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Prem Kumar Singh, *The M.S.University of Baroda*
Darshee Baxi, *The M.S.University of Baroda*
Ankita Doshi, *The M.S.University of Baroda*
Ramachandran A V, *The M.S.University of Baroda*

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Antihyperglycaemic And Renoprotective Effect of *Boerhaavia diffusa* L. in Experimental Diabetic Rats

Prem Kumar Singh, Darshee Baxi, Ankita Doshi, and Ramachandran A V

Abstract

The present study evaluates the efficacy of ethanolic extract of *Boerhaavia diffusa* L (BD) administered orally at a dose of 500mg/kg body weight for a period of 30 days to alloxanized diabetic rats and its efficacy compared with the standard hypoglycaemic drug metformin. Diabetic animals showed glycemic dysregulation, altered ionic balance, increased levels of serum markers of kidney function, and reduced Na^+-K^+ ATPase activity and endogenous antioxidant status. Administration of BD not only maintained the ionic balance and renal Na^+-K^+ ATPase activity but also significantly minimized diabetic hyperglycaemia. The renal antioxidant status (GPx, Catalase, SOD and GSH) remained in the near normal range and LPO level lower than the non-diabetic level. These effects are comparable to the changes brought about by metformin treatment and even better. Over all, the present study provides evidence for BD to be a potent renoprotective and antihyperglycaemic agent in diabetic animals.

KEYWORDS: Boerhaavia diffusa L., Diabetic nephropathy, metformin, ATPase, renal dysfunction, hyperglycaemia

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INTRODUCTION

A World Health Organization prediction suggests a targeted increase in diabetics in both developed and developing countries in the coming years. India, China and USA are predicted to be the countries with the largest number of diabetics by the year 2025 (Ramachandran, 2007; Mohan *et al.,* 2007). Uncontrolled hyperglycaemia is identified as one of the major factors contributing to development of diabetic nephropathy in animal models while, chronic and untreated diabetes in human subjects reportedly lead to enlargement of kidney with increase in RNA content and an increase in protein synthesis (Wahab *et al*., 2001). Considerable loss of electrolytes is also a feature observed in such patients. Hyperglycemia induced glucose oxidation and non-enzymatic glycation of proteins cause excessive production of free radicals in diabetics and, subsequent degradation of glycated proteins results in cellular damage, compromised endogenous antioxidant status and development of insulin resistance (Maritim *et al.*, 2003). All these factors together expectantly promote complications of Diabetes mellitus (DM).

Recent reports suggest a vascular origin for most of the diabetic complications including diabetic nephropathy. Moreover, patients with DM are at an increased risk of cardiovascular diseases and this risk is potentiated many folds by the coexistence of hypertension (Turner *et al*., 1998). Abnormalities of sodium metabolism at all physiological levels characterize hypertension that accompanies DM. Nevertheless, hypertension that accompanies Type I diabetes appears to be more of a renal phenomenon (Elliol *et al*., 2001). Apart from the disturbance in electrolyte balance that characterize metabolic diseases like diabetes, there is also an associated alteration in Na^+ - K^+ ATPase activity, thought to be linked to several complications of DM (Totan and Greaby , 2002). Traditionl Indian Ayurvedic system of medicine represents one of the oldest with more than 100 medicinal plants mentioned in this system, including the folklore medicines for treatment of diabetic complications (Kar *et al*., 2003). Herbal medicines have been in use even in other parts of the world other than India for management of Diabetes (Marles and Farnsworth, 1995). *Boerhaavia diffusa* L. is a small perennial creeping herb distributed all over India and in many other countries. The *Boerhaavia diffusa* plant contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins, Punarnavine, boeravinone A.F, ursolic acid, punarnavoside, liirodendrin which have been isolated and studied in detail for their biological activity (Basu *et al.*, 1947; Mishra and Tiwari, 1971; Surange and Pendse, 1972; Lami *et al.,* 1990; Aftab *et al.*, 1996). This plant has reported anti inflammatory, diuretic and hepatoprotective effects (Bhalla *et al*., 1968; Chopra,

1969; Chandan *et al*., 1991). Recently, Pari *et al*. (2004) and Rao *et al.* (2004) have even reported an antidiabetic effect of the aqueous extract of this plant.

