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Research Article

ATTENUATION OF HYPERGLYCEMIA IN STREPTOZOTOCIN-INDUCED DIABETIC RATS BY *BAUHINIA VARIEGATA* FLOWER EXTRACT

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ABSTRACT

To evaluate the anti-hyperglycemic activity of ethanolic extract of Bauhinia variegata flower (BVE) using streptozotocin (STZ) model and anti-oxidant activity of BVE evaluated by in-vitro methods. Ethanolic extract of Bauhinia variegata flower (BVE) 200 and 400 mg/kg body weight was administered orally according to different groups of animals once a daily for 21 days. Blood glucose levels were estimated at 0, 7th, 14th and 21st day by glucometer (one-touch) and on 21st day biochemical parameters (lipid profile) and histopathological examination of isolated organ (pancreas) was also estimated. The anti-oxidant activity of BVE was performed by in-vitro methods by help of DPPH scavenging activity and hydrogen peroxide scavenging (H_2O_2) assay. A significant reduction in blood glucose was observed in diabetic animals treated with BVE at different doses when compared with diabetic rats. Levels of triglycerides, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) were decreased while administrating BVE at different doses, compared with their control values in diabetic animals. Also a significant anti-oxidant activity was observed in selected methods for reducing power and scavenging DPPH and H_2O_2 radicals. The results show that BVE possesses significant anti-hyperglycemic and anti-oxidant activity. The anti-hyperglycemic activity might be attributed to stimulating effect on glucose utilization and anti-oxidant property.

KEYWORDS: *Bauhinia variegata; DPPH scavenging activity; hydrogen peroxide scavenging (H_2O_2) assay; anti-hyperglycemic; streptozotocin.*

INTRODUCTION

Diabetes is a type of defect in which body's ability to convert glucose to energy is impaired. According to the World Health Organization (WHO) criteria, the prevalence of known diabetes was 5.6% and 2.7% among urban and rural areas, respectively [1]. Oral hypoglycemic agents that could effectively control the abnormalities of carbohydrate, lipid, and protein metabolism that occur in patients with diabetes have been used for over half a century. Only metformin was approved for use in the United State in early 1995. Metformin also exerts little or no effect on basal insulin release by the pancreas or isolated islets of non-diabetic animals [2]. Alpha-glucosidase inhibitors, such as Acarbose, found in the mid-1990, has rationalized and simplified the treatment of diabetes. It is a competitive inhibitor of the major α-glucosidase enzymes in the brush border of the mucosal cell of the small intestine. It inhibits the digestion of the complex carbohydrates in the upper jejunum so that they are digested throughout the length of the small intestine. The major effect of this drug is to reduce postprandial rise in plasma glucose [3]. Thiazolidinedione (TZDs) analogues (Glitazones or TZDs), a new class of anti-diabetic drugs represented by ciglitazone, have been shown to be effective anti-hyperglycemic compounds in animal models of non-insulin dependent diabetes mellitus (NIDDM) [4].

Rakta Kanchan (*Bauhinia variegata* Linn.) commonly known as Kanchnar in Sanskrit and Mountain Ebony in English. The various parts of the plant viz., flower buds, flowers, stem, stem bark, leaves, seeds and roots are practiced in various indigenous systems of medicine and are popular among the various ethnic groups in India for the cure of variety of ailments [5]. Most of the diseases can be cured by existing drugs. Still there is never ending search for finding new drugs in the hope that it would yield drugs with lesser side effects and better therapeutic benefits than the existing drugs. *Bauhinia variegata* flowers are used to treat diabetes traditionally [6].

MATERIALS AND METHODS

Experimental Animals:

Young adult male wistar rats 7-8 weeks old, weighing 150-200 gm were housed in polypropylene cages in standard

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environmental conditions, 12 h light and 12 h dark cycle at 25 ± 2°C. Standard laboratory pellet diet and water ad libitum were given to the animals. Animals were acclimatized to the laboratory condition for at least 15 days prior to the experiment and were maintained in a well ventilated animal house. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) animals and the care of the laboratory was taken as per the CPCSEA regulation (Reg.No. UIP/IAEC/ April-2015/03).

Reagents:

Streptozotocin was purchased from Sigma Alderich Co., USA. Standard drug Metformin obtained from Sun Pharmaceutical Industries Ltd., Vadodara, Gujarat. 2,2-diphenylpicrylhydrazyl (DPPH) obtained from Hi-Media, Bangalore. All other chemicals used were of analytical grade and were purchased form Scientific Corporation, Allahabad (U.P.), India.

