in the level of urea.

Significant variation in enzyme marker of liver, viz., ALT was recorded, but there was no change in levels of bilirubin and AST (Table 4).

Effect of Bougainvillea spectabilis on Biomarkers of Oxidative Stress in Blood

It was observed that after inducing diabetes, the levels of GSH, SOD, and catalase were decreased significantly in the diabetic control group (DC) as compared to the normal control group (NC). Treatment with aqueous extract of *B spectabilis* for 28 days significantly increased the GSH level and activity of SOD (Table 5). Data of the present study also revealed a significantly increased concentration of TBARS, a measure of lipid peroxidation in diabetic animals. A significant reduction in the level of TBARS was observed in the *B spectabilis*–treated group. The decreased level of TBARS was also observed in the glibenclamide-treated group (Table 5), but there was no significant change in the activity of catalase.

Effect of Bougainvillea spectabilis on Biomarkers of Oxidative Stress in Tissues

Induction of diabetes significantly reduced the activities of GR, GST, and GPx in liver and kidney tissues of diabetic animals as compared to normal ones. The 28-day treatment with aqueous extract of *B spectabilis* increased the activities of these enzymes, although not significant statistically (Tables 6 and 7).

Effect of Bougainvillea spectabilis on DNA Damage by Comet Assay

The level of oxidative DNA damage was significantly higher in the diabetic group than in normal control group (Figure 1). Comets with long tail were clearly observed in case of diabetic control group, which indicated DNA damage due to oxidative stress caused by streptozotocin, whereas in normal animals no such comets were observed. In the *B spectabilis*–treated diabetic group, the number of comets in the slides was observed with shorter tail length compared to the diabetic control group. These observations clearly indicated that there is some degree of recovery of the DNA damage in the treated group animals (Figure 1).

Table 3. Effect of *Bougainvillea* Aqueous Extract on Kidney Function*.

Groups	Urea (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)
NC	18.15 ± 0.45	3.25 ± 0.05	0.59 ± 0.01
DC	37.45 ± 0.91[a]	5.73 ± 0.21[a]	1.15 ± 0.05[a]
Bougainvillea	36.97 ± 1.40[ac]	4.60 ± 0.31[abc]	0.66 ± 0.05[b]
Glibenclamide	30.98 ± 1.16[ab]	3.80 ± 0.06[ab]	0.68 ± 0.45[b]

Abbreviations: NC, normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group; and [c]in comparison to the glibenclamide treated group.

Table 4. Effect of *Bougainvillea* Aqueous Extract on Liver Function*.

Group	Bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)
NC	0.56 ± 0.03	14.50 ± 0.50	13.8 ± 2.3
DC	0.76 ± 0.07[a]	50.50 ± 2.22[a]	53.95 ± 4.0[a]
Bougainvillea	0.73 ± 0.11[c]	44.67 ± 2.85[a]	42.71 ± 2.4[ab]
Glibenclamide	0.55 ± 0.03[b]	40.10 ± 1.83[ab]	39.58 ± 0.7[ab]

Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group; and [c]in comparison to the glibenclamide treated group.

Histological Observations

Pancreas. Breakdown of microanatomical features such as extensive β-cell degranulation and decreased cellular density with an indistinct border between the exocrine and endocrine regions were observed in diabetic rats. Whereas normal rats showed distinct granules filling the entire islet of Langerhans that were stained purple, alpha cells were seen mostly at the periphery. For the *B spectabilis*–treated group, there was a remarkable improvement in the islet of Langerhans with distinct cellularity changes, and the majority of cells showed viable islets of Langerhans with an increase in granulation. In the allopathy treated group, β-cells were normally preserved compared to that of diabetic group and there was no vacuole (Figure 2).

Table 5. Effect of *Bougainvillea* Aqueous Extract on Biomarkers of Oxidative Stress in Blood*.

