Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20173110

Original Research Article

Protective effect of *Berberis asiatica* root on biochemical and histopathological changes in streptozotocin-induced diabetic Wistar rats

Mohd Muddassir Husain Khan[1], Chetan Rastogi[1,2], Sachin Gupta[3]*, Shravan Kumar Paswan[1,4], Pritt Verma[1,4], Talha Jawaid[2], Ch. V. Rao[1]

[1]Department of Pharmacognosy and Ethnopharmacology, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India
[2]Department of Pharmacology, Hygia Institute of Pharmaceutical Education and Research, Lucknow, Uttar Pradesh, India
[3]Department of Pharmacology, Advance Institute of Biotech and Paramedical Sciences, Kanpur, Uttar Pradesh, India
[4]Amity Institute of Pharmacy, Amity University, Lucknow, Uttar Pradesh, India

Received: 17 June 2017
Revised: 24 June 2017
Accepted: 27 June 2017

***Correspondence to:**
Mr. Sachin Gupta,
Email: sachincsjm@rediffmail.com

ABSTRACT

Background: This study was designed to evaluate the effect of *Berberis asiatica* root extract (BAE) against streptozotocin induced elevated blood glucose level and other liver and kidney functions changes in adult male Wistar rats.

Methods: Thirty male Wistar rats were divided into five groups of six rats in each (Groups I-V). Group I and Group II served as normal control and disease control, respectively. Group III received standard anti-diabetic drug glibenclamide (5mg/kg), while Group IV and Group V received the low dose (250mg/kg) and high dose (500mg/kg) of BAE. Serum blood glucose, SGOT, SGPT, ALP, total bilirubin, BUN, serum creatinine, TC, TG, HDL-C, LDL-C, and VLDL-C were estimated using standard methods. After collection of samples for biochemical evaluation, the pancreas from each animal was isolated and examined for histological changes.

Results: BAE and glibenclamide treated disease rats showed significant ($p < 0.05$) decrease in blood glucose concentration. Treatment with BAE at 250mg/kg and 500mg/kg in Group IV and V and standard drug glibenclamide in Group III showed significant ($p < 0.05$) reduction in the level of liver function substances such as SGOT, SGPT, ALP and total bilirubin as compared to disease group, as well as showed significant ($p < 0.05$) decrease in renal panel. Liver function parameters were significantly ($p < 0.05$) improved in groups treated with BAE. Histopathological analysis revealed the protective effect of BAE against streptozotocin induced damage to islets of Langerhans.

Conclusions: This study showed the destruction of islets of Langerhans and elevation in blood glucose level as well as alteration in other biochemical parameters were ameliorated by the effect of *Berberis asiatica* extract.

Keywords: Anti-diabetic, Anti-oxidant, *Berberis asiatica*, Berberine

INTRODUCTION

Diabetes mellitus is a disease of global importance which affects millions of people worldwide. A number of patients with polygenic disorder worldwide are expected

to double between 1994 and 2025 to affect more than 225 millions of people.[1] Diabetes mellitus is considered as one of the five leading causes of death in the world. Regarding one hundred fifty million individuals tormented by diabetes worldwide, that is nearly 5 times over the estimate ten years ago and may this double by the year 2030. India leads the way with its largest number of diabetes subjects in any given country. It has been the estimated that the number of diabetes patients in India is expected to increase 57.2 million by the year 2030.[2]

Currently there are over 150 million diabetics worldwide and this number is likely to increase 300 million or more by the year 2025 due to increasing in sedentary lifestyle, consumption of energy-wealthy diet, and fatness.[3] Diabetic Mellitus is characterized by hyperglycemia due to disturbance in the group of metabolism of carbohydrates, fat, and protein, resulting from defects in insulin secretion action or both.[4] The insulin insensitivity and insulin deficiency in several animal models of diabetes mellitus lead to a decrease in blood glucose utilization by the liver and muscles thus the animal tissue.[5,6]

Diabetes mellitus is a chronic metabolic risk disorder which is characterized by an increased risk of mortality and prevalence of cardiovascular disease. Atherosclerotic cardiovascular disease is the main source of mortality and morbidity in diabetes patients.[7]

Recently, there has been a growing interest in finding out the hypoglycemic agents from natural products, especially those derived from plants.[8] Many traditional plants have been used successfully throughout the world for antidiabetic activity. The World Health Organization (WHO) has recommended the traditional plant treatment for diabetes warrant further evaluation.[9] Moreover; today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principle for treatment.[10]

Streptozotocin is a glucosamine nitrosourea compound with similar properties to that of other alkylating agents of the nitrosourea class which is toxic to cells.[11] GLUT2 is highly expressed in beta cells which internalize the streptozotocin (STZ) and causes toxicity to beta cells more than any other cell.[12,13]

Berberis asiatica (Family *Berberidaceae*) is a pretty evergreen thorn shrub, of 1.8 to 2.4m in height with light brown rough bark and oblong-ovoid edible berries. It commonly occurs in the dry outer areas of Himalaya, Assam, Madhya Pradesh, and Mount Abu.[14] It is commonly known as Kilmora or Kingora and contains many alkaloids such as berberine, palmatine, jatrorrhizine, columbamine, tetrahydropalmatine, berbamine, and oxyberberine and oxyacanthine.[15] Ethanolic (50%) extract of roots are reported to possess spasmolytic and anticancer activity.[16]