Collection of plant material:

The flowers of *Bauhinia variegata* were collected during January 2015 from the local surroundings in Allahabad, Uttar Pradesh. Further taxonomic identification was conducted by Dr. G.P. Sinha, Scientist-E/Head of Office, Botanical Survay of India, CRC, Allahabd (U.P.) Voucher specimen no. BSI/CRC/Tech./2014-15/571. A plant specimen was deposited in the herbarium of Botanical Survay of India, CRC, Allahabd (U.P.) for future reference.

Preparation of plant extract:

The freshly collected flowers (2.5 kg) of *Bauhinia variegata* were first air dried and then dried in tray dryer under controlled conditions and powdered. The powdered flowers (1.2 kg) were extracted with petroleum ether to remove fatty substances. The mark was further extracted with 95% ethanol by hot percolation method. The extract was filtered and concentrated under vacuum at 40°C and stored in desiccator. The percentage yield of ethanolic extract (BVE) was found to be 20.8% w/w.

Physicochemical analysis: [7]

Physicochemical analysis was performed for loss on drying, total ash, water soluble ash acid insoluble ash, sulphated ash and methanol soluble extractive value.

Preliminary phytochemical investigation: [7]

Preliminary phytochemical investigation revealed the presence of alkaloids, tannins, flavonoids and glycosides in ethanolic extract of *Bauhinia variegata* flower.

Determination of oral acute toxicity study:

The lethal median dose (LD_{50}) determination was performed in rats by Organisation for Economic Co-operation and Development (OECD) Guideline 423. A single dose of the extract (i.e., 5 mg/kg, 50 mg/kg, 300 mg/kg, or 2000 mg/kg) in the appropriate quantity of water was administered orally by gavage to different groups of rat (three rat in each group). However, all the animals were deprived of food for 2 hours prior to dosing and 4 hours after dosing. For the first 12 hours, the animals were initially monitored continuously for any adverse effects for 4 hours and then monitored at 1-hour intervals. They were later monitored twice daily for any abnormal changes throughout the study period (which lasted 14 days) [8]. The lethal median dose (LD_{50}) of the 95% ethanolic extract of ethanolic extract of *Bauhinia variegata* flower was 2000 mg/kg. One-tenth of the maximum dose of the extract tested for acute toxicity was selected for pharmacological activity (i.e., 200 mg/kg and its double strength of 400 mg/kg of body weight).

Preparation of extract dose:

Weighed quantity of ethanolic extract of *Bauhinia variegata* was suspended in water and administered orally to experimental animals. Suspension of extract was prepared freshly. The extract was administered at a dose of 200 and 400 mg/kg of body weight.

In-Vitro Antioxidant Activity:

DPPH scavenging activity:

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-bpicrylhydrazyl; DPPH) was characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gave rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH was mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gave rise to the reduced form with the loss of this violet color.

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals was monitored. The sample extract (0.2 ml) was diluted with methanol and 2 ml of DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation as given below:

$$\% \text{ inhibition of DPPH radical} = ([A_{br} - A_{ar}) / A_{br}] \times 100$$

where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place [9].

Hydrogen peroxide scavenging (H_2O_2) assay:

Human beings are exposed to H_2O_2 indirectly via the environment nearly about 0.28 mg/kg/day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapor or mist and through eye or skin contact. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH^-) that can initiate lipid peroxidation and cause DNA damage in the body.

The ability of plant extracts to scavenge hydrogen peroxide was estimated by the solution of hydrogen peroxide (40 mM) which was prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extract (20–60 µg/mL) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

where A_i is the absorbance of control and A_t is the absorbance of test[9].

Experimental induction of diabetes:

A freshly prepared solution of streptozotocin (50 mg/kg, body weight) in 0.1M citrate buffer (pH 4.5) was injected intraperitonealy in a volume of 1 ml/kg. STZ-injected animals exhibited massive glycosuria and hyperglycemia within 2 days.

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Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration 96 h after the injection of STZ. The rats with blood glucose level >200 mg/dL were considered to be diabetic and were used in the experiment [10].

Experimental design and procedure:

Five groups of rats were used to study the effect of 95% ethanolic extract of *Bauhinia variegata*. Each group consisted of six rats and experimental designs were as follows:

Group I: The control rats, which received vehicle normal saline solution.

Group II: Diabetic control rats, which received the vehicle normal saline solution.

