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Antidiabetic, anti-hyperlipidemic and antioxidant activities of *Bauhinia variegata* flower extract

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ABSTRACT

To evaluate antidiabetic, *anti*-hyperlipidemic and antioxidant activities of ethanolic extract of *Bauhinia variegata* flower. Ethanolic extract of *B. variegata* was administered orally to Streptozotocin (STZ) induced diabetic rats once daily for 21 days. Blood glucose levels were estimated at day 0, 7, 14 and 21 by glucometer (one touch) and lipid profile and histopathological examination of isolated organs (kidney, liver and pancreas) were also estimated on 21 day. The anti-oxidant activity of *B. variegata* was evaluated by performing 1,1-diphenylpicrylhydrazyl (DPPH) and hydrogen peroxide scavenging (H_2O_2) assays. *B. variegata* flower extract showed reduction in blood glucose level (90.00 mg/dL) at highest dose 400 mg/kg when compared with diabetic control rats (224.50 mg/dL). The levels of triglycerides, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) were restored while administrating *B. variegata*. In addition, the percentage inhibition of *B. variegata* was 86.60% and 68.47% at 100 µg/ml for DPPH and H_2O_2 radicals, respectively, which was near to standard BHT i.e. 91.63% (DPPH) and 73.42% (H_2O_2). It can be concluded from the present study that *B. variegata* possesses significant antidiabetic, *anti*-hyperlipidemic and antioxidant activities.

1. Introduction

Diabetes mellitus is a major cause of morbidity and mortality in the adult population and having profound impact on the quality of the human life. It may results to hypoglycemia, hyperglycemia, renal dysfunction, and cardiovascular complications (Sreedharan, 2018). It has estimated that there was a global prevalence of 425 million people with diabetes in 2017 that is expected to rise to 629 million by 2045 (Forouhi and Wareham, 2018). It represents a set of autoimmune, metabolic and genetic disorders. Etiologically diabetes can be categorized into Type 1 and Type 2; generally, type 2 diabetes (T2DM) is found in 95% of population, and 5% have type 1 diabetes (T1DM) (American Diabetes Association, 2018). T1DM is characterized by absolute insulin deficiency resulting from an autoimmune destruction of pancreatic β-cells whereas T2DM is characterized by chronic hyperglycaemia due to defective insulin synthesis, secretion and/or action (Hurtado, 2018). Factors responsible for T2DM are obesity, age, ethnicity, family history, physical inactivity and diet.

The body generates free radicals due to metabolic processes while antioxidant systems are present in the body to disarm them. This homeostasis gets disturbed due to excess free radical production, depletion of antioxidants or both. Thus, when body's antioxidant system is inadequate, cells get exposed to high levels of free radicals i.e. reactive oxygen species (ROS), reactive nitrogen species (RNS) or reactive sulphur species (RSS); the condition called oxidative stress (Corrochano et al., 2018). Oxidative stress is responsible for cell injury such as protein and lipid peroxidation, DNA fragmentation, racemization or decarboxylation of amino acids, enzyme dysfunction, breakdown of carbohydrates and aggravates various chronic diseases like diabetes, cancer, rheumatism and heart diseases (Li et al., 2015). Excess concentration of glucose in blood is one of the most important causes of diabetes secondary disorders like angiopathy, cataract, neuropathy, deficiency in the antioxidant defence system and lipid profile disorders (Ahangarpour et al., 2019). There is a strong consistent relationship between oxidative stress-induced hyperglycemia and progression of diabetic complications in patients with diabetes mellitus (Ozkaya et al., 2011).