Different parts of the plant are used in the variety of ailments, such as fruits or berries are given as laxative to youngsters, stems in rheumatism and the root possesses powerful antimalarial activity. Stewing made up of the basis brings down fever. The dried extract is used as a purgative in children, as a blood-purifier, antipyretic, antiseptic and for external application in conjunctivitis. It's conjointly been counseled for stomach and duodenal ulcers and for hemorrhoids, both locally and internally.[16] In the Unani system; it is used in Hansen's disease and in leprosy. A yellow dye obtained from the roots and stems is of great value in tanning and for coloring leather.[17] It inhibits the intestinal ion secretion, smooth muscle contraction, ventricular tachyarrhythmia's, and causes reduction of inflammation, elevation of platelet count in patients with primary and secondary thrombocytopenia and stimulation of bile secretion and bilirubin discharge.[18-22] Evidence also suggests that intravenous berberine administration can play a role in preventing the onset of ventricular tachyarrhythmia and sudden coronary death after myocardial ischemic damage.[23] Studies have been carried out on its role in the treatment of diabetes mellitus but its mechanism of action has not been elucidated.[24] Studies have been carried out for lipid lowering action of berberine in a murine diabetic model induced by Streptozotocin (STZ). However, there is no data available on the glucose lowering effect of *Berberis asiatica* plant; hence the present study is an attempt in this direction.

METHODS

The plant material i.e. roots of *Berberis asiatica* was purchased from Regional Research Institute (RRI), Central Council for Research in Ayurveda and Siddha (CCRAS), Uttaranchal (India) in the month of February. The plant material was identified and authenticated by Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (NBRI), Lucknow.

Extraction and isolation

Fresh plant roots were chopped into small pieces and were dried in sunlight. Then the dried roots were coarsely powdered using an electric grinder.

Further, it was defatted with petroleum ether at 60-80^{0}C. 600g of plant material was extracted with 1.5 l of 50% aqueous ethanol for 72 hrs. Finally, the extract was concentrated using rotary evaporator (4001; Heidolph Instruments, Schwabach, Germany) at a reduced pressure and temperature (50±2^{0}C) until a viscous and sticky mass obtained.[25]

Chemicals

Streptozotocin and glibenclamide were purchased from Sigma Aldrich (Bangalore, India).

Animals

Adult male albino rats (160-200g) of either sex were procured and kept under controlled conditions of temperature 27±2°C and ratio 44%-56%, light/dark cycles of 12 h severally for one week before and through the experiments. Animals were provided with a standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though the water was allowed *ad libitum*. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals.[26]

Induction of experimental diabetes

Diabetes would be induced by single intraperitoneal injection of a freshly prepared solution of streptozotocin (SLR, Bangalore) (60mg/kg body weight) in 0.1 M citrate buffer having the pH 4.5. Diabetes would be confirmed in the animals by elevated plasma level of glucose after 24 hrs of injection. Rats with fasting blood glucose level >200mg/dl will be selected for the experimentation.[27]

Experimental design

Healthy male Wistar rats (n=30) were divided into five groups, containing six rats in each and the preventive study was conducted for 15 days. All animals were weighed before and after the study period. All groups received regular rat food and drinking water *ad libitum*.

Except for group I, all animals were received streptozotocin (SLR Bangalore) (60mg/kg body weight) in 0.1 M citrate buffer having the pH 4.5 throughout the study period. Group I and group II served as normal control and disease control, respectively. Group III received standard anti-diabetic drug, glibenclamide (5mg/kg), while Group IV and group V received the low dose (250mg/kg) and high dose (500mg/kg) of ethanolic extract of roots of *Berberis asiatica* plant (BAE). The treatment was given orally once daily throughout the study period for 15 days.

Various biological samples like blood, urine, pancreases were collected at the end of the treatment period for the analysis of different biochemical parameters.[28]

- Group I (Control): Normal control (Distilled water p.o once daily)
- Group II (Disease): Diabetic control (Streptozotocin, 60mg/kg ip)
- Group III (Standard): Diabetic control + Glibenclamide (5mg/kg p.o for 15 days)
- Group IV (Test 1): Diabetic control + BAE-LD (250mg/kg p.o for 15 days)

Group V (Test 2): Diabetic control + BAE-HD (500mg/kg p.o for 15 days)

Biochemical parameters

Analysis of urine samples

All the animals were kept in the individual metabolic cages and the urine sample of 24 h was collected on the 15th day. The urine samples were acidified with a drop of concentrated hydrochloric acid and stored at -20^{0}C for determination of glycosuria with Uro-dip 10c (Erba Diagnostics Mannheim, Mumbai, India) and urine volume was measured by measuring cylinder (Axiva, Delhi, India).

Blood serum and plasma preparation

The blood samples were collected by puncturing the retro-orbital venous plexus from each animal in centrifuge tubes without anticoagulant and allowed to clot at room temperature. The serum was separated by centrifugation at 10000 rpm for 10 min in refrigerated research centrifuge (Sigma 3K30, UK). The plasma and serum samples were divided into storage tubes according to their required amount of biochemical tests. They were quickly stored at -20^{0}C for further analysis.

Biochemical measurement

After 15 days of the treatment, fasting blood samples were collected from all the groups in the heparinized tube. Blood samples were also collected at the time interval of 30, 60, 90 and 120 min after administration of glucose at a concentration of 2mg/kg of body weight.[29]

Blood glucose level will be estimated by using glucose oxidase-peroxidase reactive strips and a glucometer (GOD-POD). The estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen (BUN), serum creatinine, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) using Vitros-250, fully dry biochemistry auto-analyzer (Johnson and Johnson, U.S.A).