Group III: Diabetic rats, which were treated with extract 200 mg/kg body weight.

Group IV: Diabetic rats, which were treated with extract at 400 mg/kg body weight.

Group V: Diabetic rats, which were treated with the standard drug metformin at 100 mg/kg body weight.

Anti-hyperglycemic activity:

All animals were allowed to adapt to cages for 3 days, after which they were fasted overnight. Diabetes was induced in rats by intraperitoneal injection of streptozotocin at a dose of 50 mg/kg, dissolved in normal saline. After streptozotocin treatment, all animals were given free access to food and water. Blood glucose levels were measured 2 days after streptozotocin injection and used as parameters to match pairs of diabetic rats with similar level of severity. Only rats with fasting blood glucose levels higher than 200 mg/dL were considered to be diabetic and were used in the experiment. The mean blood concentration of glucose in normoglycemic rats was around 74 mg/dL. Animals were randomly assigned to four different groups i.e. Group II to V. Group I animals served as normal control (6 normal rats). All treatments started 2 days after Streptozotocin injection. Vehicles and the drugs were administered orally using oral gavage tube daily for 3 weeks. Blood samples were collected for the measurement of blood glucose level from the tail vein on 0, 7th, 14th and 21st day. Values of sample treated were compared with that of the standard group which was treated with metformin (100 mg/kg) [11].

Biochemical parameters:

Lipid profile:

On the 21st day, the animals were fasted for 12 h and then under mild ether anesthesia, animals were sacrificed and blood samples were collected. Blood was collected immediately into blood collection tubes for analysis of biochemical parameter viz. lipid profile (total cholesterol, triglycerides, HDL, LDL and VLDL). The collected blood samples were immediately centrifuged at 2500 rpm for 15 min. The serum separated was collected in fresh serum tubes and stored in refrigerator (2-4°C) after tightly capped[11]. The instrument at which biochemical parameters were performed was Siemens Dimension RxL Max (S.No.221816-AX) at United Diagnostics and Research, Allahabad.

Histopathological study:

The animals were sacrificed by cervical dislocation method. The animals were dissected and pancreas was exposed and perfused with cold saline phosphate buffer of pH 7.4 for histopathological examination[11]. Blood free pancreas was taken out and placed in containers separately filled with formalin (10% v/v). Incubation was done at 37°C under controlled conditions for histopathological estimation. The histopathology was performed at United Diagnostics and Research, Allahabad.

Statistical analysis:

The data obtained from animal experiments are expressed as mean ± SD (standard deviation). Statistical comparisons were performed by one-way ANOVA followed by Newmans-Keuls method using GraphPad Prim version 3.03 software for Windows, GraphPad Software, Inc. La Jolla, CA, USA.

RESULT

Physicochemical analysis:

The results of physicochemical analysis of crude powder of *Bauhinia variegata* flower found to be loss on drying 4.8% w/w, total ash 6.7% w/w, water soluble ash 4.6% w/w, acid insoluble ash 0.5% w/w, sulphated ash 2.5% w/w, methanol soluble extractive value 3.2% w/w.

Preliminary phytochemical screening:

Qualitative phytochemical tests showed presence of Carbohydrate, proteins and Amino acids, Flavonoid test and tannins and phenolic compounds.

In-Vitro Antioxidant Activity:

Table No. 1: Free radical scavenging activity of ethanolic extract of *Bauhinia Variegata* flower (BVE)

Drug	Concentration (µg/ml)	DPPH scavenging activity (% inhibition)	Hydrogen peroxide scavenging (H_2O_2) assay (% inhibition)
BE	25	52.36	54.12
BE	50	72.48	61.75
BE	100	86.60	68.47
BHT	25	83.13	68.32
BHT	50	86.84	71.56
BHT	100	91.63	73.42