It is worth evaluating the efficacy of BD extract in this context, on diabetes associated secondary complications of electrolyte imbalance and renal oxidative stress related to glycemic dysregulation. Thus, the present study addresses the efficacy of BD extract on these aspects compared to Metformin, a standard hypoglycaemic drug. Maximal hypoglycemic effect shown in a previous dose dependant study formed the basis for the herein employed dosage of 500mg/kg body weight.

MATERIAL AND METHODS

PLANT MATERIAL

Fresh leaves of *Boerhaavia diffusa* L. (BD) were collected from Rajpardi village near Bharuch district, Gujarat, India and authenticated by Prof. M.Daniel (Head, Department of Botany, The M.S.University of Baroda).

PREPARATION OF PLANT EXTRACT

The collected leaves were shade dried and ground to a fine powder in an electronic mixer. The powder was sieved and then subjected to extraction in 95% ethanol in a Soxhlet apparatus (Borocil Glass Works, Mumbai, India) at 60 ° C for 10 hrs yielded a green colored extract. After cooling and filtration, the filtrate was concentrated at 65° C in a rotavapour to obtain a dry powder. This powder was stored in a refrigerator maintained at 4 ° C until further use (Narendhikannan *et al.,* 2006).

EXPERIMENTAL ANIMALS

Female albino *Wistar* rats of 200-250g body weight maintained in the animal house of the department under a 12:12 light and dark cycle at 21-23°C served as the experimental animals. The animals were maintained in accordance with CPCSEA guidelines and the animal experiments were approved by the animal ethical committee of Department of Zoology, The M.S.University of Baroda, Vadodara (Approval no 827/ac/04/CPCSEA). Throughout the experimental period, the animals received standard rat chow (M/S Pranav Agro limited, Baroda) and water *ad libitum.* Induction of diabetes by alloxan was as per our previous studies (Singh *et al.,* 2010 a, b).

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EXPERIMENTAL DESIGN

Animals for experimentation consisted of five groups of six rats each.
Group I: (C)
Normal rats administered double distilled water for 30 days.
Group II: (C+E)
Normal rats administered with the herbal (BD) extract (500mg/kg of body weight) orally for 30 days.
Group III: (D)
Diabetic rats administered with double distilled water as vehicle daily for 30 days.
Group IV: (D+E)
Diabetic rats administered with the BD extract (500mg/kg of body weight) orally for 30 days.
Group V: (D+Mt)
Diabetic rats administered with Metformin (500mg/kg of body weight) orally for 30 days.
Fasting blood glucose level was assayed at regular intervals while, food and water intake and body weight were recorded on daily basis.

GLUCOSE TOLERANCE TEST (GTT)

At the end of four weeks of treatment, animals were fasted and subjected to GTT as described previously (Singh *et al.,* 2010 b).

INSULIN RESPONSE TEST (IRT)

At the end of four weeks of treatment, recording of temporal glycemic response to a challenge of insulin constituted IRT (Singh *et al.,* 2010 b).

BIOCHEMICAL ASSAYS

At the end of the experimental period, animals were food deprived and sacrificed by decapitation. Blood was collected from the jugular vein, serum separated and, various parameters assayed in the same. The kidneys were dissected out, washed in saline, blotted dry, and weighed.

Blood glucose levels were estimated by the glucose oxidase method (Trinder, 1969) using kit obtained from Aggape Diagnostics. Insulin was assayed using an ELISA based assay kit (Rat Insulin ELISA kit from Mercodia, Sweden). Serum levels of sodium, potassium and magnesium were estimated by flame photometry. Serum calcium, urea and creatinine levels were determined using reagent kits obtained from Bio-Invitro Diagnostic, DiaSyS Diagnostic system-Germany and Nicholas Piramal India Limited respectively.

Na^+ - K^+ ATPase activity in kidney was estimated by the method of Floreani & Bonetti (1981) and the resultant phosphate released was assayed by the method of Fiske and Subbarow (1925). Tissue protein content was estimated by the method of Lowry *et al*. (1951).

Beneficial effect of the plant extract on oxidative stress in both diabetic and non-diabetic rats was determined by assaying enzymatic and non-enzymatic antioxidant status. Lipid Peroxidation (LPO) was determined by the method of Beuge and Aust (1978), glutathione content (GSH) by Beutler *et al.* (1963), SOD activity by Marklund and Marklund (1974), catalase activity by Sinha (1972) and GPx activity by Rotruck *et al*. (1973).