Natural antioxidants obtained from plant matrices play an important role in protecting the body against damage from reactive oxygen species (Wang et al., 2018). *Bauhinia* is large genus comprises of 300–350 species of different trees and plants mainly found in tropical regions (Farag et al., 2015). Extracts from leaves and stem-bark of *Bauhinia* possess active constituents used for the treatment of diabetes, inflammation, hyperlipidemia, infections, pain, HIV, wound and

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bacterial infections (Cagliari et al., 2018). *Bauhinia variegata* Linn. belongs to the family Caesalpiniaceae. Wide range of glycosylated and aglycone flavonoids have been isolated from *Bauhinia variegata* L., reported in several studies (Reddy et al., 2003) (Mohamed et al., 2009). Several studies reported that genus *Bauhinia* possesses antioxidant and anti-diabetic activities due to presence of polyphenol and flavonoid contents (Menezes et al., 2007) (Ahmed et al., 2012). Thus, the present study was designed to determine antidiabetic, antihyperlipidemic and antioxidant activities of *B. Variegata* flower extract.

2. Materials and methods

2.1. Drugs and chemicals

Streptozotocin (STZ) purchased from Sigma Aldrich Co., USA. Metformin was obtained from Sun Pharmaceutical Industries Ltd., Vadodara, Gujarat. Hydrochloric acid, Potassium hydroxide, Ferric chloride, Iodine, Nitric acid, Sulphuric acid, Lead acetate, Zinc chloride, Tannic acid, Potassium dichromate, Sodium hydroxide, tri-Sodium Citrate, Copper sulphate were obtained from Merk specialties Pvt. Ltd., Mumbai. All other chemicals and reagents were of analytical grade and distilled water was used throughout the study.

2.2. Collection, identification and extraction

The flowers of *Bauhinia variegata* were collected in the month of January 2015 from the local surroundings of Allahabad, Uttar Pradesh. It was identified by Dr. G.P. Sinha, Scientist-E/Head of Office, Botanical Survey of India, CRC, Allahabad (U.P.). A plant specimen was deposited in the herbarium of Botanical Survey of India, CRC, Allahabad (U.P.) for future reference (BSI/CRC/Tech./2014–15/571). The air dried and powdered flowers (1.2 kg) of *B. variegata* 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.

2.3. In vitro antioxidant activity

2.3.1. DPPH scavenging activity

1,1-diphenylpicrylhydrazyl (DPPH) radical scavenging activity was evaluated by method according to Argolo et al. (2004) with slight modification. 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:

$$\text{Scavenging activity (\%)} \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{X } 100$$

Where As− Absorbance of sample, Ac− Absorbance of control.

2.3.2. Hydrogen peroxide scavenging (H_2O_2) assay

Hydrogen peroxide scavenging (HPS) ability was determined by Elizabeth and Rao (1990) with slight modification. 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 ethanolic extract of *B. variegata* (20–60 µg/mL) was mixed in distilled water and then hydrogen peroxide was added. The absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows:

$$\text{Scavenging activity (\%)} \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{X } 100$$

Where As− Absorbance of sample, Ac− Absorbance of control.

2.4. In vivo evaluation

2.4.1. Experimental animals

Adult male Wistar rats 7–8 weeks old, weighing 150–200 gm were obtained from inbred animal house of C.D.R.I., Lucknow. The animals were housed in polypropylene cages in standard environmental conditions, 12 h light and 12 h dark cycle at 25 ± 2 °C. Animals were fed with standard pelleted diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) animals (UIP/IAEC/April-2015/03).

2.4.2. Acute toxicity study

A toxicity study was performed according to Organization 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) was administered orally by gavage to the animals and animals were initially monitored continuously for any adverse effects for 4 h and then monitored at 1-h intervals. They were later monitored twice daily for any abnormal changes throughout the study period (which lasted 14 days). The lethal median dose (LD50) of *B. variegata* was found non-toxic up to 2000 mg/kg.

2.4.3. Experimental protocol

Animals were divided into five groups each containing six animals. Group I was control group received saline solution, Group II was diabetic control group received STZ only, Group III was reference group received metformin 100 mg/kg, Group IV and V were treated group received 200 and 400 mg/kg of ethanolic extract of *B. variegata*.