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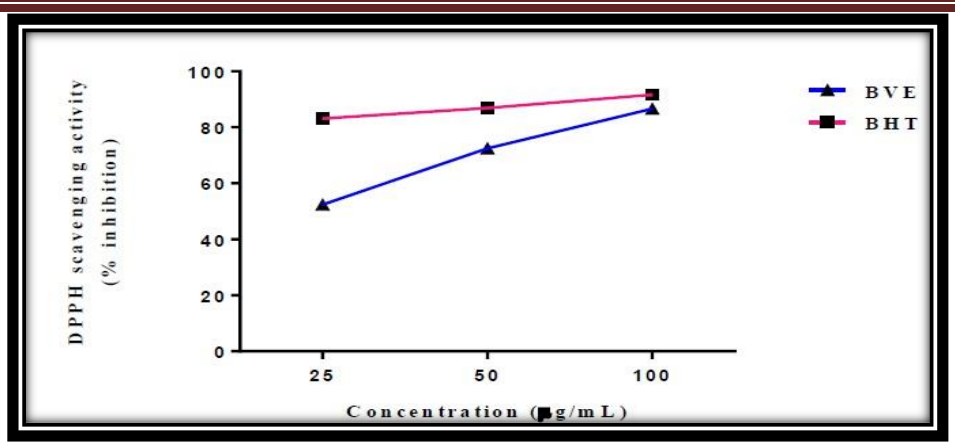

Fig. 1: Graph showing inhibition of DPPH radical by BVE and BHT

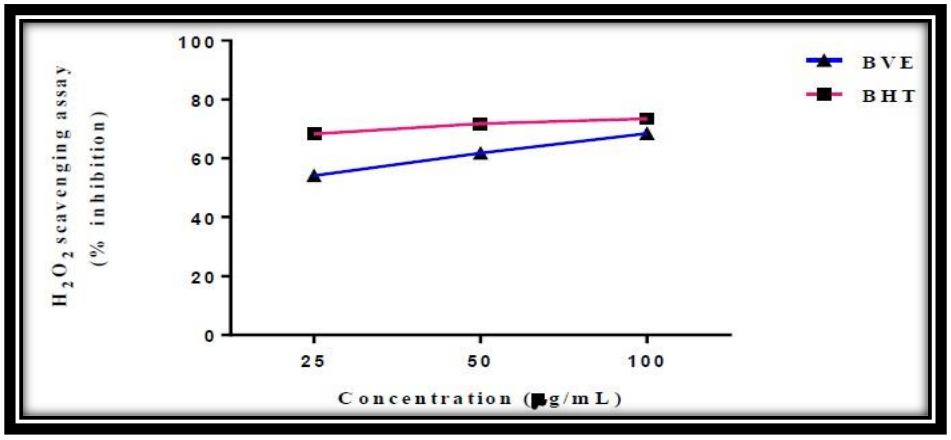

Fig. 2: Graph showing scavenging of hydrogen peroxide (H2O2) by BVE and BHT

Anti-hyperglycemic activity:

Table No. 2: Effects of ethanolic extract of *Bauhinia Variegata* flower (BVE) on blood glucose levels in streptozotocin-induced diabetic rats (mean ± SD)

Treatment (dose)	0 day (mg/dL)	7th day (mg/dL)	14th day (mg/dL)	21st day (mg/dL)
Normal Control	74.16±5.46	74.83±3.97	74.0±4.73	74.0±2.49
Diabetic Control	247.83±18.81z	237.16±17.12z	230.33±16.41z	224.50±14.54z
Metformin (100 mg/kg)	256.16±13.07	184.50±10.59***	127.66±16.88***	83.16±15.97***
BVE (200 mg/kg)	249.50±12.81	184.66±8.89***	149.33±12.04***	104.83±9.39***
BVE (400 mg/kg)	252.66±13.74	180.50±11.11***	123.66±12.14***	90.00±6.92***

*The data represents the mean ± SD for six rats per group. zp<0.001 as compared to normal control; ***p<0.001 as compared to diabetic control.*

Biochemical Parameters:
Lipid profile:

Table No. 3: Effect of ethanolic extract of *Bauhinia Variegata* flower on the total cholesterol, triglyceride, HDL, LDL and VLDL (mean ± SD)

Treatment (dose)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Normal Control	70.66±3.20	80.50±2.43	30.83±2.48	35.2±2.10	14.47±0.70
Diabetic Control	153.00±3.35z	130.5±3.02z	20.83±2.14	79.07±1.57z	30.60±0.67z
Metformin (100 mg/kg)	97.50±2.43	90.66±2.16***	34.05±1.87	38.67±2.83***	19.50±0.49***
BVE (200 mg/kg)	96.00±2.28	94.66±3.44***	32.50±2.88	42.97±6.01***	19.20±0.46***
BVE (400 mg/kg)	93.33±1.51	90.5±1.87***	32.00±2.83	39.83±2.88***	18.67±0.30***

*The data represents the mean ± SD for six rats per group. zp<0.001 as compared to normal control; ***p<0.001 as compared to diabetic control.*