Group	GSH (mg/mL)	SOD (μM/min/mg protein)	Catalase (μM/min/mg protein)	TBARS (n mole of MDA/mL of blood)
NC	2.8 ± 0.15	3.5 ± 0.2	12.7 ± 1.39	191.7 ± 5.35
DC	0.83 ± 0.05[a]	0.7 ± 0.13[a]	4.3 ± 0.48[a]	388.9 ± 11.96[a]
Bougainvillea	1.43 ± 0.25[a]	1.61 ± 0.11[abc]	10.09 ± 1.37	316.5 ± 17.48[abc]
Glibenclamide	1.8 ± 0.135[ab]	2.4 ± 0.09[ab]	10.3 ± 1.63[b]	263.3 ± 4.53[ab]

Abbreviations: GSH, reduced glutathione; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group; and [c]in comparison to the glibenclamide treated group.

Table 6. Effect of *Bougainvillea* Aqueous Extract on Biomarkers of Oxidative Stress in Liver Tissue*.

Groups	GR	GPx	GST
NC	29.50 ± 2.5	0.45 ± 0.1	40.50 ± 1.5
DC	17.19 ± 3.19[a]	0.26 ± 0.05[a]	10.50 ± 2.5[a]
Bougainvillea	17.75 ± 1.15[a]	0.38 ± 0.03	21.83 ± 2.57[a]
Glibenclamide	23.76 ± 5.24	0.47 ± 0.02[b]	30.07 ± 4.93[b]

Abbreviations: GR, glutathione reductase (μmol of NADPH oxidized/min/mg protein); GPx, glutathione peroxidase (μmol of GSH consumed/min/mg protein); GST, glutathione transferase (amount produced/mmol of CDNB-GSH conjugate/min); normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group.

Table 7. Effect of *Bougainvillea* Aqueous Extract on Biomarkers of Oxidative Stress in Kidney Tissue*.

Groups	GR	GPx	GST
NC	28.75 ± 0.75	0.49 ± 0.04	22.50 ± 1.5
DC	19.49 ± 0.67[a]	0.26 ± 0.03[a]	14.50 ± 0.5[a]
Bougainvillea	20.7 ± 1.25[a]	0.40 ± 0.01[b]	18.75 ± 2.55[c]
Glibenclamide	26.0 ± 1.00[b]	0.43 ± 0.09[b]	29.45 ± 5.45[b]

Abbreviations: GR, glutathione reductase (μmol of NADPH oxidized/min/mg protein); GPx, glutathione peroxidase (μmol of GSH consumed/min/mg protein); GST, glutathione transferase (amount produced/mmol of CDNB-GSH conjugate/min); normal control group; DC, diabetic control group.
*The values are mean ± SEM of 6 animals in each group and significantly different ($P < .05$): [a]in comparison to normal control; [b]in comparison to diabetic control group; and [c]in comparison to the glibenclamide treated group.

Kidney. The kidney tissue of diabetic rats showed adhesion of glomerulus and Bowman's capsule as well as reduced lumen of renal tubules, whereas kidney tissue of normal rats displayed a compact glomeruli and renal tubules. In *B spectabilis* and glibenclamide-treated groups, well-formed Bowman's capsule was clearly seen (Figure 3).

Liver. In liver tissue of diabetic rats, some hypertrophy of hepatocytes with lucent cytoplasm was noted. Phagocytosis of yellowish brown pigment by Kuffer's cells was seen in the liver of diabetic rats. Narrowing sinusoids were also seen. The liver tissue of normal rats showed normal hepatocytes and maintained sinusoidal spaces with well-formed chord arrangements. In the *B spectabilis*–treated group, well-formed hepatocytes and central vein with maintained plates were seen. Sinusoidal space with Kuffer's cell was also maintained. The pathomorphological alteration observed in diabetic rats becomes apparently normal with the concentric arrangement of the hepatocytes with the vesicular nuclei after the treatment of glibenclamide. Hexagonal hepatocytes were noted with concentric arrangement and clear sinusoids (Figure 4).