Plasma insulin level would be estimated using auto analyzer (Roche Diagnostic, U.S.A.). Hemoglobin and glycosylated hemoglobin (HbA1c) would be measured by using HPLC Bio-Rad method (U.S.A.).

Hexokinase, lactate dehydrogenase (LDH), glucose-6-phosphatase and fructose -1, 6-bisphosphatase would be assayed by chemical method. The malondialdehyde level, glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) would be assessed by double beam UV spectrometer (Labindia, Mumbai, India).[30-33]

Histopathological analysis

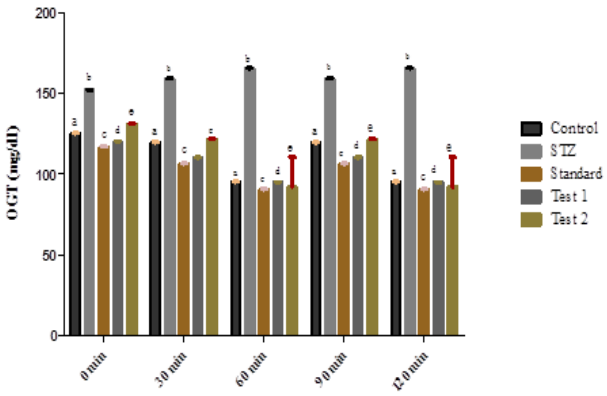
After collection of samples for biochemical evaluation, the pancreas from each animal was isolated after dissection under ether anesthesia followed by cervical dislocation and pancreas were transferred to 10% solution of buffered formalin (pH 7.4). The tissues were embedded in paraffin and the sections were cut off, stained with hematoxylin-eosin. The slides were then examined for histological changes under the light microscope.

Statistical analysis

All the statistical comparison between the groups were made by means of one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. P <0.05 regarded as significant using, GraphPad Prism 5.03 software (CA, USA). The data expressed are the mean±standard error of mean (S.E.M.).

RESULTS

The blood glucose levels in normal and disease groups of rats after oral administration of glucose were shown in Figure 1. The blood glucose values in normal rats rise to peak values 60 min after the glucose load and decreased to near normal levels at 120 min in diseased rats, the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. BAE and glibenclamide treated disease rats showed significant (p <0.05) decrease in blood glucose concentration at 60 and 120 min compared with disease group of rats. An elevated level of urine sugar in group II leads to glycosuria. Whereas, there was no glycosuria observed in the groups treated with *Berberis asiatica* and glibenclamide (Group III, IV and V) when compared to the normal group (Group I).



p <0.05 compared with normal;
*** p <0.05 compared with disease;
*OGT-Oral glucose tolerance

Figure 1: Effect of BAE on OGT in STZ induced diabetes in rats.

Liver function was assessed by measuring the serum SGOT, SGPT, ALP and total bilirubin which is shown in Table 1. The serum SGPT, SGOT, ALP and total bilirubin were significantly (p <0.05) elevated in disease group (Group II) when compared with normal group (Group I) which indicate the liver damage. While treatment with BAE at 250mg/kg and 500mg/kg in Group IV and V and standard drug glibenclamide in Group III showed significant (p <0.05) reduction in the level of liver function substances such as SGOT, SGPT, ALP and total bilirubin as compared to disease group (Group II).

Table 1: Effect of BAE on liver function analysis parameters in STZ induced diabetic study.

Categories	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	TBL (mg/dl)
Control	32.31±0.5437	45.15±0.1607	228.4±0.09866	0.3400±0.04612
Disease	72.73±0.6467$^{###}$	135.6±0.1289$^{###}$	289.6± 1.392$^{###}$	0.6500±0.01155$^{###}$
Standard	30.58±0.1289***	55.65±0.1358***	221.4±0.09342***	0.3900±0.06377**
STZ+BAE-LD	48.54±0.1017***	95.65±0.1055***	264.4± 0.1017***	0.5200±0.005774
STZ+BAE-HD	39.27±0.1478***	68.38±0.1331***	247.8± 8.296***	0.4400± 0.06904*

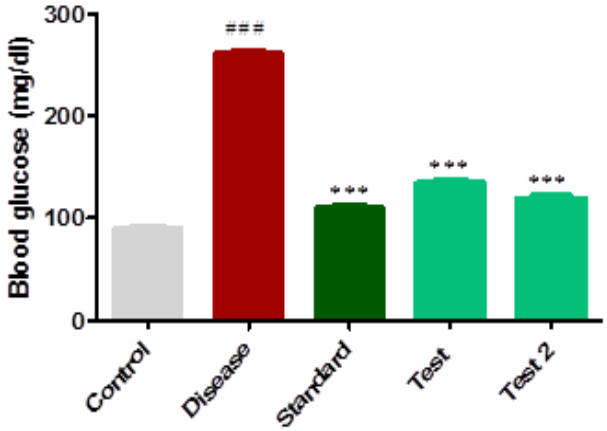
Table 2: Effect of BAE on renal function analysis parameters in STZ induced diabetic study.

Categories	Creatinine (mg/dl)	BUN(mg/dl)
Control	0.3400±0.04612	26.09±0.1436
Disease	0.6500±0.01155$^{###}$	51.49±0.1556$^{###}$
Standard	0.3600±0.04524**	23.09±0.1470***
STZ+BAE-LD	0.4800±0.01653	39.65±0.1358***
STZ+BAE-HD	0.4400±0.06904*	30.62±0.1313***

Kidney function markers were assessed by measuring serum BUN and creatinine which is shown in Table 2. BUN and creatinine were significantly (p <0.05) increased in disease group (Group II) when compared with normal group (Group I) indicating marked renal damage. While treatment with BAE at 250mg/kg and 500mg/kg in Group IV and V respectively and standard drug glibenclamide treatment in Group III showed significant (p <0.05) decrease in renal damage as compared to disease group (Group I).