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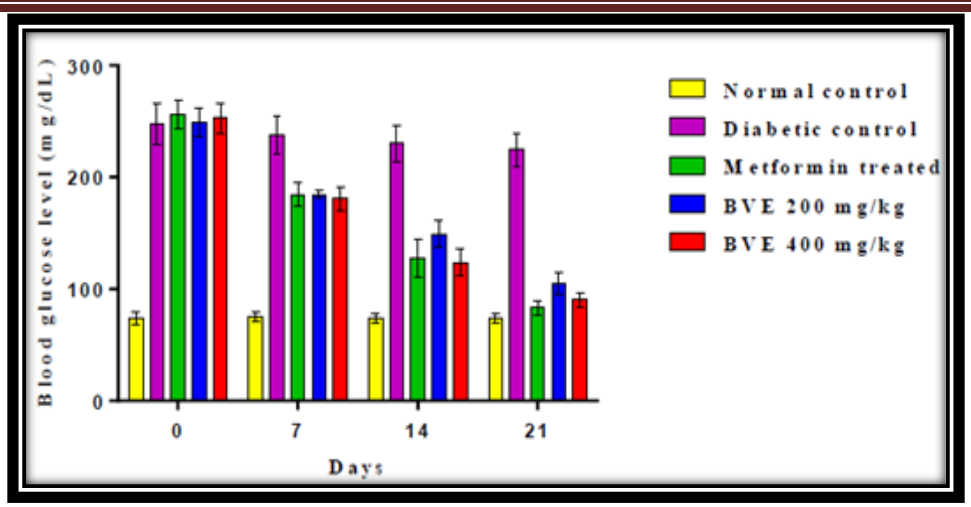

Fig. 3: Graph showing blood glucose levels in STZ-induced diabetic rats in 21 days

