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ANTI DIABETIC ACTIVITY 0F *BOERHAAVIA DIFFUSA* AGAINST ALLOXAN INDUCED DIABETIC RATS.

MRS.U.KANAGAVALLI[1]*, MS.B.BHUVANESHWARI[1] AND DR.A.MOHAMED SADIQ[2]

1 PG & Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, G.B.Nagar, Kalavai.
2 Principal, Adhiparasakthi College of Arts And Science, G.B.Nagar, Kalavai.

ABSTRACT

The main objective of the present study was Antidiabetic activity of aqueous extract of leaves of *Boerhaavia diffusa* in alloxan induced diabetic rats was assessed. After the treatment, blood samples were collected and the serum was subjected to estimate different biochemical parameters viz. blood glucose, insulin, cholesterol, urea, glycosylated haemoglobin, triglycerides and protein levels. There was a steep decrease in protein, insulin and elevated level of blood glucose, urea, glycosylated hemoglobin, cholesterol and triglycerides are seen. It becomes normal after exposure of aqueous leaf extract of *Boerhaavia diffusa*. It may be concluded that *Boerhaavia diffusa* might be used in the treatment of diabetics.

KEYWORDS: ***Boerhaavia diffusa*, Antidiabetic acticvity, Alloxan induced rats.**

MRS.U.KANAGAVALLI
PG & Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, G.B.Nagar, Kalavai.

***Corresponding author**

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia with several long term health complications if not checked early due to insulin deficiency. Currently, IDF estimates a total of 371 million cases of diabetes worldwide with 71million cases of diabetes only in India. An expenditure of 471 billion USD is spent in the year 2012 for treatment of diabetes. WHO estimates an increase of 3.9 billion diabetes cases by 2030.[1] The disease is a major degenerative ailment in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders.[2] As diabetes aggravates and β -cell function deteriorates, the insulin level begins to fall below the body's requirements and causes prolonged and more severe hyperglycemia.[3] Hyperglycemia induces long term complications of diabetes such as cardiovascular complications and microvascular complications such as retinopathy, nephropathy and neuropathy and foot ulcer.[4] Several approaches are presently available to reduced the hyperglycemia including insulin therapy which suppresses glucose production and augments glucose utilization and several drawbacks like insulin resistance,[5] anorexic nervosa, brain atrophy and fatty liver[6] after chronic treatment; treatment by sulfonylurea, which stimulates pancreatic islet cell to secrete insulin; metformin, which acts to reduce hepatic glucose production; a-glucosidase inhibitors, which interfere with glucose absorption. Unfortunately, all of these therapies have limited efficacy and various side effects and thus searching for new classes of compounds is essential to overcome these problems. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease.[7] *Boerhaavia diffusa* L. (Nyctaginaceae), a species of flowering plant in the four o'clock family commonly known as 'Punarnava' in the Indian system of medicine, is a perennial creeping herb found throughout the waste land of India[15] Alloxan was one of the most widely used chemical diabetogens during initial research work on experimental diabetes. It is a cyclic urea analog of chemical composition 2,4,5,6-tetraoxo- hexa hydropyrimidine.[8] Alloxan induces diabetes in animals and impairs glucose induced insulin secretion from *β* cells of Islets of Langerhans of Pancreas. It has been reported that alloxan rapidly and selectively accumulates in *β* cells in comparison with non- β cells. Several reports directly or indirectly indicated that alloxan affects the membrane potential and ion channels in β cells.[9] In the present investigation, aqueous extract of leaves of *Boerhaavia diffusa* was used to evaluate the antidiabetic activity in normal and alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection of plants

Boerhaavia diffusa linn were collected from Adhiparasakthi Agricultural College, Kalavai, Vellore district, Tamilnadu and authenticated by Prof.P.Jayaraman, Institute of herbal botany plant anatomy research centre, Chennai. The authentication certificate number was PARC/2014/2078.

Processing of plant materials

The leaves were washed in running tap water and cut into small bits to facilitate drying. The pieces of plant material were dried for 12hrs in a hot air oven (Model: HIPL-024A) at 60°C. The dried plant material (leaves) was taken separately and grounded using an electric blender to obtain a fine powder. The powder was further passed through a 2mm sieve to obtain finer particles. The powdered samples were stored in a clean glassware container until needed for analysis. Preparation of plant extract— *Boerhaavia diffusa* leaves (500g) were chopped into small pieces, extracted with 1500 ml water by the method of continuous hot extraction at 60°C for 6 hr and evaporated. A dark semi-solid (greenish–black) material was obtained (22.5 g). It was stored at 4°C until used. When needed, the residual extract was suspended in distilled water and used in the study[16].