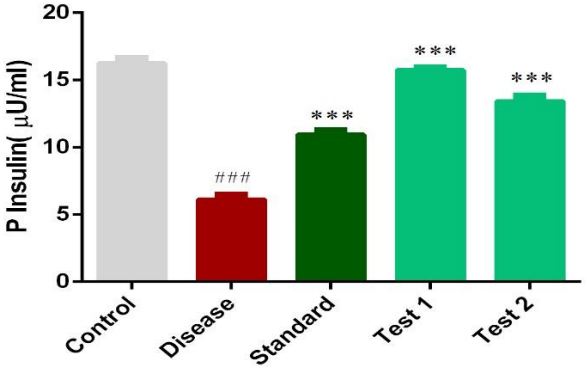
Serum lipid profile was assayed by measuring TC, TG, HDL-C, LDL-C and VLDL-C is shown in Table 3. The serum TC, TG, LDL-C, and VLDL-C were significantly increased in disease group (Group 2) when compared with normal group (Group I).Whereas serum HDL-C was significantly decreased in disease group (Group II) when compared with normal group (Group I) while the treatment with BAE at 250mg/kg and 500mg/kg in Group IV and V respectively, and treatment with standard drug glibenclamide to group III showed significantly (P <0.05) decrease serum TC, TG, LDL-C and VLDL-C as compared to disease group (Group II), whereas serum HDL-C was significantly (p <0.05) increased as compared to disease group (Group II).

The level of blood glucose, plasma insulin, hemoglobin, and glycosylated hemoglobin and urine sugar is plotted in Figures 2, 3, 4 and 5 respectively. The blood glucose, glycosylated hemoglobin, and urine sugar levels were significantly (p<0.05) higher in disease group (Group II) when compared with normal group (Group I).



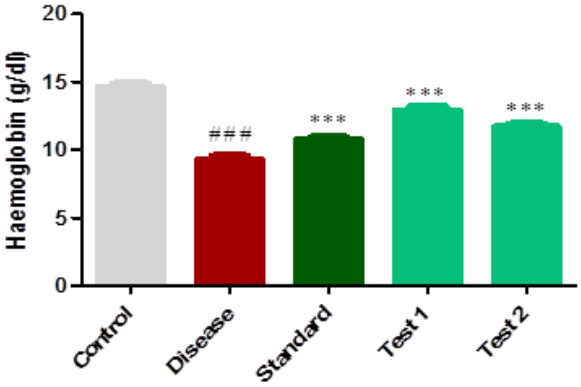
p <0.05 compared with normal;
*** p <0.05 compared with disease.

Figure 2: Effect of BAE on blood glucose in STZ induced diabetes in rats.



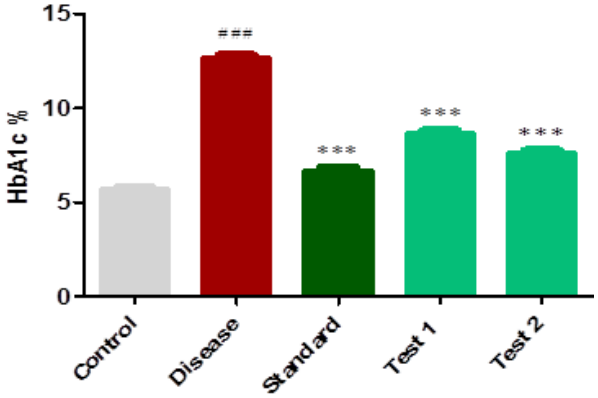
p <0.05 compared with normal;
*** p <0.05 compared with disease.

Figure 3: Effect of BAE on plasma insulin in STZ induced diabetes in rats.



p <0.05 compared with normal;
*** p <0.05 compared with disease.

Figure 4: Effect of BAE on haemoglobin in STZ induced diabetes in rats.



p <0.05 compared with normal;
*** p <0.05 compared with disease.

Figure 5: Effect of BAE on HbA1c in STZ induced diabetes in rats.

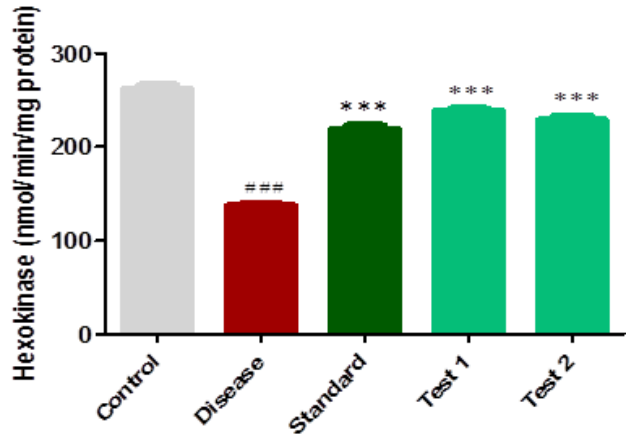
Plasma insulin and hemoglobin were significantly (p<0.05) decreased in disease group (Group 2) when compared with normal group (Group I). While the treatment with BAE at 250mg/kg and 500mg/kg in Group IV and V respectively, and standard drug glibenclamide treatment in group III showed significantly (p <0.05) decreased blood glucose, glycosylated hemoglobin, and urine sugar as compared to disease group (Group II). Also, the plasma insulin and hemoglobin were significantly (p<0.05) increased in group IV and V, as compared to disease group (Group I).