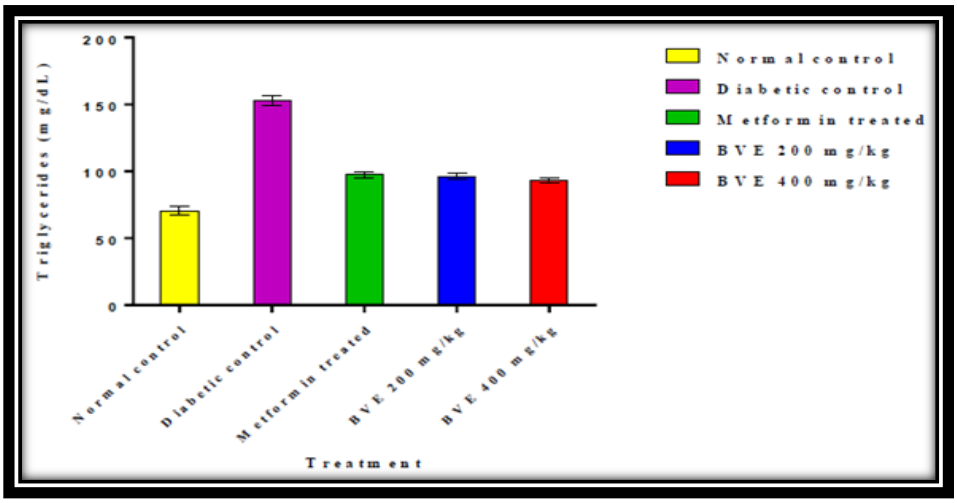

Fig. 4: Graph showing triglycerides levels in STZ-induced diabetic rats

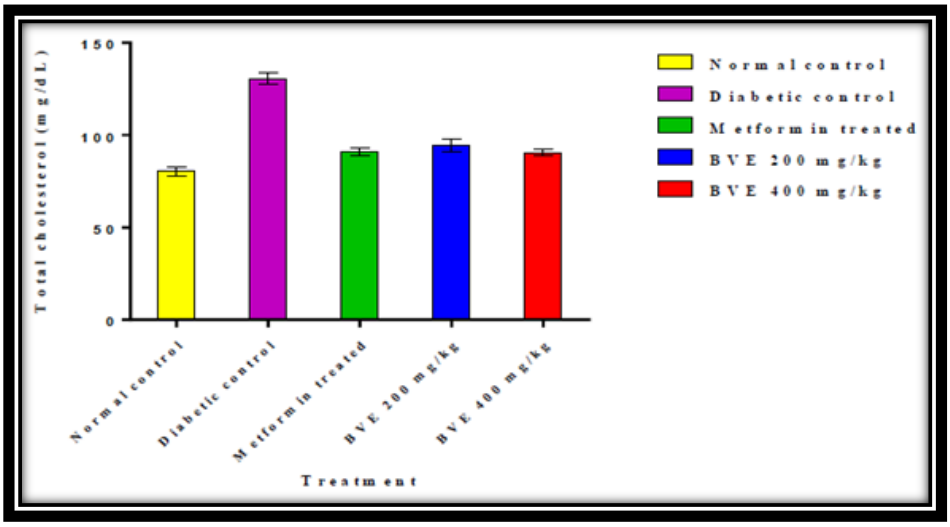

Fig. 5: Graph showing total cholesterol levels in STZ-induced diabetic rats

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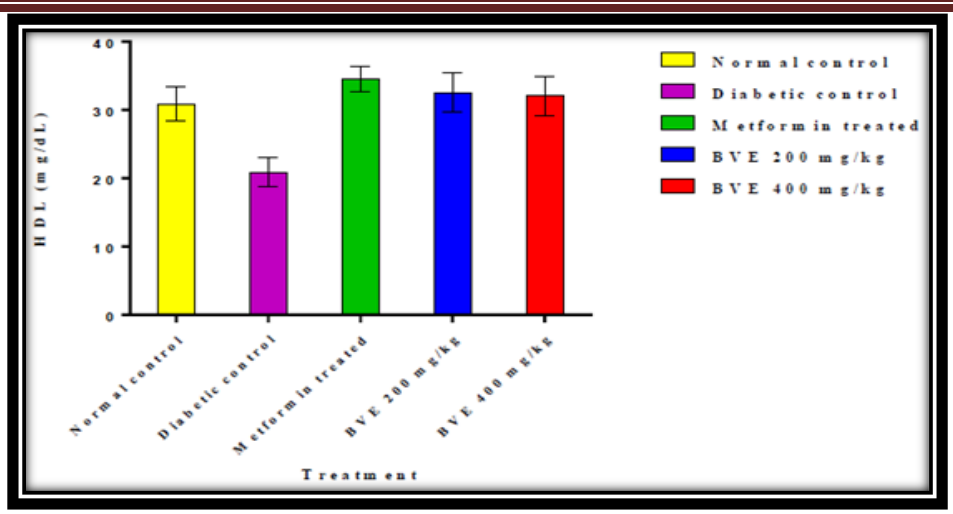