2.4.4. Induction of diabetes

Diabetes was induced by intraperitoneal injection of freshly prepared solution of Streptozotocin (50 mg/kg, body weight) in 0.1 M citrate buffer (pH 4.5) in a volume of 1 ml/kg. STZ-injected animals exhibited massive glycosuria and hyperglycemia within 2 days. 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 diabetic and used in the experiment as reported by Elbanna et al. (2017).

2.4.5. Antidiabetic activity of B. variegata

All treatments were started after 2 days of STZ 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, 7, 14 and 21 day. The blood glucose level was determined by glucometer (one touch). Values of sample treated were compared with that of the diabetic control group.

2.4.6. Anti-hyperlipidemic activity of B. variegata

On day 21, animals were fasted for 12 h and then under mild ether anesthesia, they were sacrificed and blood samples collected into blood collection tubes for analysis of lipid profile (total cholesterol, triglycerides, HDL, LDL and VLDL) (Siemens Dimension RxL Max). 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 as reported by Thiruvenkatasubramaniam and Jayakar (2010).

2.4.7. Histopathological study

The animals were sacrificed by cervical dislocation method and vital organs such as liver, kidney and pancreas were dissected, exposed and perfused with cold saline phosphate buffer of pH 7.4 for histopathological examination. Tissues were placed in containers separately filled with formalin (10% v/v). Incubation was done at 37 °C under controlled conditions for histopathological estimation.

Table 1
Antioxidant activity of ethanolic extract of *Bauhinia variegata*.

Antioxidant activity	*B. variegata* (µg/mL)			Butylated hydroxytoluene (µg/mL)		
	25	50	100	25	50	100
DPPH scavenging activity (% inhibition)	52.36	72.48	86.60	83.13	86.84	91.63
H_2O_2 scavenging activity (% inhibition)	54.12	61.75	68.47	68.32	71.56	73.42

2.4.8. Statistical analysis

Data are represented as mean ± SD (n = 6). Statistical comparisons were performed by one-way ANOVA followed by Newmans-Keuls method using GraphPad Prism version 3.03 software, Inc. La Jolla, CA, USA.

3. Result and discussion

3.1. Antioxidant activity

DPPH free radical scavenging activity and Hydrogen peroxide scavenging (H_2O_2) activity are represented in Table – 1. *Bauhinia variegata* flower extract significantly reduced DPPH and H_2O_2 activity in a dose dependent manner. DPPH and H_2O_2 radical scavenging activity at highest dose (100 µg/mL) were 86.60 and 68.47 µg/mL, respectively in comparison to standard Butylated hydroxytoluene i.e. 91.63 and 73.42 µg/mL, respectively. DPPH radical get reduced to corresponding hydrazine. The purple DPPH radical upon reaction with donors of hydrogen changes to yellow color (Kedare and Singh, 2011). 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 (Phaniendra et al., 2015). Antioxidant activity of *B. variegata* found in this study is also supported by the work of Argolo et al. (2004). DPPH has been used as a reagent for screening of antioxidant activity of small molecules (Soler-Rivas et al., 2000). It has also reported by many researchers that antidiabetic activity may be associated with antioxidant properties (McCune and Johns, 2002) (Sabu and Kuttan, 2002).

3.2. Antidiabetic activity of B. variegata

The effect of oral administration of ethanolic extract of *Bauhinia variegata* on blood glucose level of animals in 21 days is shown in Table – 2. *B. variegata* flower extract showed reduction in blood glucose level (90.00 mg/dL) at highest dose 400 mg/kg when compared with diabetic control (224.50 mg/dL) and normal control groups (74.00 mg/dL). However, reference group reducing activity was slightly greater than test groups i.e. 83.16 mg/dL. The results obtained in this study is supported by the study of Frankish et al. (2010), they reported that ethanolic extract of *B. variegata* showed insulin secretion in insulin secreting cell line. It has also reported that insulin like proteins have isolated from leaves of *B. variegata* that possess blood glucose lowering activity (Rashid, 2014). Antidiabetic activity of *B. variegata* extracts was reported in many studies that also justify our results (Khot et al., 2012) (Kulkarni and Garud, 2015).