The estimation of carbohydrate metabolizing enzymes such as hexokinase, LDH, glucose-6-phosphatases (G-6-P) and fructose-1, 6-bisphosphatase was shown in Figures 6, 7, 8 and 9 respectively. The level of the enzyme such as LDH, G-6-P, and fructose-1, 6-bisphosphatase were significantly (p<0.05) increased in disease group (Group II) when compared with normal group (Group I), while hexokinase level was significantly (p<0.05) decreased in disease group (Group II). While the treatment with BAE at 250mg/kg and 500mg/kg in group IV and V

respectively and standard drug glibenclamide treated group (Group 3) showed significantly decreased levels of LDH, G-6-P, and F-1, 6-bis-P as compared to disease group (Group II), whereas hexokinase level was significantly ($p<0.05$) increased as compared to disease group (Group II).

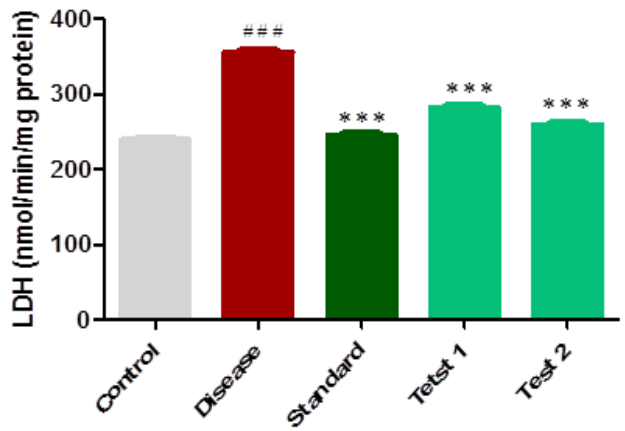
Table 3: Effect of BAE on lipid profile analysis parameters in STZ induced diabetic study.

Categories	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
Control	35.65±0.1358	24.80±0.4053	12.60±0.1242	13.65±0.1358	19.44±0.1510
Disease	72.42±0.2638$^{###}$	48.56±0.1409$^{###}$	8.580±0.1333$^{###}$	39.81±1.255$^{###}$	32.69±0.1549$^{###}$
Standard	38.65±0.1358***	29.38±0.1800***	11.71±0.1401***	16.61±0.1401***	20.59±0.1252***
STZ+BAE-LD	48.47±0.08327***	36.47±0.1070***	14.46±0.07638***	26.07±0.1056***	25.49±0.1003***
STZ+BAE-HD	43.62±0.2269***	31.61±0.1428***	12.70±0.1328***	20.52±0.1325***	23.67±0.1407***



p <0.05 compared with normal;
*** p <0.05 compared with disease.

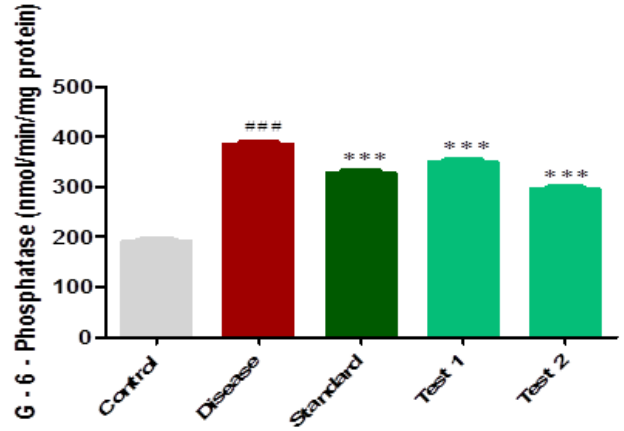
Figure 6: Effect of BAE on hexokinase in STZ induced diabetes in rats.



p <0.05 compared with normal;
*** p <0.05 compared with disease.

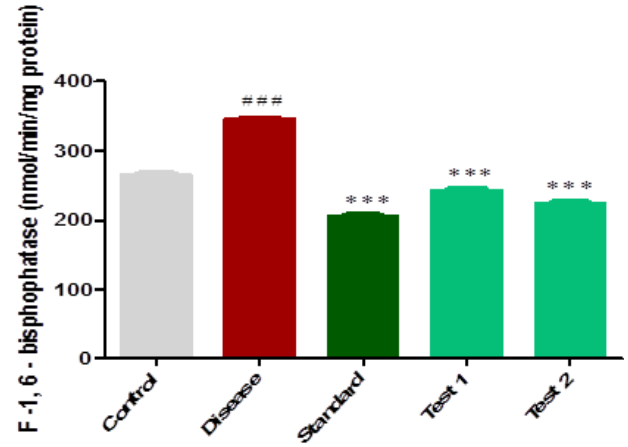
Figure 7: Effect of BAE on LDH in STZ induced diabetes in rats.

The antioxidant effect was assayed by measuring the level of MDA, GSH, SOD and CAT which is shown in Table 4.



p <0.05 compared with normal;
*** p <0.05 compared with disease.

Figure 8: Effect of BAE on G-6-phosphatase in STZ induced diabetes in rats.



p <0.05 compared with normal;
*** p <0.05 compared with disease.

Figure 9: Effect of BAE on F-1,6-biphosphatase in STZ induced diabetes in rats.