Fig. 6: Graph showing HDL levels in STZ-induced diabetic rats

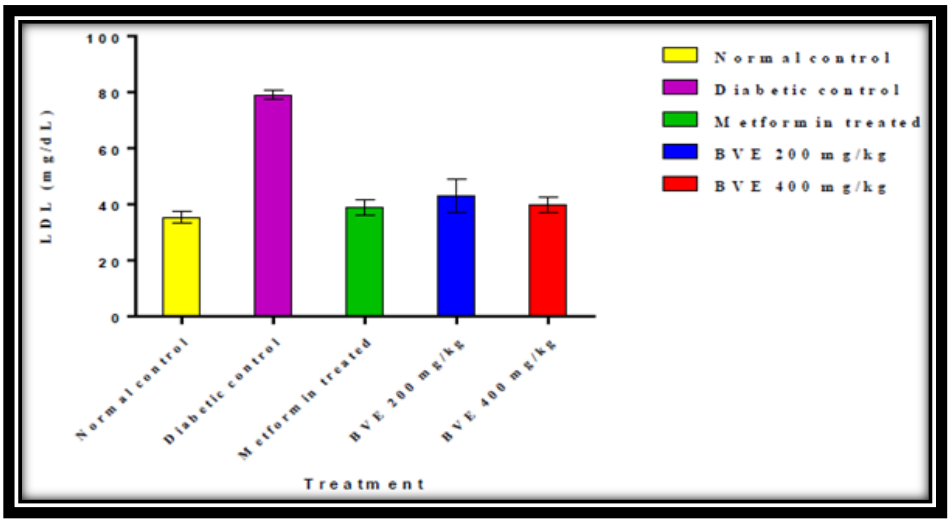

Fig. 7: Graph showing LDL levels in STZ-induced diabetic rats

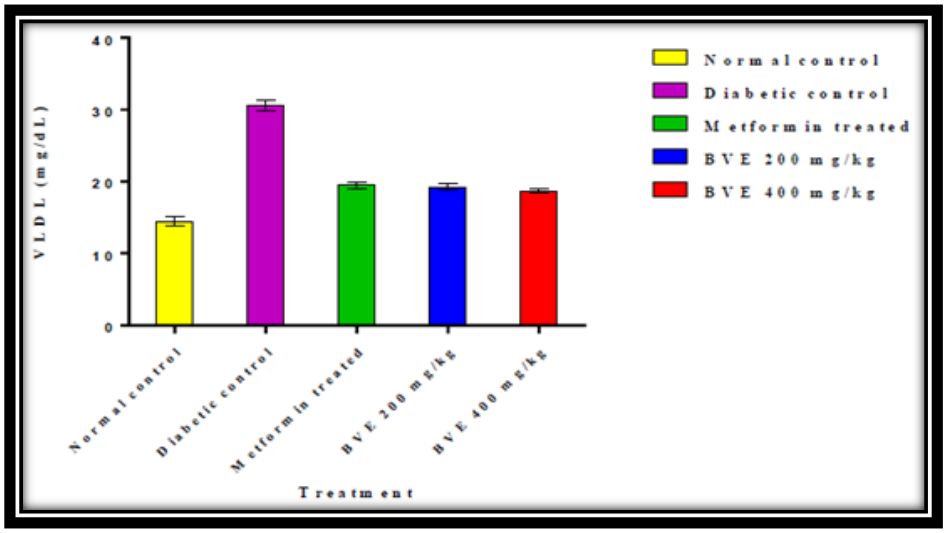

Fig. 8: Graph showing VLDL levels in STZ-induced diabetic rats

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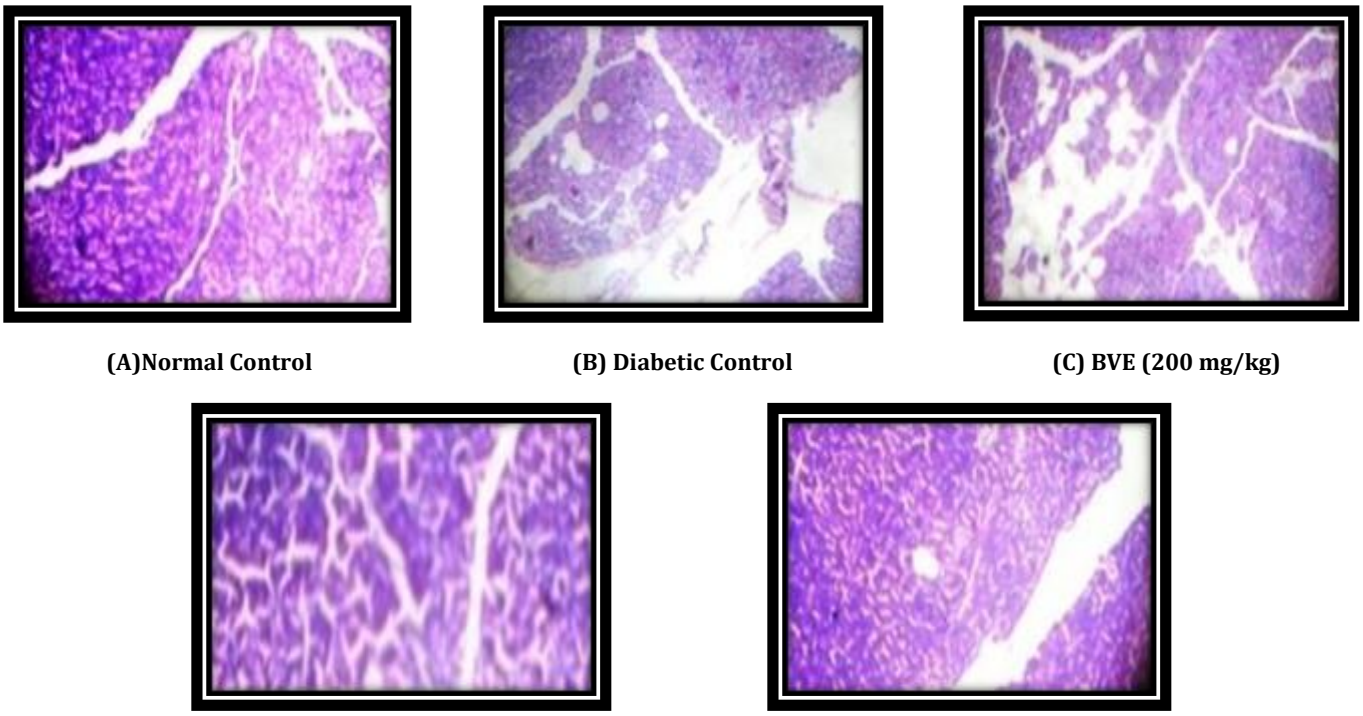
Histopathological Study:



(A)Normal Control **(B) Diabetic Control** **(C) BVE (200 mg/kg)**

(D) BVE (400 mg/kg) **(E) Metformin Treated**

Fig. 9: Histopathology of pancreas in experimental rats after 21 days of treatment

Microscopically examined pancreas section of normal control group (A) showed normal architecture with acini of serous epithelial cells. No fibrosis or inflammation was seen. Diabetic control group (B) showed diminition in the diameter of islets and islets cell population. BVE group at 200 mg/kg (C) showed normal islets but have a mild and diffused infiltrate of lymphocytes within the stroma. BVE group at 400 mg/kg (D) the islets showed depletion of acinar cells but there is no evidence of inflammation. Prominent recovery of the islets cell population is observed. The metformin treated group (E) showed that diameter and number of islets significantly recovered and the architecture was restored to approximately normal architecture.

DISCUSSION

Diabetes mellitus is a metabolic disease. It is characterized by hyperglycemia associated with impairment in insulin secretion/action along with altered carbohydrate, protein and lipid metabolism. To maintain the normal blood glucose levels the insulin functions either by suppression of glucose output from liver or by the stimulation of glucose uptake and its metabolism.

BVE produced hypoglycemia and improved glucose tolerance in normal rats in spite of counter regulatory factors avoiding reduction in blood glucose levels. Since it is reported that pale violet flowers of *Bauhinia variegata* contain anthrocynin glycosides i.e. cyanidine-3-glucoside, malvidin-3-glucoside, malvidin-3-diglucoside, and peonidin-3-diglucoside[12]. Therefore hypoglycemic activity of BVE and may be mediated via stimulation of the GLUT4 transporter activity in fat cells[13] and reducing retinol binding protein 4 (RBP4) expression[14]. The reduction in expression of RBP4 is correlated with decreased levels of TNF-α in white adipocytes as well, which is related to an anti-inflammatory state. These anti-inflammatory effects, via inhibiting c-Jun NH2-terminal kinase activation, can possibly protect fat cells from damages associated with a pro-diabetic diet[15,16]. STZ is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce DM in animals. It interferes with cellular metabolic oxidative mechanisms. The present data suggested that BVE significantly reduced hyperglycemia in single dose 21 days anti-hyperglycemic study. DM is often linked with abnormal lipid metabolism. The impairment of insulin secretion causes enhanced metabolism of lipids from the adipose tissue to the plasma. It has been demonstrated that insulin deficiency in diabetes leads to a variety of disruptive changes in metabolic and regulatory processes, due to which the lipids get accumulated. The extract supplementation resulted in significant attenuation in TG, TC, HDL, VLDL, and LDL. These effects may be due to low activity of cholesterol biosynthesis enzymes or low levels of lipolysis. BVE administration reinstated dyslipidemic markers to near-normal values. Histopathological analysis confirms anti-hyperglycemic potential of BVE.

CONCLUSION

The aim of this research work was to evaluate the ethanolic extract of flower of *Bauhinia variegata* (BVE) for anti-hyperglycemic and anti-oxidant activitiy. The physicochemical analysis and preliminary phytochemical screening were carried out. The oral acute toxicity study (LD_{50}) was carried out according to OECD guide lines 423 for extract (BVE), they were found to be safe up to 2000 mg/kg body weight. The evaluation

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of anti-hyperglycemic activity was carried out for ethanolic extract of flower of *Bauhinia variegata* at two doses 200 and 400 mg/kg of body weight. Anti-hyperglycemic activity was evaluated by streptozotocin-induced model with metformin as a standard drug. There was a significant reduction in blood glucose levels in extract treated animals when compared to diabetic control group. The BVE produced prominent anti-hyperglycemic activity in streptozotocin-induced model. The above study has been justified by histopathological analysis which confirms anti-hyperglycemic potential of BVE. There was a significant reduction in total cholesterol, triglycerides, LDL and VLDL level in extract treated animals when compared to diabetic control animals. The flavonoids present in the extract may be responsible for the anti-oxidant activity. The results show that BVE possesses significant anti-hyperglycemic and anti-oxidant activity. The anti-hyperglycemic activity might be attributed to stimulating effect on glucose utilization and anti-oxidant property.

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