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Research Article

Effect of Ethanolic Extract of Leaves of *Boerhavia diffusa* on Carbohydrate Metabolising Enzymes, Renal and Hepatic Markers in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The present study was formulated to evaluate the effect of *Boerhavia diffusa* by using *in vivo* methods in normal and streptozotocin (STZ) induced diabetic rats. Diabetes mellitus was induced by single intraperitoneal injection of STZ (60 mg/kg body weight) in male Wistar rats. Various parameters such as carbohydrate metabolizing enzymes, renal and hepatic markers were studied in the normal and diabetes induced rat. The ethanolic extract of leaves of *Boerhavia diffusa* (ELBD) at a dose of 500 mg/kg body weight and glibenclamide, a standard oral hypoglycemic drug at a dose of 10 mg/kg body weight were administered orally to the diabetic induced groups for 45 days. The diabetic induced groups treated with the ELBD restored the elevated levels of renal and hepatic markers to normal levels. It also altered the activities of carbohydrate metabolizing enzymes to near normal. Thus, from the present study it can be concluded that the ethanolic extract of leaves of *Boerhavia diffusa* possess a favourable antidiabetic effect.

Keywords: *Boerhavia diffusa,* hypoglycemic, glibenclamide, intraperitoneal.

INTRODUCTION

Diabetes mellitus a common manifestation of metabolic disorder occurs due to high consumption of carbohydrates, fats and proteins. Hyperglycaemia an associate with hyperlipidemia in the late phase of life is also prone to diabetes. It is a complex endocrine disorder resulting in macro (heart attack, stroke and peripheral vascular disease) and micro (retinopathy, neuropathy and nephropathy) vascular complications[1]. Medicinal plants have become popular to cure diseases because of its ease of availability, safety and lesser side effect when compared to the currently available synthetic drugs[2].

Boerhavia diffusa Linn. (Family: Nyctaginaceae) is widely distributed in tropical and sub-tropical areas of Asia, Africa, America and Australia. The whole part of the plant has a numerous medicinal properties and has a long history of use by the indigenous and tribal people in Ayurvedic and Unani medicines. In Sanskrit it is called as Punarnava, Spreading hog-weed in English and Mukarati keerai in Tamil. The plant is a perennial creeping or climbing herb. The root and the whole plant of *Boerhavia diffusa* are used in traditional medicine for the treatment of diabetes, stress, dyspepsia, abdominal pain, jaundice, heart disease, inflammation, enlargement of spleen and bacterial infections[3]. The present study was carried out to investigate the efficacy of ethanolic extract of leaves of *Boerhavia diffusa* (ELBD) on the antihyperglycemic activity in streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

The experimental plant *Boerhavia diffusa* was grown in the herbal garden of Avinashiligam Institute for Home Science and Higher Education for Women, Coimbatore and duly authenticated from Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, with the authentication number BSI/SRC/5/23/2013-14/Tech/1041. 15gm of fresh leaves of *Boerhavia diffusa* was extracted by soaking in 95% ethanol in Soxhlet apparatus for 72 hours. The extract obtained is filtered and the solvent was removed by using rotary evaporator apparatus and used for the study. Rats were given oral dose of the extract after 3 days of induction of diabetes.

Drugs and Chemicals

Streptozotocin and glibenclamide were obtained from Sigma-Aldrich, Chemico Co, USA. All the other chemicals and solvents used were purchased from standard commercial suppliers and are of analytical grade.

Experimental Animals

Male Wistar rats of 8–10 weeks old, weighing 150-200g used for the study were procured from KMCH College of Pharmacy, Coimbatore, India. The rats were acclimatized for a week under laboratory conditions. Animals were housed in polyethylene cages in an animal room maintained under standard environmental conditions like ambient temperature (25±2° C), relative humidity 50%-60% and 12 hours light-dark cycle. They were fed with normal laboratory diet and water *ad libitum*. The animal experiments were carried out in accordance with the CPCSEA guidelines. The experimental animal protocol

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Table 1. Levels of Hepatic Markers in the Serum of Experimental Rats

Groups	AST	ALT	ALP	Total Bilirubin (mg/dl)	Total protein (g/dl)
Control	54.33 ± 4.04	27.73 ± 4.53	69.00 ± 5.00	0.62 ± 0.18	7.90 ± 0.56
STZ	80.67 ± 3.06	67.00 ± 6.24	127.00 ± 5.57	1.89 ± 0.55	4.80 ± 0.56
STZ + ELBD	64.00 ± 4.58	42.33 ± 5.51	84.00 ± 3.00	0.52 ± 0.15	7.30 ± 0.53
STZ + Glibenclamide	66.00 ± 4.58	45.67 ± 4.73	87.00 ± 5.57	0.58 ± 0.12	6.93 ±0.51

Values are mean ± SD (n=6 rats in each group)
(One Way ANOVA followed by Dunnet's multiple comparison test)

satisfied the guidelines for animal experimentation approved by the Institutional Animal Experimentation Committee (approval no: KU/IAEC/Ph.D/127/2013).