The MDA level was significantly ($p <0.05$) increased in disease group (Group II) when compared with normal group (Group I). Whereas GSH, SOD and CAT levels

were significantly decreased in disease group (Group II) when compared with normal group (Group I) while the treatment with BAE at 250mg/kg and 500mg/kg in Group IV and V respectively, and standard drug glibenclamide to group III showed significantly ($p < 0.05$) increased GSH, SOD and CATAs compared to disease group (Group II). Whereas the MDA level was significantly ($p<0.05$) decreased as compared to disease group (Group II).

Table 4: Effect of BAE on LPO, GSH, SOD and CAT analysis parameters in STZ induced diabetic study.

Categories	LPO (mmol/L)	GSH (µ/ml)	SOD (U/mgproteine)	CAT (U/mgproteine)
Control	0.3400±0.04612	141.4±0.08552	147.4±1.064	44.53±0.3286
Disease	0.8300±0.02708$^{###}$	69.29±0.06137$^{###}$	81.07±4.484$^{###}$	17.88±0.3506$^{###}$
Standard	0.3900±0.06377***	94.07±0.8090***	120.8±138.6***	30.60±0.1178***
STZ+BAE-LD	0.4800±0.01653***	136.1±1.063***	138.6±0.1337***	39.56±0.09852***
STZ+BAE-HD	0.4400±0.06904***	115.4±0.1013***	130.4±0.1074***	35.68±0.2226***

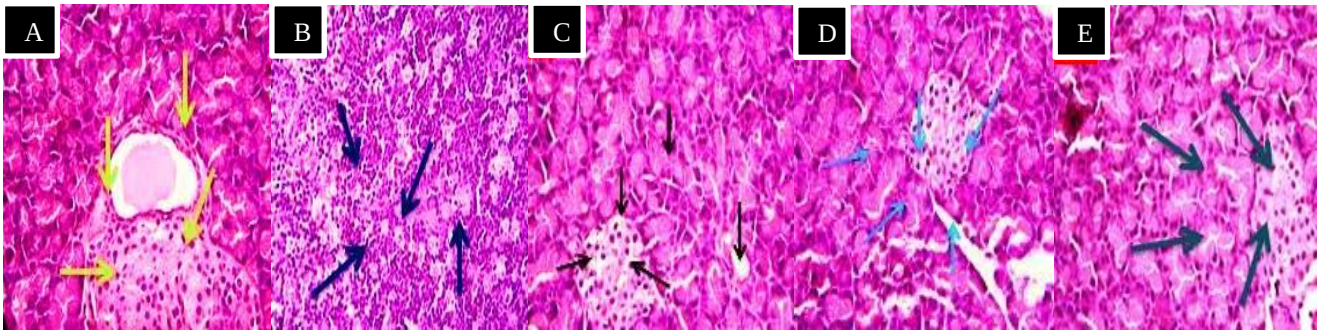


Figure A: Normal group showing normal architecture of islets of langerhans.
Figure B: Necrotized islets of langerhans.
Figure C: Glibenclamide treated rats showing normal islets of langerhans.
Figure D: Test 1 group showed little collagen fibers around the islets of langerhans.
Figure E: Diabetic rats treated with test 2 shows near to normal architecture of islets of langerhans with regeneration of islets.

Figure 10: Effect of *Berberis asiatica* and glibenclamide (5mg/kg/po/day/15days) in streptozotocin (STZ) (60mg/kg/i.p./single dose) treated rats. Pancreatic histology after 15 days of treatment.

Histopathological study of isolated pancreas

Histopathological examination of diabetic pancreas shows the decrease in cells upto 35% of this means degeneration of cell. Treatment with BAE 250mg/kg unconcealed that cell degeneration was up to 45% and with BAE 500mg/kg solely half-hour whereas commonplace drug showed solely 75% degeneration of cell. Maximum protection (75%) was seen in commonplace drug followed by treatment with BAE 500mg/kg (70%), however, no important distinction discovered (Figure 10).

DISCUSSION

Type-2 diabetes identified patients don't require hormone treatment to stay alive. The inability of peripheral tissues to respond insulin is called as insulin resistance.[34,35] Major characteristics of type-2 polygenic disease embody impaired utilization of aldohexose and resistance to the flexibility of hormone to stimulate aldohexose uptake and disposal in tissues.[36] The *Berberis asiatica* extract (BAE) significantly improved the glucose tolerance test in diabetic rats at 500mg/kg dose orally.

The increase in the liver function substances like SGOT, SGPT, ALP and total bilirubin were observed in diabetic control rats (Group II). The unseaworthy increase of the liver enzymes such as SGOT and SGPT in blood were as a result of destroying of liver cells, indicate that the liver damage is probably present. An elevated level of bile in the blood results from the slow production or blocked in bile flow that leads to improper digestion of fats and elevation of enzyme like alkaline phosphate (ALP).[37] Total Bilirubin is the breakdown product of old RBCs. Hyperbilirubinemia occurs due to hemolysis by blockage of the bile duct and liver disease. Treatment with *Berberis asiatica* plant extract and standard drug reduced the elevated levels of liver function substances.

In the current study, the STZ-induced diabetic renal disorder showed a considerable inflation in creatinine and

urea levels in (Group II). Treatment with *Berberis asiatica* plant extract and standard drug reduced the elevated levels of kidney function substances. Diabetic rats exhibited abnormalities in lipid metabolism as evidenced by the elevated levels of cholesterol, triglycerides, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol and low levels of HDL-C in group II.[38,39] Treatment with *Berberis asiatica* plant extract and standard drug reduced the elevated levels of TC, TG, LDL-C and LDL-C, whereas it showed the improvement in the level of HDL-C.

