

Antidiabetic and Antioxidants Activity of *Boerhavia diffusa* L. in Alloxan Induced Diabetic Rats

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Abstract

The present study is aimed to gain an insight about the effects of ethanolic extract of *Boerhavia diffusa* L roots against alloxan induced diabetic rats. Rats were administered 200 mg/kg/day and 500 mg/ kg/day p.o or vehicle [0.3% CMC] for a duration of 26 days. Diabetes was induced by administering a single dose of alloxan 120 mg/kg.

Blood glucose and antioxidant levels were checked. Ethanolic extract of *B. diffusa* exhibited significant decrease in blood glucose level and upregulation of antioxidant status in diabetic animals when compared to positive control diabetic rats. Hence, we propose in the manuscript that *Boerhavia diffusa* L. possess antidiabetic and antioxidant activities.

Keywords: Alloxan; *Boerhavia diffusa*; Antidiabetic; Antioxidant

Introduction

Diabetes is a metabolic disease associated with increase in the blood glucose level. Till now there is no effective drug therapy to cure diabetes. Allopathic drugs available in market for the management of diabetes are either costly or ineffective and possess obnoxious side effects [1]. A lot of research is being done to find a safe and effective antidiabetic drug [2]. WHO has also advised to screen plant for the conditions where allopathic medicine is either ineffective or not safe [3]. Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyruacil) is the most commonly used chemical to induce diabetes in experimental animals. Alloxan induces diabetes by destroying β - cells in pancreas when administered intravenously, intraperitoneal or subcutaneously [4]. Herbal drugs have been reported to be effective in the treatment of [5]. Secondary metabolites like flavonoids, gallotannins, amino acids and other related polyphenols present in the plants are responsible for the therapeutic activities like antidiabetic, antihyperlipidemic and antioxidant activity [6,7]. *Boerhavia diffusa* L. has been widely used in Indian system of medicine in treatment of various diseases [8]. It has been used as a diuretic, anti-inflammatory, anti-oxidative, anti-arthritic, spasmolytic, antibacterial, analgesic, immunity Booster, and anti-ageing [9]. The present study was carried out to find antidiabetic and antioxidant activity of *Boerhavia diffusa*.

Materials and Methods

Plant collection and extraction

Fresh roots of *Boerhavia diffusa* were obtained from Jalandhar, Punjab, India. The plant was authenticated at Department of Pharmacognosy, CT Institute of Pharmaceutical Sciences. Shade-dried and pulverized roots 1.5 kg were successively extracted with 95% ethanol employing continuous hot extraction process with soxhlet apparatus at a temperature below boiling point of the solvent for 6 hours. The extract was final dried in high vacuum and a yield of 105g of extract was obtained.

Phytochemical screening

The ethanolic extract of *Boerhavia diffusa* was subjected to various qualitative test to detect the presence of secondary metabolites like tannins, alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins and steroids [10].

Estimation of total phenolic content

Total phenolic content was assessed by Folin-Ciocalteu reagent using gallic acid as reference. The extract was oxidized with 10% Folin-Ciocalteu reagent (Merck, Germany), and was neutralized [11].

Animals

Wistar albino rats (200 - 250g) were obtained from Panacea Biotec Ltd, Lalru (140501), India. Animals were housed in colony cages in a well-ventilated room under an ambient temperature of $22 \pm 3^\circ\text{C}$ and 40 - 65% relative humidity, with artificial photoperiod (12-h light/12-h dark cycle). They were provided with standard rodent pellet diet (Nutrilab Rodent, Tetragon Chemie, India) and purified water *ad libitum* (RIOS, USA). Experimental animals were acclimatized for 7 days to the laboratory conditions prior to experimentation. Institutional Animal Ethical Committee (IAEC-CTIPS/2015/VII/0050 (PCL-M) approved the study protocol.

Chemicals

Alloxan monohydrate was purchased from Loba Chemie Ltd, India. Glibenclamide was purchased from Sanofi India Ltd. Diagnostic kits were purchased from the ERBA diagnostic Mannheim GmbH India. All the chemicals used in the study were of analytical grade and were procured from Sigma Aldrich, India.

Induction of diabetes

Rats were fastened for 12 hours before induction of diabetes as done by Joy and Kuttan (1999) with a minor modification. Alloxan was freshly prepared in 0.5% CMC and was administered at a dose of 120 mg/kg, intraperitoneally in rats. Glucose solution (5% w/v) was immediately administered orally to alloxan treated rats in order to prevent transient hypoglycemia [4]. Confirmation of diabetes was confirmed by measuring of blood glucose after four days of administration of alloxan. Rats with blood glucose above 220 mg/dl were considered for the study.

Experimental designs

Rats were randomized into 5 groups with each group containing six animals. Solvent 0.5% CMC/EtBD (200 and 500 mg/kg)/glibenclamide (GLB) (10 mg/kg) was administered orally for a period of 26 days.