Induction of Diabetes

Diabetes mellitus was induced in the rats after 18 hours fasting by single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight in 0.01 M sodium citrate buffer (pH 4.5)[4].The rats injected with STZ were given 20% glucose solution overnight to overcome drug induced hypoglycemia. The control rats were injected with same volume of isotonic saline. After 3 days, fasting blood glucose was measured and rats with fasting blood glucose level > 200 mg/dl were used for the study.

Experimental Design

The male Wistar rats were divided into four groups of six animals each. The grouping of the animals is as follows:

Group I: Untreated control rats which received standard pellet diet and water throughout the experimental period

Group II: STZ treated rats which served as diabetic control

Group III: STZ rats which received ELBD (500 mg/kg body weight) orally for 45 days

Group IV: STZ rats which received known antidiabetic drug, glibenclamide (10 mg/kg body weight) orally for 45 days

At the end of the study (after 45 days) the overnight fasted rats were anesthetized using ether anesthesia and blood samples were collected by cardiac puncture and used for the biochemical analysis such as serum aspartate transaminase (AST) and alanine transaminase (ALT)[5], alkaline phosphatase (ALP)[6], total bilirubin[7], total protein[8], blood urea[9], uric acid[10], creatinine[11], glucokinase[12], glucose-6-phosphate dehydrogenase[13], fructose-1,6 bisphosphatase[14] and glucose-6-phosphatase[15]. The animals were later sacrificed by cervical decapitation; liver is dissected, washed immediately in ice-cold saline and homogenized in Tris-HCL buffer (0.1 M, pH 7.5). The homogenate was centrifuged at 10,000 rpm to remove debris and the supernatant was used as to estimate the carbohydrate metabolizing enzymes.

Statistical Analysis

Results were expressed as mean ± SD for six rats in each group. All the data were analysed with SPSS student software. Statistical significance was determined by One-way Analysis of variance (ANOVA). A value of $p<0.05$ or less were considered statistically significant.

RESULTS AND DISCUSSION

Activities of Enzymic Markers of Hepatic Injury in the Serum of Experimental Rats

Table 1 shows the effect of the ELBD on various enzymic markers of hepatic injury namely serum glutamate oxaloacetate transaminase / serum aspartate amino transferase (SGOT/AST), serum glutamate pyruvate transaminase / serum alanine amino transferase (SGPT/ALT), alkaline phosphatase (ALP), total bilirubin and total protein in the normal and diabetic rats.

There was a significant increase in the levels of SGOT/AST, SGPT/ALT and ALP in the serum of STZ induced rats when compared to control rats. Administration of ELBD (500 mg/kg b.w.), glibenclamide (10 mg/kg b.w.) to the STZ induced rats caused a significant decrease in the levels of AST, ALT and ALP in the serum of ELBD/glibenclamide treated rats when compared with the STZ induced rats.

Liver enzymes such as AST, ALT and ALP are markers for liver function and integrity. AST and ALT were used as markers to assess the extent of liver damage in STZ induced diabetic rats. Elevated levels of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by STZ.

The level of total bilirubin was significantly increased in STZ rats when compared to the control group. Treatment with the ELBD and drugs glibenclamide significantly reduced their levels which are in par with that of the control group. The levels of total protein in the STZ induced rats showed a decrease in the total protein when compared with control group. The treatment of rats with ELBD and standard drugs glibenclamide for 45 days caused a significant increase in the level of protein. The reduction

Table: 2. Serum Urea, Uric Acid and Creatinine Level in Experimental Rats

Groups	Urea	Uric acid	Creatinine
Control	25.67 ± 5.68	7.85 ± 0.46	1.09 ± 0.32
STZ	61.33 ± 8.14	18.12 ± 0.85	2.19 ± 0.93
STZ + ELBD	29.81 ± 5.71	9.87 ± 1.03	1.58 ± 0.77
STZ + Glibenclamide	31.00 ± 6.00	10.17 ± 0.68	1.63 ± 0.68

Values are mean ± SD (n=6 rats in each group)
(One Way ANOVA followed by Dunnet's multiple comparison tests)

Table 3: Activities of Glycolytic and Gluconeogenic Enzymes in Experimental Rats

Groups	Glucokinase (U/mg protein)	Glucose 6 Phosphate dehydrogenase (U/mg protein)	Fructose 1,6 bis phosphatase (U/mg protein)	Glucose 6 Phosphatase (U/mg protein)
Control	45.55 ± 6.59	139.1 ± 4.05	235.0 ± 26.69	387.9 ± 22.19
STZ	22.16 ± 3.96	71.7 ± 7.13	543.8 ± 13.20	698.0 ± 18.63
STZ + ELBD	38.97 ± 5.07	125.7 ± 4.36	346.4 ± 16.79	410.1 ± 12.15
STZ + Glibenclamide	37.43 ± 5.40	123.7 ± 4.41	357.8 ± 6.49	414.5 ± 6.29

Values are mean ± SD (n=6 rats in each group)
(One Way ANOVA followed by Dunnet's multiple comparison tests)

in the levels of total protein may be attributed to localized damage in the endoplasmic reticulum resulting in the loss of Cytochrome P450, leading to its functional failure with a decrease in protein synthesis[16]. The rise in protein levels in the ELBD, glibenclamide treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis.