Discussion

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia and is associated with hyperlipidemia and oxidative stress. A single intraperitoneal injection of streptozotocin given to normoglycemic rats produced hyperglycemia in 48 hours by producing cytotoxic effect on pancreatic β-cells with blood glucose level of 200 to 250 mg/dL.[31] Glibenclamide is a sufonylurea and widely used as a reference drug for treatment of type 2 diabetes mellitus.

In the present study, we have observed that *B spectabilis* at a dose of 100 mg/kg body weight significantly decreased the blood glucose level in diabetic rats. The possible mechanism of action of aqueous leaf extract of *B spectabilis* might be correlated with the reminiscent effect of insulin, which is due to the presence of D-pinitol.[12] D-pinitol does not directly increase the insulin activity but it might be associated with the process that links insulin with glucose transport.[32] We observed a significant increase in the level of triglycerides in diabetic rats, which might be due to the deficiency of insulin. Insulin activates lipoprotein lipase activity, which hydrolyzes triglycerides.[33] Administration of aqueous leaf extract of *B spectabilis* for 28 days restored the levels of total cholesterol, VLDL-cholesterol, and triglycerides near to the normal level, thus regaining lipid functioning similar to that of the normal control group.

ALT and AST are metabolic marker enzymes that represent the status of normal functioning of liver, such as hepatocellular integration, bile production, and protein synthesis. When oxidative stress increases, these enzymes get leaked into the blood stream, which is indicative of hepatic tissue damage.[34] In the present study, ALT and AST activities increased 2-fold in diabetic rats relative to normal. Oral administration of aqueous leaf extract of *B spectabilis* caused a significant decrease in the activity of ALT but we did not find any significant reduction in AST and bilirubin levels, indicating that the extract is not associated with considerable hepatoprotective function.

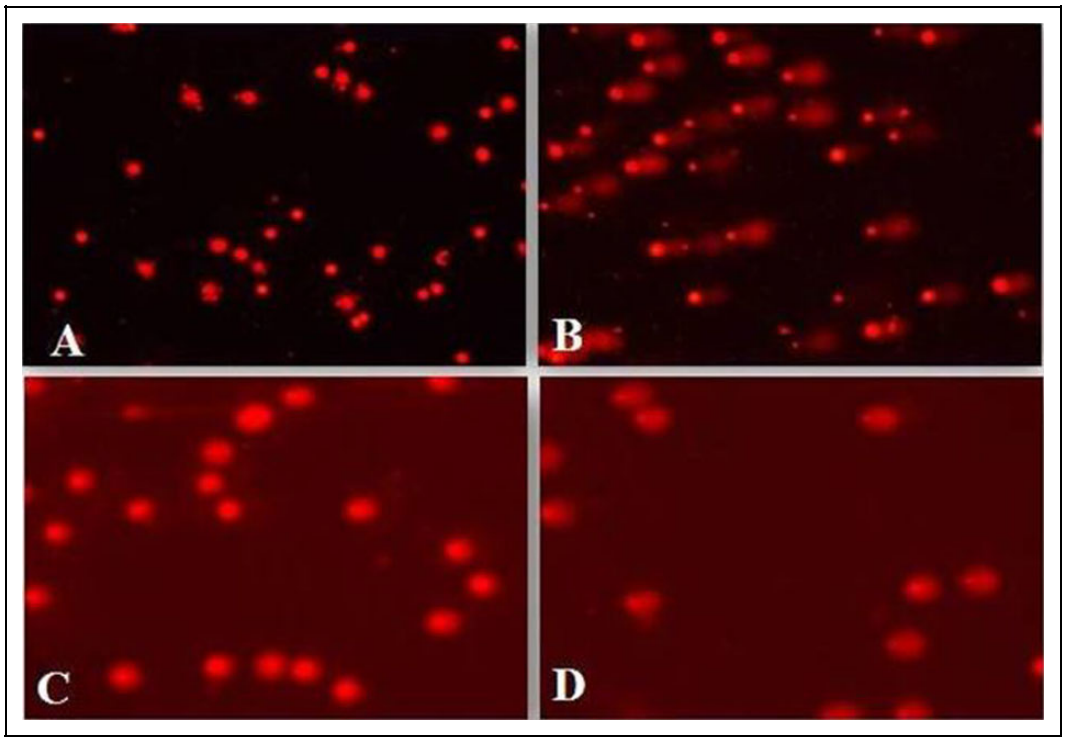

Figure 1. Comet images by Comet assay: (A) Normal control; (B) Diabetic control; (C) *Bougainvillea*-treated diabetic group; (D) Glibenclamide-treated diabetic group.