Table 3
Effect of ethanolic extract of *Bauhinia variegata* lipid profile of animals.

Groups	Treatment	Triglycerides (mg/dL)	TC (mg/dL)
NC	0.5 ml	70.66 ± 3.20	80.50 ± 2.43
DC	0.5 ml	153.00 ± 3.35z	130.5 ± 3.02z
Metformin	100 mg/kg	97.50 ± 2.43	90.66 ± 2.16***
BV-1	200 mg/kg	96.00 ± 2.28	94.66 ± 3.44***
BV-2	400 mg/kg	93.33 ± 1.51	90.5 ± 1.87***

The data represents the mean ± SD for six rats per group. p < 0.001 as compared to normal control; ***p < 0.001 as compared to diabetic control. NC = Normal control, DC = Diabetic control, BV = *B. variegata* extract, TC = Total cholesterol.

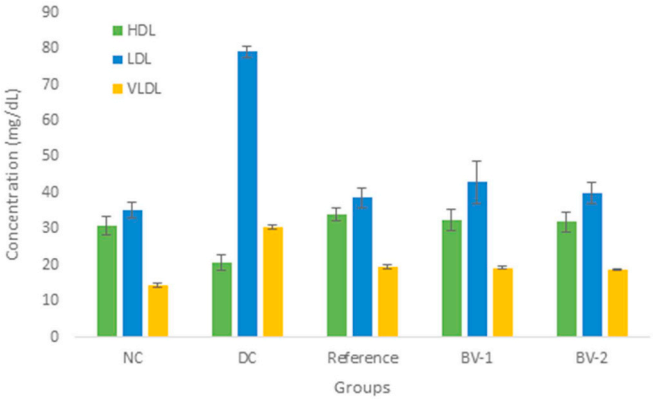

Fig. 1. Lipoprotein levels of *B. variegata* extract
NC = Normal control, DC = Diabetic control, BV = *B. variegata* extract, LDL = Low density lipoprotein, HDL = High density lipoprotein, VLDL = Very low density lipoprotein.

3.3. Anti-hyperlipidemic activity of B. variegata

The effect of oral administration of ethanolic *B. variegata* on lipid profile of animals in 21 days is presented in Table 3. *B. variegata* extract restored lipid levels in animals, Triglyceride and Total cholesterol levels of BV-2 were 93.33 and 90.5 mg/dL, respectivley at day 21 in comparison to diabetic control (153.00 and 130.5 mg/dL, respectively) and normal control groups (70.66 and 80.50 mg/dL, respectivley), which

Table 2
Effects of *B. variegata* extract on blood glucose levels in Streptozotocin-induced diabetic rats.

Groups	Treatment	Days			
		0	7	14	21
		Concentration (mg/dL)			
NC	0.5 ml	74.16 ± 5.46	74.83 ± 3.97	74.0 ± 4.73	74.0 ± 2.49
DC	0.5 ml	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***
BV-1	200 mg/kg	249.50 ± 12.81	184.66 ± 8.89***	149.33 ± 12.04***	104.83 ± 9.39***
BV-2	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. p < 0.001 as compared to normal control; ***p < 0.001 as compared to diabetic control. NC = Normal control, DC = Diabetic control, BV = *B. variegata* extract.