STATISTICAL ANALYSIS

All data were expressed as Mean ± SE and the statistical significance was evaluated using One Way ANOVA followed by Bonferroni`s Multiple Comparisons test using Graph Pad Prism Version 3.0 for Windows, Graph Pad software, San Diego CA /USA .

RESULTS

Body and kidney weights and, food and water intake

Body weight, relative kidney weight and water and food intake of control and experimental rats are depicted in Table 1. Control diabetic rats showed significant ($p<0.001$) decrement in the body weight over a period of 30 days as compared to non-diabetic control rats, which showed an increase. There was considerable weight gain in extract and metformin treated diabetic rats. Kidney weight, which redorded an increase in diabetic rats, showed significant decrement on supplementation with extract or metformin, relatively more with the former. The increased food and water intake characteristic of diabetic rats got significantly reduced on supplementation with extract or metformin though more significantly with latter.

Blood glucose and Insulin

At the end of 30 days, there was significant decrement in blood glucose level in extract treated diabetic rats with concomitant increase in insulin titre. These changes were comparable to those seen in rats treated with metformin. Extract treatment of non-diabetic control rats had no significant effect on glucose level (Table 2).

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GTT and IRT

The glucose tolerance curve that revealed a higher positioning in diabetic rats showed significant improvement on treatment with both extract and metformin, with the curves of both these groups of rats showing lower positioning compared to diabetic rats (Fig.1). The area under curve that was greater in diabetic rats showed significant decrement in extract and metformin treated groups. However, the extract treated non-diabetic control rats showed a similar area under curve as the non-treated control rats (Fig 2).

The insulin response curve and the area under curve showed no significant differences in extract treated non-diabetic rats while, extract and metformin treatment of diabetic rats showed a lowered positioning of the curves, with the curve of metformin group being relatively better (Fig.3). However, the area under curve showed a greater decrease in extract treated diabetic rats, better than metformin (Fig 4).

Serum Urea and Creatinine

Both these serum markers of kidney dysfunction showed significant increase in diabetic rats (Table 3). Administration of extract or metformin was successful in reversing these changes to near normal levels though relatively much better with extract. However, there were no noteworthy changes in non-diabetic control rats treated with extract.

Na^+, K^+, Mg^{++}, Ca^{++} levels and Na^+ - K^+ ATPase activity

Table 4 represents the changes in serum levels of sodium, potassium, magnesium and calcium and, Na^+ - K^+ ATPase activity in kidney in all the experimental groups. There was increase in serum sodium and potassium levels but decrease in magnesium level in diabetic rats. Renal Na^+ - K^+ ATPase activity also showed decrement in diabetic rats. The extract and metformin treated rats showed reversal of levels of serum ionic levels and renal Na^+ - K^+ ATPase activity, with the changes being comparatively better with extract treatment.

Renal Oxidative stress and antioxidant status

Levels of LPO and of enzymatic (GPx, Catalase and SOD) and non-enzymatic (GSH) antioxidants in kidney are shown in Table 5. Lipid peroxidation was significantly ($P<0.001$) increased in diabetic rats. Both extract and metformin were equally effective in decreasing the levels of lipid peroxidation in diabetic

rats. Correspondingly, the levels of both enzymatic and non-enzymatic antioxidants showed significant reduction in diabetic animals. Supplementation of diabetic animals with either extract or metformin was equally effective, though the former being relatively better, in increasing the levels of endogenous antioxidants.

Table 1: Changes in body weight, relative weight of kidney and food and water intake.

GROUPS	INITIAL WEIGHT(g)	FINAL WEIGHT(g)	FOOD INTAKE (g/animal/day)	WATER INTAKE (ml/animal/day)	KIDNEY WEIGHT (g/100gbw)
C	246±1.156	270±2.890	18.333±0.882	37.667±0.372	1.45±0.016
C + Ex	240±10.029	262.5±12.53	15.556±2.005	37.488±2.005	1.25±0.150
D	236.25±2.393	215± 3.952 [c]	33.916±0.853 [c]	114.71±0.853 [c]	1.87 ±0.064 [c]
D + Ex	185 ±12.990 [e]	192.5±7.5	30.889±0.853	98.91±0.853 [•]	1.57±0.110 [e]
D + Mt	220±10.029	230±20.059	24.565±2.005	55.65±2.005 [e]	1.69±0.270 [e]