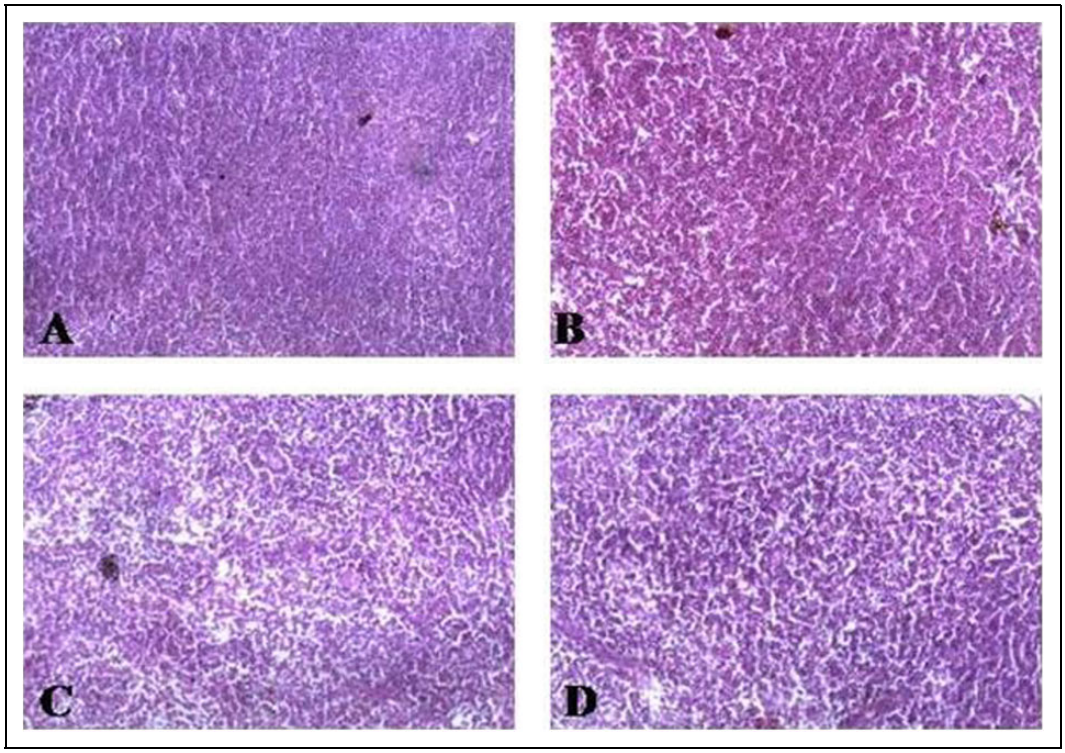

Figure 2. Histopathologic changes of the pancreas. (A) Normal control (the pancreas architecture was well preserved); (B) Diabetic control (streptozotocin-treated rats show extensive β-cell degranulation and decreased cellular density); (C) *Bougainvillea*-treated diabetic group (in streptozotocin + *Bougainvillea* treated rats, remarkable improvements in the islet of Langerhans were observed); (D) Glibenclamide-treated diabetic group (streptozotocin + glibenclamide treated rats, β-cells were normally preserved) (100×).