Administration of methanolic extract of leaves of *Dillenia indica* showed a decline in the liver marker enzymes which is responsible for liver damage. The restoration of AST, ALT, ALP to normal levels indicates the revival of insulin secretion[17]. STZ induced diabetic rats showed a decrease in the levels of serum transaminases on administration with *Carica papaya* leaf extract[18].

Renal Function Parameters in Experimental Rats

The influence of the extract on the levels of serum urea, uric acid and creatinine in STZ induced diabetic rats is given in Table 2.

There was a significant increase in the levels of serum urea, uric acid and creatinine in STZ induced rats when compared with the normal rats. Administration of ELBD (500 mg/kg b.w.) and glibenclamide showed a significant decrease in the levels of serum urea, uric acid and creatinine in all the treated groups when compared to control rats which may be due to the derangement of kidney functions.

Negative nitrogen balance with enhanced proteolysis in tissues and decreased protein synthesis can contribute to elevated levels of serum urea and creatinine[19]. Administration of ELBD and glibenclamide has significantly reduced the serum urea, uric acid and creatinine levels in STZ induced rats. Thus showing that ELBD improved renal morphology and function in STZ conditions.

Administration of methanolic extract of leaves of *Dillenia indica* showed a decrease in the serum urea and creatinine levels[17]. Maheswari et al. have demonstrated significant decrease in urea, uric acid and creatinine levels in the serum of diabetic rats treated with a polyherbal formulation- Dibolin and glibenclamide[20]. Thus the results suggest that the ELBD could play a protective role in restoring the diabetic complications by improving renal levels.

Activities of ELBD on Carbohydrate Metabolising Enzymes

The activities of various carbohydrate metabolizing enzymes namely glucokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase (G6PD) and fructose 1,6 bis phosphatase in the liver of experimental rats are tabulated in Table 3.

The activity of hepatic glucokinase and glucose-6-phosphate dehydrogenase was found to be dropped in STZ induced diabetic rats when compared to control rats which might be due to insulin deficiency. However, the experimental rats treated with ELBD (500 mg/kg b.w.) resulted in augmentation in the enzyme level, which was similar to glibenclamide administration.

Increased hepatic glucose production, decreased hepatic glycogen synthesis and glycolysis, are the major symptoms in type 2 diabetes that results in hyperglycemia. Hepatic glucokinase is the sensitive indicator of the glycolytic pathway in diabetes and an important regulator of glucose storage and disposal in the liver. Glucokinase an insulin-dependent enzyme phosphorylates glucose to glucose-6-phosphate playing a pivotal role in the maintenance of glucose homeostasis[21].

Glucose-6-phosphate dehydrogenase catalyzes the first step of pentose phosphate pathway and is an important site of metabolic control. This enzyme is thought to be regulated by the ratio of NADPH and NADP. The reduced activity of G6PD noticed in the liver of STZ diabetic rats, suggests decrease in production of 6-phospho gluconate resulting in decreased production of NADPH by HMP shunt. This cycle is an alternate source of energy impairment of glycolytic pathways and Krebs cycle. In diabetic condition the activity of the enzyme was significantly decreased leading to impaired utilization of glucose to target tissues[22].

The activity of fructose 1,6 bis phosphatase and glucose-6-phosphatase was found to increase significantly in STZ induced diabetic rats when compared to normal control rats. However, diabetic rats treated with ELBD (500 mg/kg b.w.) resulted in significant decrease of this enzyme, which was similar to glibenclamide treatment.

The gluconeogenic enzymes fructose 1,6 bis phosphatase and glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis[23].

The STZ induced rats on treatment with *Paederia foetida* leaf extract has showed a rise in the hexokinase levels with a simultaneous decline in the levels of Glucose-6-phosphatase and Fructose 1,6 bis phosphatase in a dose dependent manner in[1].

There was a significant normalization in the hexokinase and glucose-6-phosphatase levels and a rise in the levels of hexokinase upon treatment with methanolic extract of *Merremia emarginata* and drug glibenclamide[24]. The

results could be substantiated with the observation of Rasineni et al. who reported that administration of *Catharanthus roseus* leaf powder stimulates the activity of the enzymes hexokinase and Glucose-6-phosphate dehydrogenase in the diabetic rats[25].

CONCLUSION

To conclude, the results of the present shows that oral administration of ethanolic extract of leaves of *Boerhavia diffusa* exhibited a synergistic action on the hepatic and renal tissues in the streptozotocin treated rats. This investigation shows the potential of *Boerhavia diffusa* for use as a natural oral agent with antihyperglycemic property. Further pharmacological investigations are needed to prove the exact mechanism of antihyperglycemic effect of the extract.

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