Data are expressed as Mean±SE

[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, • p< 0.01, [e] p< 0.001 when compared to Diabetic Control

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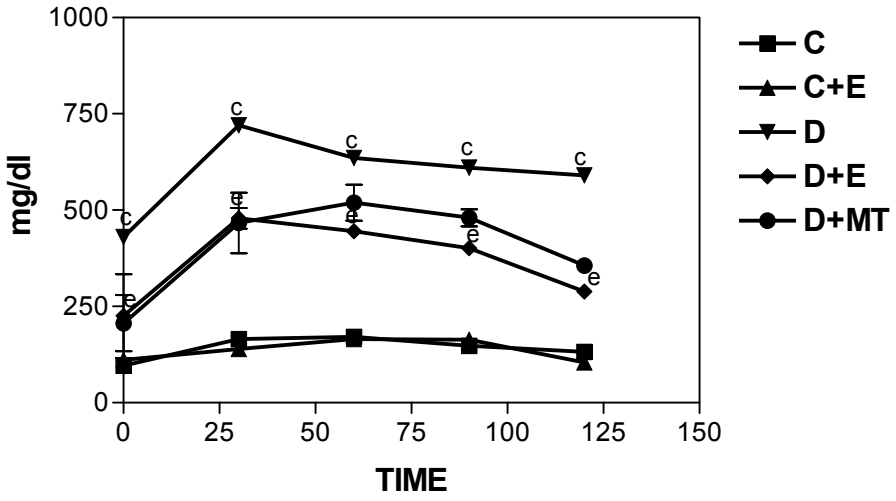
Table 2: Blood glucose levels during the four week of extract or metformin treatment in non-diabetic and diabetic rats.

GROUPS	BLOOD GLUCOSE (mg/dl)						INSULIN
	0 DAY	15 DAYS AFTER ALLOXAN INJECTION	I WEEK (AFTER TREATMENT)	II WEEK	III WEEK	IV WEEK	
C	82±2.11	95±2.01	98±5.1	92±4.56	94±3.2	96.0 ±1.15	0.34±0.01
C + Ex	90±1.22	98±1.31	102.4±5.4	98±8.7	105±11.35	111.50± 4.19	0.39±0.02[b]
D	87±1.31	385±12.25	389±18.4	394±22.54	410±26.55	430.00± 6.93	0.18±0.01[C]
D + Ex	89±2.31	354±14.10	302±21.1	258±25.45	210±18.22	195.00± 10.10	0.32±0.01•
D + Mt	88±1.11	324±12.7	310±17.32	267±12.25	224±15.78	206.5± 72.68	0.29±0.02

Data are expressed as Mean±SE

[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, • p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Fig 1: Glucose tolerance curves of non-diabetic and diabetic rats treated with extract or metformin.



Data are expressed as Mean±SE
[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, [*] p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Fig 2: Showing area under curve (AUC) of non-diabetic and diabetic rats treated with extract or metformin.

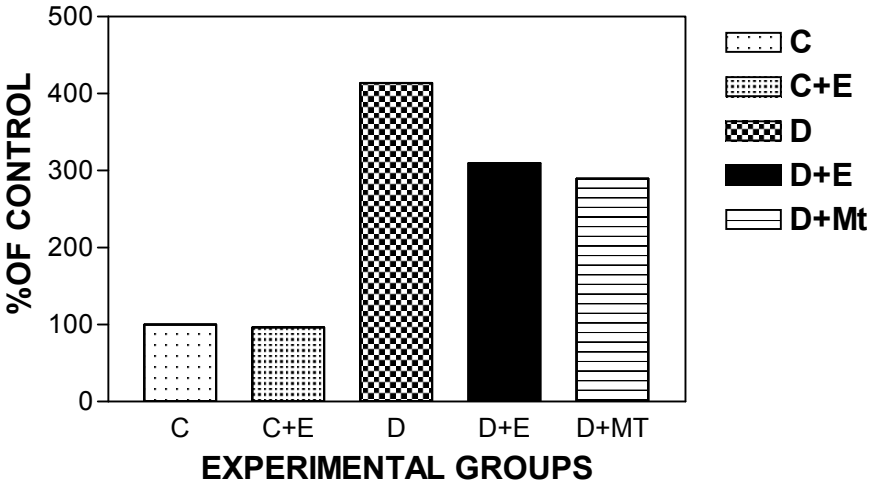


Fig 3: Insulin response curves of non-diabetic and diabetic rats treated with extract or metformin.