- Normal control: 0.5% CMC p.o.
- Positive control: Diabetic rats received 0.5% CMC p.o
- GLB: Diabetic rats received GLB (10 mg/kg/day p.o.)
- EtBd 200: Diabetic rats received Et BD (200 mg/kg, p.o.)
- EtBd 500: Diabetic rats received Et BD (500 mg/kg, p.o.)

Biochemical measurements

The animals were put on fasting on 26th day, blood was collected from retro orbital plexus under anesthesia and centrifuged at 2500 rpm to get the serum. Biochemical parameters like serum blood glucose [2], thiobarbituric acid reactive substances (TBARS) [12], reduced glutathione (GSH) [13], superoxide dismutase (SOD) [14], catalase [15] were determined. All the animals were sacrificed under anesthesia on completion of the study.

Statistics

Statistical analysis was performed using GraphPad Prism, 4.03 San Diego, U.S.A. Data were expressed as mean \pm SEM. Mean difference was analyzed by one-way ANOVA (ANOVA) followed by Duncan's multiple range test (DMRT). $P \leq 0.05$ was fixed as the statistical significance criterion.

Results and Discussion

Preliminary phytochemical studies

The phytochemical screening has confirmed the presence of secondary metabolites alkaloids, flavonoids carbohydrates, anthraquinones and to some lesser extent terpenoids and tannins in *Boerhavia diffusa* roots. The complete details of the presence of the phytochemicals in crude extract were presented in table 1.

Phytoconstituents	Name of the test	Result
Alkaloids	Hager's test	+++
Anthraquinones	Chloroform layer test	+++
Cardiac glycosides	Killer- Killani's test	-
Flavonoids	Modified ammonia test	+++
Reducing sugars	Fehlings test	+++
Saponins	Frothing test	-
Steroids	Salkowski test	-
Tannins	FeCl ₃ Test	++
Terpenoids	Modified salkowski test	++
Amino acids	Ninhydrin test	-

Table 1: Phytochemical compositions of ethanolic *B. diffusa* root extract.
+++ : Highly Present; ++ : Moderately Present; + : Slightly Present; - : Absent.

Total phenolic content

The total phenolic content of *B. diffusa* extract was calculated as 153.27 (in mg/g, Gallic acid equivalents).

Effect on blood glucose in diabetic rats

The rats indicated significant ($P < 0.001$) rise in blood glucose upon administration of alloxan when compared with normal control rats. Alloxan destroys the insulin secreting beta cells in pancreas as a result it affects the uptake of the glucose in tissues. Alloxan generates free radicals which damage the β cells [16]. The diabetic rats showed a significant ($P < 0.001$) decrease in the blood glucose upon administration of EtBD and GLB (Table 2). The mechanism of the antidiabetic activity of *Boerhavia diffusa* may be because of antioxidant phytochemicals like flavonoids, polyphenols, and tannins, which kills the free radicals. It also promotes the release of the insulin Our results agree with the result stated by Chude., *et al* [17]. A progressive metabolic connection with increase in glycolysis and decrease in gluconeogenesis is activated by EtBD proposes a promising biological mechanism by which glucose equilibrium is controlled. The action of EtBD was found to be better than GLB [18].

Date	Normal	Positive control	GLB	EtBd 200	EtBd 500
0	60.2 ± 2.09	59.45 ± 1.89	57.88 ± 2.12	58.4 ± 1.92	60.11 ± 2.32
4	65.33 ± 1.76	253.5 ± 6.29 ^a	262.67 ± 8.71 ^a	260.83 ± 5.70 ^a	259.33 ± 2.41 ^b
40	61.5 ± 1.87	305.52 ± 7.34 ^a	73.16 ± 2.57 ^{b,c}	92.67 ± 5.19 ^a	85.33 ± 3.51 ^{b,c}

Table 2: Effect on blood glucose in diabetic rats.

All data are expressed as mean ± SEM (n = 6). Vehicle: 0.5% CMC.

^aP < 0.001 diabetic control compared with control.

^bP < 0.001 EtBd 200 mg/kg and glibenclamide 10 mg/kg compared with diabetic control.

^cP < 0.001 EtBd 500 mg/kg or glibenclamide 10 mg/kg compared with EtBd 200 mg/kg.

Effect on antioxidants in diabetic rats

Marked increase in TBARS and hydroperoxides was found in the liver and kidneys of the diabetic rats. Administration of EtBd 200, EtBd500 and GLB significantly reduced TBARS and hydroperoxides as indicated in table 3. Glutathione is a tripeptide antioxidant existing in most of the cells [19]. Downregulation of GSH has been considered to indicate oxidative stress in diabetes [20]. In addition to its antioxidant activity, it kills free radicals and repairs the cellular damage done by radicals [21,22]. EtBd 200 and EtBd500 and GLB improved the levels of GSH in diabetic rats. A fall in GSH was observed in diabetic control animals.