Chronic internal secretion deficiency and internal secretion unfitness are the foremost causes of the decreased viscous aldohexose utilization and accumulated aldohexose production in many animal models of two polygenic disorders. As a result of which internal secretion decreases the viscous aldohexose output by activating polysaccharide synthesis and metastasis, and by inhibiting gluconeogenesis.[5] Glycosylated hemoglobin was found to elevate within the patients with DM, and therefore the increased quantity is directly proportional to the abstinence blood sugar level.[40] Throughout polygenic disorder, the surplus aldohexose gift within the blood reacts with hemoglobin. Therefore, the entire hemoglobin level is decreased in diabetic rats.[41] Since STZ causes selective destruction of ß-cells of islets of Langerhans leading to the marked decrease in internal secretion levels, it's rational that polysaccharide levels in tissues (skeletal muscle and liver) decrease as they depend upon internal secretion for inflow of aldohexose.[42] The augmentation of blood glucose and glycosylated hemoglobin and reduced level of the plasma insulin and hemoglobin in group II when treated with *Berberis asiatica* plant extract and standard drug, reduced the elevated levels of blood glucose and glycosylated hemoglobin and improved the level of plasma insulin and hemoglobin.

The gluconeogenic accelerator aldohexose-6-phosphatase could be a crucial accelerator of glucose equilibrium. As a result of this, it catalyzes the last word organic chemistry reaction of each glycogenolysis and gluconeogenesis. Aldohexose production is raised in the diabetic state is related to the impaired suppression of the gluconeogenic accelerator laevulose 1, 6-bisphosphatase.[43] LDH in anaerobic glycolysis catalyzes the conversion of pyruvate to lactate which subsequently is converted to glucose in gluconeogenic reflux in diabetic condition. An increase in the activity of LDH was observed.[44] The hexokinase synthesis is decreased due to the low level of mRNA coding for hexokinase and insulin administration stimulate transcription of hexokinase mRNA synthesis and thus enhanced the synthesis and activity of the enzyme.[45] The increase in the level of LDH, G-6-P and fructose-1, 6-biphosphate and reduce the level of hexokinase was observed in group II. While treatment with *Berberis asiatica* plant extract and standard drug reduced the elevated levels of LDH, G-6-P and fructose-1,6-biphosphate and glucose and improved the level of hexokinase.

The MDA is reactive aldehyde which is the major electrophonic species known to elicit the stress of toxic nature in cells and to form covalent protein adduct which is referred as advanced lipoxidation end product found to be akin to advanced glycation end product.[46] SOD catalyses superoxide anions which are the important reactive oxygen species in cells and involved in membrane damage. The elevation of GSH and SOD activates the endogenous compensatory mechanism for prolonged overproduction of free radicals and oxidative stress.[47] CAT is also an oxidative enzyme located in peroxisome which decomposes H_2O_2 to H_2O and O_2.[48] These entire defensive antioxidant enzymes works in conjunction with each other and thus are able to protect from free radicals mediated oxidative damage. It was found that there was increased lipoxidation LPO and reduced the level of SOD, GSH and CAT in disease group (Group II). Whereas, treatment with *Berberis asiatica* plant extract and standard drug reduced the elevated levels of LPO and improved the level of SOD, GSH, and CAT in group III, IV and V.

Microscopic evaluation of pancreas revealed the high percentage of cellular degeneration which clearly indicates that the diabetes is induced by STZ. The groups treated either with BAE and standard drug showed a higher percentage of protection in pancreatic cells and also reduces the chances of diabetes to get elevated.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow and Mr. Lalu Prasad.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee with approval number (IAEC/ CPCSEA/07/2014)

REFERENCES

1. McGinley C, Shafat A, Donnelly AE. Does antioxidant vitamin supplementation protect against muscle damage? Sports Med. 2009;39:1011-32.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diab Care. 1998;2:1414-31.
3. Yajnik CS. The insulin resistance epidemic in India: fetal origins, later lifestyle, or both? Nutri Rev-Washing. 2001;59:1-9.
4. Nyholm B, Pørksen N, Juhl C, Gravholt C, Butler P, Weeke J, et al. Assessment of insulin secretion in relatives of patients with type 2 (non-insulin-