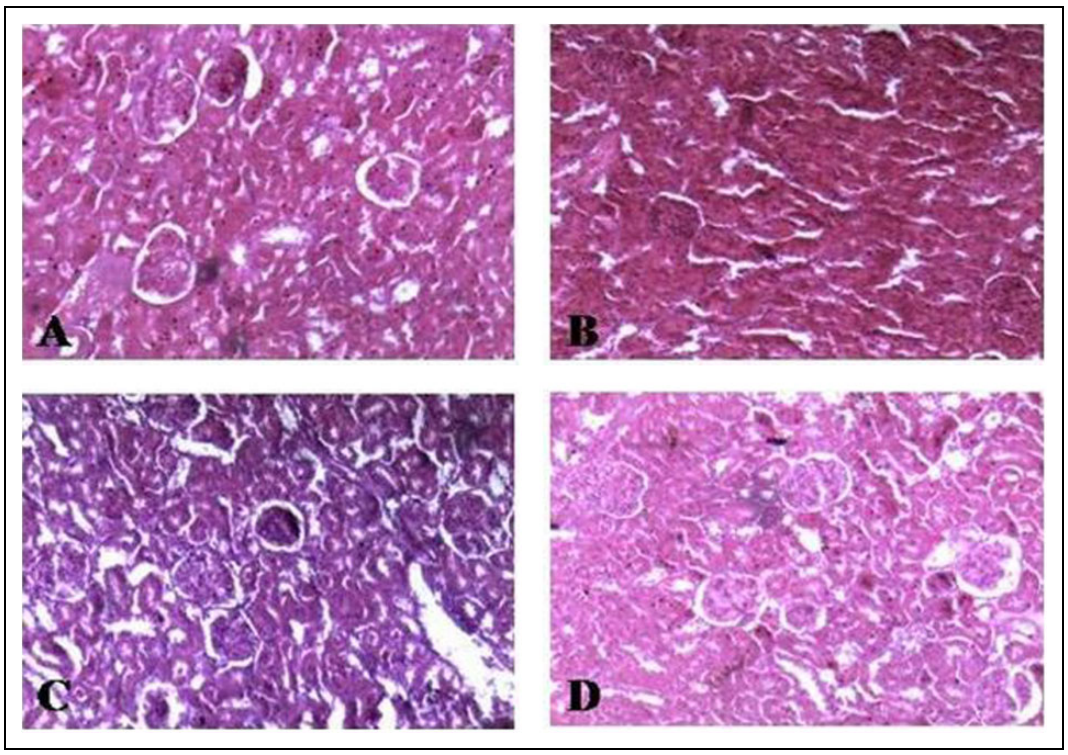

Figure 3. Histopathologic changes of the kidney. (A) Normal control (the kidney architecture was well preserved); (B) Diabetic control (streptozotocin-treated rats show adhesion of glomerulus and Bowman's capsules); (C) *Bougainvillea*-treated diabetic group; (D) Glibenclamide-treated diabetic group (in streptozotocin + *Bougainvillea* treated rats as well as in glibenclamide treated rats, the kidney architectures were well preserved) (100×).