Groups		TBARS	Hydroperoxide	Reduced glutathione
		(mM/100mg tissue)	(mg/100 mg tissue)	
Normal	Liver	0.85 ± 0.002 ^a	76.21 ± 2.81 ^a	46.2 ± 2.06 ^a
	Kidney	1.62 ± 0.007 ^{ab}	55.32 ± 2.21 ^a	33.12 ± 2.14 ^a
Positive control	Liver	2.09 ± 0.04 ^b	105.4 ± 103.2 ^b	22.14 ± 0.81 ^b
	Kidney	1.61 ± 0.03 ^c	82.31 ± 80.21 ^c	20.75 ± 0.72 ^b
GLB	Liver	1.72 ± 0.06 ^d	89.72 ± 4.32 ^d	40.12 ± 1.43 ^c
	Kidney	1.91 ± 0.11 ^d	62.45 ± 3.21 ^d	26.43 ± 1.20 ^c
EtBd 200	Liver	1.36 ± 0.07 ^c	88.32 ± 3.25 ^c	43.2 ± 2.03 ^c
	Kidney	1.5 ± 0.03 ^{bd}	67.12 ± 2.19 ^d	28.63 ± 2.15 ^c
EtBd 500	Liver	1.82 ± 0.05 ^c	82.11 ± 4.72 ^c	40.21 ± 1.80 ^c
	Kidney	1.9 ± 0.11 ^{bd}	62.79 ± 3.01 ^d	24.36 ± 1.47 ^c

Table 3: Effect of EtBd on TBARS, hydroperoxide and reduced glutathione in liver and kidneys in diabetic rats.

Values not sharing a common superscript letter differ significantly at P<0.05 (DMRT) Duncan procedure,

Range for level 2.91, 3.07, 3.17, 3.22.

SOD is a key shield enzyme which catalyzes the dismutation of superoxide radicals [23]. Catalase, a hemeprotein catalyzes the reduction of H₂O₂ and guards the tissue from hydroxyl radicals which can damage the tissues [24]. Downregulation of SOD and catalase can produce harmful effects due to increase of free radicals. Administration of EtBd 200,500 and GLB augmented the activity of SOD and catalase in diabetic rats.

GPx an enzyme with selenium, and GST catalyze the reduction of H₂O₂ and hydroperoxides to non-toxic products their activity was found to decrease in diabetic rats [25]. Decrease in the levels is found to be responsible for accumulation of toxic products which lead to

oxidative changes. Administration of EtBd200,500 and GLB enhanced the levels of GPx and GST in diabetic animals as indicated in table 4. The powerful free radical scavenging and upregulating the antioxidant status by *Boerhavia diffusa* may be due to secondary metabolites which protected against oxidative damage in alloxan induced diabetes [18].

Groups		Glutathione-S-transferase ^A	Superoxide dismutase ^B	Catalase ^C	Glutathione peroxidase ^D
Normal	Liver	6.23 ± 0.42 ^a	6.42 ± 0.31 ^a	73.14 ± 4.25 ^a	6.42 ± 0.32 ^a
	Kidney	5.47 ± 0.22 ^a	15.01 ± 0.74 ^a	34.25 ± 2.05 ^a	4.52 ± 0.27 ^a
Positive control	Liver	3.29 ± 0.16 ^b	4.23 ± 0.15 ^b	45.97 ± 1.99 ^b	4.49 ± 0.18 ^b
	Kidney	2.71 ± 0.9 ^b	10.3 ± 0.47 ^c	24.12 ± 0.79 ^b	2.30 ± 0.14 ^c
GLB	Liver	5.02 ± 0.24 ^d	5.49 ± 0.2 ^d	64.17 ± 2.80 ^c	5.23 ± 0.20 ^c
	Kidney	3.83 ± 0.11 ^c	11.84 ± 0.53 ^c	26.83 ± 1.34 ^d	3.67 ± 0.18 ^d
EtBd 200	Liver	4.92 ± 0.29 ^d	5.82 ± 0.22 ^c	65.36 ± 2.87 ^c	5.36 ± 0.23 ^c
	Kidney	5.01 ± 0.23 ^d	13.92 ± 0.6 ^d	25.6 ± 1.58 ^c	3.92 ± 0.21 ^a
EtBd 500	Liver	5.67 ± 0.32 ^d	5.67 ± 0.18 ^c	66.31 ± 3.06 ^c	5.7 ± 0.19 ^c
	Kidney	4.75 ± 0.21 ^d	13.75 ± 0.56 ^d	29.42 ± 1.62 ^c	4.21 ± 0.21 ^a

Table 4: Effect of EtBd on Glutathione-S-transferase, Superoxide dismutase, Catalase and Glutathione peroxidase in liver and kidneys in diabetic rats.

Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Duncan procedure, Range for level 2.91, 3.07, 3.17, 3.22.

A= μ moles of CDNB-GSH conjugate formed/min/mg protein.

B= One unit of activity was taken as enzyme reaction which gave 50% inhibition of NBT reduction in one min.

C= μ mole of H_2O_2 consumed/min/mg protein.

D= μ g of GSH consumed/min/mg protein.

Conclusion

The ethanolic extract of *Boerhavia diffusa* has possess antidiabetic and antioxidant activity. Further studies need to be done to elucidate the mechanism of action which will help in projecting this plant as an therapeutic target in diabetics research

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Conflict of Interest Statement

Authors declare no conflict of interest.

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