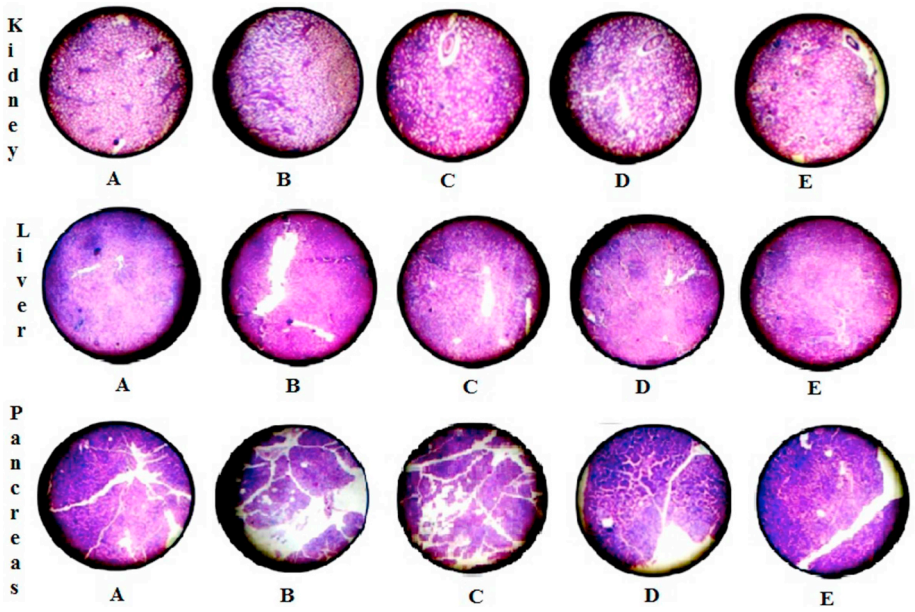

Fig. 2. Histopathological study of *B. variegata* flower extract
A = Normal control, B = Diabetic control, C = BV-1, D = BV-2, E = Reference group.

was near to reference group, metformin (97.50 and 94.66 mg/dL, respectively) as shown in Table – 3. Likewise, lipoprotein levels of BV-2 was greater than BV-1 i.e. 39.83 and 18.67 mg/dL for LDL and VLDL, respectively in comparison to diabetic control group i.e. 79.07 and 30.60 mg/dL for LDL and VLDL, respectively (Figure – 1). However, HDL level was increased with the treatment of BV-2 i.e. 32 mg/dL in comparison to diabetic control group (20.83 mg/dL). The results obtained in the study was favored by a study in the authors reported that methanolic extract of *B. variegata* leaves exhibited antihyperlipidemic activity (Kumar et al., 2011). Several reported works have shown that HDL cholesterol is inversely proportional to total cholesterol and other lipoproteins. Reduction in plasm HDL cholesterol may lead to atherosclerosis results in ischaemic heart disease (Dhulasavant et al., 2010). It has reported that flavonoids present in *B. variegata* increases HDL concentration and decrease LDL and VLDL levels in hypercholesteremic rats (Patel et al., 2009).

3.4. Histopathological studies

Figure – 2 shows histopathological condition of all treated groups. Histopathological studies revealed that *B. variegata* normalized damaged tissues and reduced inflammation. Individual tissues of kidney, liver and pancreas were observed after treatment. It was found that kidney of extract treated group (400 mg/kg) showed prominent recovery of glomeruli in comparison to normal control group where glomeruli appeared normal. Similarly, in liver and pancreas also extract treated group (BV-2 mg/kg) exhibited portal tract with normal lobular architecture with minimum fatty changes and the islets showed depletion of acinar cells but there is no evidence of inflammation, respectively.

4. Conclusion

It can be concluded from the study that flower extract of *B. variegata* (Linn.) resulted in significant reduction of blood glucose level, cholesterol, triglyceride, LDL, VLDL, free radicals level but also increases the HDL level. The radical scavenging activity of *B. variegata* was 86.60% (DPPH) and 68.47% (H2O2) at 100 µg/mL relative to BHT i.e. i.e. 91.63% (DPPH) and 73.42% (H2O2). Studies on the isolated flower extract are needed to elucidate mechanisms by which *B. variegata* exerted protective effects on diabetes, hyperlipidemia and oxidative stress.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101142.

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