Animals

Healthy albino wistar rats weighing 150 - 200 g was used for the present study. They were housed in polypropylene cages under controlled conditions of temperature (25 ± 2^0C) with a 12-h lightedark cycles. All the animals were acclimatized for 7 days before the study. They were fed with standard pellet diet obtained from Sai-Durga feeds and foods, Arcot and water ad libitum. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee number - APCAS/IAEC/ 2011/12).

Chemical used

Alloxan was obtained in the powder form from in merck company. Other chemicals were used were of A.R. Grade chemicals of research lab brand were procured from a local chemical dealer, Galaxy Scientific Company, Vellore, Tamil Nadu India.

Preparation of Alloxan solution

At first body weight of rats were measured. Then required amount of Alloxan was measured according to the body weight by following the dose of 150 mg of Alloxan per 1000 gm of body weight. Then calculated amount of Alloxan was dissolved in 0.1 ml of sterile normal saline water.

Induction of diabetes

For induction of diabetes in Wistar rats, 150 mg/kg of alloxan monohydrate dissolved in normal saline was administered intraperitoneally in overnight fasted rats.[11] After 1 h, the animals were fed with standard pellet and

water ad libitium. After 72 h, the blood glucose levels were estimated and rats having a blood glucose level more than 180 mg/dl were selected for the study.

Experimental designs

Antidiabetic activity evaluation

Experimental design:In the investigation, a total of 24 rats (18 diabetic surviving rats and 6 normal rats) were taken and divided into four groups of 6 rats each.

Group I: Normal, untreated rats.

Group II: Alloxan treated control rats (150 mg/kg.ip).

Group III: Diabetic rats given standard drug Glibenclamide (100mg/kg of body weight).

Group IV: Diabetic rats given aqueous extract of *Boerhaavia diffusa leaf* (200 mg/kg, p.o.)).

Acute toxicity test

The method of Lorke (1983) [10] was employed in this study. Twenty mice of both sexes were randomly grouped into four groups (A–D) of five mice per group and were dosed with 100, 500, 1000 and 3000 mg/kg of the extract orally by gastric gavage. The animals were given feed and water ad libitum. They were observed over a period of 48 h for signs of toxicity and mortality

Biochemical estimations

At the end of the experiment, the animals were fasted overnight and then rats were sacrificed by the cervical decapitation and the blood samples were collected to clot and serum separated by centrifugation at 2500 rpm for 10 min. Serum glucose, total cholesterol, triglycerides, insulin and urea was determined. Serum glucose was estimated by Oxidase method.[12] Total cholesterol[13], triglycerides[14], urea and insulin were determined by the respective methods.

Statistical analysis

Data were statistically evaluated by use of one-way ANOVA, followed by post hoc Scheffe's test using version 13 of SPSS software and Microsoft office excel 2003. The values were considered to be significant if $p<0.05$ was obtained.

RESULTS

The present study was conducted to evaluate the antidiabetic activity of *BoerhaaviaDDiffusa* aqueous leaf extract in alloxan induced diabetes rats. Diabetic rats treated with *Boerhaavia diffusa* aqueous leaf extract at oral dose of 200 mg/kg of body weight for 20 days. Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 20 days after treatment. Alloxan mediated body weight reduction was significantly reversed by the aqueous extract in dose dependant fashion (at 200 mg/kg). The effect of test extract at 200 mg/kg on body weight of the animals was also found statistically not significant. Results are shown in Table No. 1

Table 1
Effect of Boerhaavia diffusa leaf extract on body weight in alloxan induced diabetic rats

Groups	Initial body weight	Initial body weight
Group I	200.50 ±2.75	209.90 ± 2.86
Group II	203.50 ± 2.89	145.00 ± 1.72
Group III	205.50 ± 2.84	197.00 ± 1.90
Group IV	204.50 ± 2.45	188.00 ± 1.50

Table 2
Effect of Boerhaavia Diffusa Aqueous extract on serum insulin in alloxan induced diabetic rats.