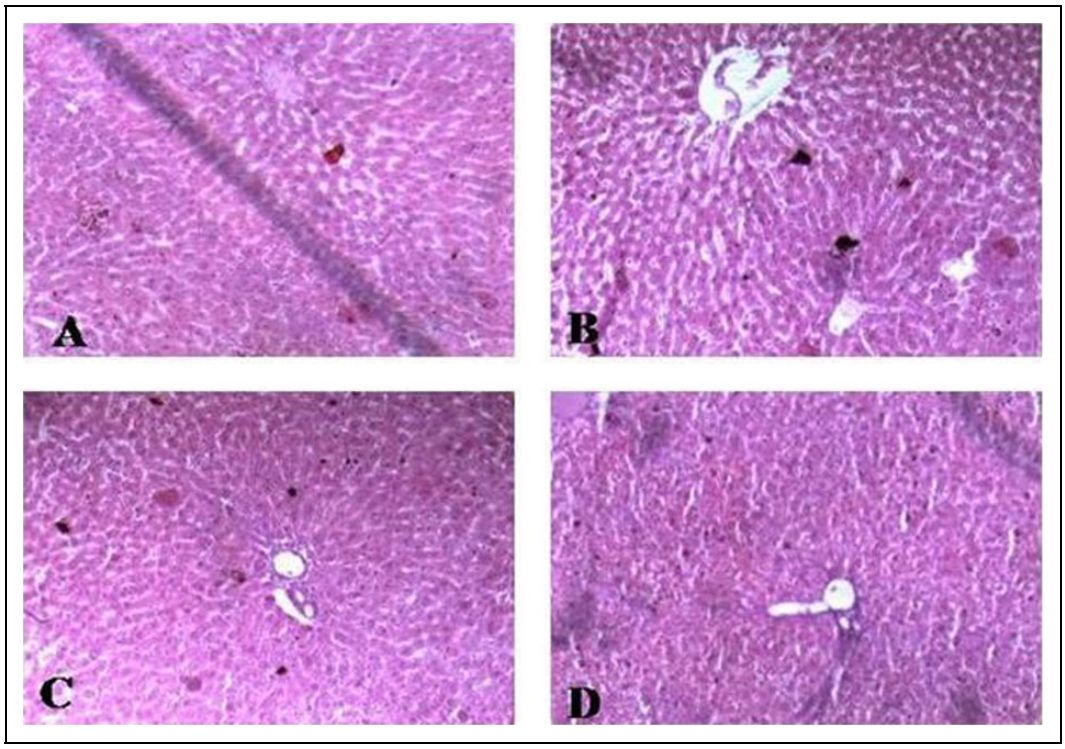

Figure 4. Histopathologic changes of the liver. (A) Normal control (in health control rats hepatic structures were well preserved); (B) Diabetic control (in streptozotocin-treated rats, degeneration of hepatocytes was observed); (C) *Bougainvillea*-treated diabetic group (section of liver tissue from *Bougainvillea*-treated diabetic rats shows well-formed hepatocytes); (D) Glibenclamide-treated diabetic group (section of liver tissue from glibenclamide treated rats shows normal architecture) (100×).

Creatinine, urea, and uric acid levels, which show the functioning of renal activity, are significantly elevated in the diabetic group. The *B spectabilis* extract significantly restored the levels of creatinine and uric acid near to normal level, while the level of urea did not significantly alter when compared to control.

Antioxidant defense enzymes protect cells from oxidative damage by scavenging free radicals and maintaining redox homeostasis. The activities of SOD, CAT, GSH, GR, GST, and GPx of diabetic rats were significantly reduced. Increased glucose flux causes the impaired antioxidant enzyme activities and increased oxidants production by multiple interacting pathways.[35,36] In the present study, a statistically significant increase in the levels of SOD and GSH in rats treated with *B spectabilis* was recorded. Significant decrease in TBARS was accomplished in rats treated with *B spectabilis* leaf extract, suggesting that aqueous leaf extract contains the antioxidant property thereby preventing the disruption of organs by protecting lipids from peroxidation under hyperglycemic conditions. Our results indicate that *B spectabilis* may act as a potent antioxidant. It has the potential to restore the blood glucose level in hyperglycemic conditions along with the normalization of altered biochemical parameters. The ingredients from *B spectabilis* thus may offer an efficient tool to combat diabetes mellitus without any side effect. However, a detailed study over the signaling mechanism behind the antioxidant effect of *B spectabilis* that allude to their antihyperglycemic role in diabetic conditions is needed to pave the way to the quest behind the clinical implications of *B spectabilis* in diabetic treatments and to render its uniquely beneficial applications for alleviating diverse complications.

The present study concluded that the aqueous extract of *B spectabilis* leaves may be useful in treating diabetes mellitus and has no toxicity. The results from the present investigation have opened new vistas for further research to elucidate the exact underlying mechanism of its hypoglycemic effect.

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Author Contributions

Pratibha Chauhan, Sunil Mahajan, and Archana Kulshrestha were involved in experimental work. Sadhana Shrivastava was associated with histopathology of tissues. Bechan Sharma, H. M. Goswamy, and G. B. K. S. Prasad were associated with study design, execution, and manuscript writing.

Declaration of Conflicting Interests

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Ethical approval

Approval was obtained from the institutional animal ethics committee of Jiwaji University, Gwalior, India (JU/IAEC/2012-119).

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