dependent) diabetes mellitus: evidence of early β-cell dysfunction. Metabol. 2000;49:896-905.
5. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. Science. 1992;258:766-70.
6. Reaven GM. Role of insulin resistance in human disease. Diabetes. 1988;37:1595-607.
7. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991;40:405-12.
8. Sabu M, Smitha K, Kuttan R. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. J Ethnopharmcol. 2012;83:109-16.
9. Organization WH. WHO Expert Committee on Diabetes Mellitus. 1980 [meeting held in Geneva from 25 September to 1 October 1979]: second report.
10. Singh RP, Padmavathi B, Rao AR. Modulatory influence of Adhatoda vesica leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. Mol Cell Biochem. 2000;213:99-109.
11. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50:537-46.
12. Wang Z, Gleichmann H. GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. Diabetes. 1998;47:50-6.
13. Schnedl WJ, Ferber S, Johnson JH. STZ transport and cytotoxicity: specific enhancement in GLUT2-expressing cells. Diabetes. 1994;43:1326-33.
14. Thakur R, Puri HS, Husain A. Major medicinal plants of India. Central Institute of Medicinal and Aromatic Plants, Lucknow; 1989.
15. Asolkar L, Kakkar K, Chakre O. New Delhi: Publications and Information's Directorate, CSIR; Glossary of Indian Medicinal Plants with active principles. 1992;1:171-3.
16. Chatterjee RB, Das Gupta AK. Plant alkaloids VI. Berberis asiatica Roxburgh. J Indian Chem Soc. 1954;31-83.
17. Bhakuni D, Shoeb A, Popli S. Studies in Medicinal Plants: Part 1 - Chemical constituents of Berberis asiatica Roxb. Ind J Chem. 1968;6:123.
18. Dhar M, Dhawan B, Mehrotra B, Ray C. Screening of Indian plants for biological activity: Part I. Ind J Exp Bio. 1968; 6:232-47.
19. Kumar E, Elshurafa AA, Elango K, Subburaju T, Suresh B. Anti-tumour effect of Berberis asiatica on dalton's lymphoma ascite. Anc Sci Lif. 1998;17:290.
20. Chopra R, Badhwar R, Ghosh S. Poisonous plants of India. Leguminosae; 1965.
21. Babu T, Kuttan G, Padikkala J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to Centella asiatica. J Ethnopharmcol. 1995;48:53-7.
22. Kuttan G, Vasudevan D, Kuttan R. 55th Annual Meeting. Soc Biol Chem; 1986:105.
23. Mary K, Girija K, Ramadasan K. Partial purification of tumour reducing principle from Helicanthis elasticus (Fam. Loranthaceae). Can Lett. 1994;81:53-7.
24. Hepper FN. Old world withania (Solanaceae): a taxonomic review and key to the species. Solanaceae III Taxonomy, Chemistry, Evolution Hawkes, Kew, UK: Royal Botanic Gardens Richmond, Surrey, UK for the Linnean Society of London, London; 1991:211-28.
25. Maithani A, Parcha V, Pant G, Dhulia I, Kumar D. Studies on phytochemical investigation and hypoglycemic evaluation of Azadirachta indica leaves extract on alloxan induced diabetic rats. J Phar Res. 2011;4.
26. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983;16:109-10.
27. Brosky G, Logothetopoulos J. Streptozotocin diabetes in the mouse and guinea pig. Diabetes. 1969;18:606-11.
28. Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res. 2005;52:313-20.
29. Joy K, Kuttan R. Anti-diabetic activity of Picrorrhiza kurroa extract. J Ethnopharmcol. 1999;67:143-8.
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt Biochem. 1979;95:351-8.
31. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Cli Med. 1963;61:882-8.
32. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Bioph. 1978;186:189-95.
33. Claiborne A. Catalase activity. CRC handbook of methods for oxygen radical research. 1985;1:283-4.
34. Arulmozhi D, Veeranjaneyulu A, Bodhankar S. Neonatal streptozotocin-induced rat model of type 2 diabetes mellitus: A glance. Ind J Pharm. 2004;36:217.
35. Murugan P, Pari L. Antioxidant effect of tetrahydrocurcumin in streptozotocin–nicotinamide induced diabetic rats. Life Sci. 2006;79:1720-8.
36. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Inv. 2000;106:171.
37. Feldman M, Friedman L, Brandt L. Sleisenger and Fordtran's gastrointestinal and liver disease. Pathophysiology/diagnosis/management. 1998;1-2:169.
38. Florence NT, Benoit MZ, Jonas K, Alexandra T, Désiré DDP, Pierre K, et al. Antidiabetic and antioxidant effects of Annona muricata (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. J Ethnopharmcol. 2014;151:784-90.

39. Ananthan R, Latha M, Ramkumar K, Pari L, Baskar C, Bai VN. Effect of Gymnema montanum leaves on serum and tissue lipids in alloxan diabetic rats. J Diab Res. 2003;4:183-9.
40. Goodarzi M, Zal F, Malakooti M, Sadeghian MSS. Inhibitory activity of flavonoids on the lens aldose reductase of healthy and diabetic rats. Acta Med Iran. 2006;44:41-5.
41. Sheela C, Augusti K. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic Allium sativum Linn. Ind J Exp Bio. 1992;30:523-6.
42. Whitton PD, Hems DA. Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats. Biochem J. 1975;150:153-65.
43. Pari L, Murugan P. Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. J Basic Cli Phy Pharmacol. 2005;16:257-74.
44. Kumar GPS, Arulselvan P, Kumar DS, Subramanian SP. Anti-diabetic activity of fruits of Terminalia chebula on streptozotocin induced diabetic rats. J Heal Sci. 2006;52:283-91.
45. Spence JT. Levels of translatable mRNA coding for rat liver glucokinase. J Bio Chem. 1983;258:9143-6.
46. Farmer EE, Davoine C. Reactive electrophile species. Curr Opi Plan Bio. 2007;10:380-6.
47. Aksoy N, Vural H, Sabuncu T, Aksoy S. Effects of melatonin on oxidative–antioxidative status of tissues in streptozotocin-induced diabetic rats. Cell Biochem Func. 2003; 21:121-5.
48. Rauscher FM, Sanders RA, Watkins JB. Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats. J Biochem Mol Toxicol. 2001;15:41-6.

Cite this article as: Khan MMH, Rastogi C, Gupta S, Paswan SK, Verma P, Jawaid T, et al. Protective effect of *Berberis asiatica* root on biochemical and histopathological changes in streptozotocin-induced diabetic Wistar rats. Int J Basic Clin Pharmacol 2017;6:1880-9.