Groups	Serum insulin (U/mL)	
	Initial	Final
Group I	19.21±0.50	18.56±0.45
Group II	8.57±0.26	6.01±0.34
Group III	8.14±0.40	18.32±0.30
Group IV	8.34±0.42	10.81±0.38

Alloxan caused significant decrease in serum insulin. Administration of *Boerhaavia diffusa* extract (200mg/kg) caused significant increase in insulin level at the end of the study

Table 3
Effect of Boerhaavia diffusa Aqueous extract on biochemical parameters blood glucose and Urea in serum

Groups	Blood glucose (mg/dl)	Blood urea (mg/dl)	Triglycerides (mg/dl)
Group I	88±0.10	27.3±0.10	150.0±0.27
Group II	170.4±0.10	62.5±0.11	220.5±0.22
Group III	80.0±0.13	24.0±0.10	145.0±0.17
Group IV	92.8±0.12	25.8±0.10	160.5±0.22

Table 3 shows exhibited significant reduction in blood glucose in comparison to untreated diabetic rats, elevated level of urea observed in diabetic rats. These are found to be corrected to near normal in the aqueous leaf extract of Boerhaavia Diffusa.

Table 4
Effect of Boerhaavia Diffusa Aqueous extract on biochemical parameters blood glucose and Urea in serumprotein, cholesterol and glycosylated hemoglobin.

Groups	Protein (g/dl)	Cholesterol (mg/dl)	Glycosylated hemoglobin (mg/g of Hb)
Group I	95.8±0.15	90.3±0.10	6.42 ± 0.34
Group II	6.6±0.11	175.5±0.11	10.63 ± 0.55
Group III	8.0±0.13	98.0±0.10	9.82 ± 0.29
Group IV	7.8±0.12	100.8±0.10	6.75 ± 0.28

.***Table 4 shows the protein level was decreased in diabetic rats because of the insufficient of insulin leads to increased protein degradation and decreased protein synthesis and cholesterol, glycosylated hemoglobin levels were significantly higher in diabetic rats. The administration of aqueous Boerhaavia diffusa extract in diabetic rats increased protein level and the cholesterol, glycosylated hemoglobin, triglyceride levels were significantly decreased as compared to diabetic rats.***

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin will be secreted.[17] However, Alloxan is an oxygenated pyrimidine derivative betacytotoxin and is known to induce diabetes mellitus in a wide variety of animal species through the damage of pancreatic β-cells.[18] When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results.[19] In the present study, results of the experiment indicated the significant antidiabetic activity of aqueous leaf extract of *Boerhaavia diffusa*. (200 mg/kg b.w.). Since, the experiment focused on exploring the competence of aqueous leaf extract of *Boerhaavia Diffusa* for the treatment of diabetes and relative complications like oxidative stress to substantiate folklore claim. The elevated glucose level was successfully controlled by the *Boerhaavia diffusa* extract and several investigators have recommended that glycosylated hemoglobin to be used as an indicator since glycohemoglobic control of diabetes since glycohemoglobin levels approach normal values in diabetics in metabolic control.[20] So in our case also the *Boerhaavia diffusa*. controlled the glycosylated hemoglobin.The diabetic hyperglycemia induces elevation of the serum levels of urea and decrease protein level which were considered as significant markers of renal function.[21] The diabetic hyperglycemia induces elevation of the plasma levels of urea, uric acid and creatinine which are significant markers of renal dysfunction and reflecting a decline in the glomerular filteration rate were significantly recovered by *Boerhaavia diffusa*. Protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin in alloxan induced diabetic rats[22], the *Boerhaavia diffusa* increased the protein level in blood. Hypercholesterolemia has been reported to occur in alloxan diabetic rats and marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots[23], the *Boerhaavia diffusa* decreased the total cholesterol in the diabetic rats. In our studies, the damage of pancreas in alloxan-treated diabetic control rats and regeneration of *β* cells by glibenclamide was observed. It was found that aqueous extract at dose (200 mg/kg) is more effective for treatment. Hence the above discussion reveals that Aqueous extracts of *Boerhaavia diffusa* at dose (200 mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (100 mg/kg). This could be due to the possibility that some *β*-cells are still surviving to act upon by *Boerhaavia diffusa* aqueous extract to exert its insulin releasing effect. Histopathological studies reinforce the healing of pancreas, by *Boerhaavia diffusa* aqueous extract, as a possible mechanism of their antidiabetic activity.

CONCLUSION

Diabetes mellitus is a metabolic disorder involving carbohydrate, protein and lipid metabolism and also due to absolute lack of insulin. The male rats were induced diabetes using alloxan, after the treatment with *Boerhaavia diffusa* the diabetic rats show significant decrease in blood glucose level which is similar to the normal rats. The result of this study show that aqueous leaf extract of *Boerhaavia diffusa* possessed antidiabetic properties as shown in its ability to reduce blood glucose level of alloxan induced diabetic rats .This conformation justifies its use in ethnomedical medicine for the treatment of diabeties.

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