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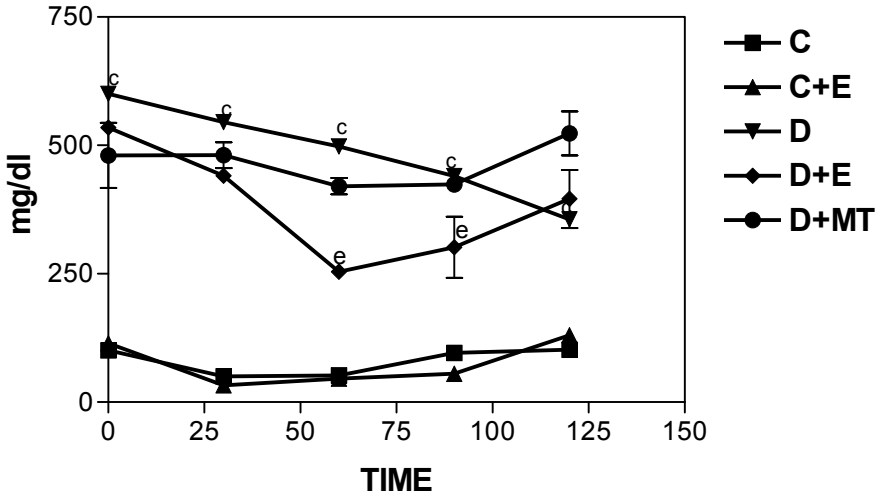

Data are expressed as Mean±SE
[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, ˙ p< 0.01, ᵉ p< 0.001 when compared to Diabetic Control

Fig 4: Showing area under curve (AUC) of non-diabetic and diabetic rats treated with extract or metformin.

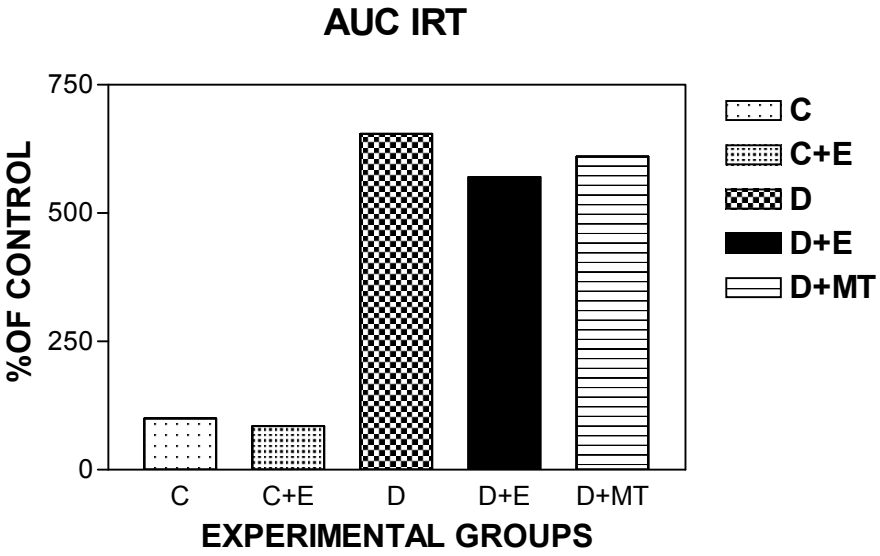

Table 3: Changes in serum urea and creatinine levels of extract or metformin treatment of non-diabetic and diabetic rats.

GROUPS	UREA (mg/dl)	CREATININE (mg/dl)
C	69.66 ± 28.27	0.6 ± 0.10
C + Ex	66.66 ± 0.33	0.63 ± 0.03
D	152.67 ± 2.90 [b]	0.8 ± 0.05 [c]
D + Ex	85.66 ± 1.45 [e]	0.63± 0.08 [e]
D + Mt	99.66 ± 1.45 [e]	0.66 ± 0.08 [e]

Data are expressed as Mean±SE

[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, ˙ p< 0.01, [e] p< 0.001 when compared to Diabetic Control

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Table 4: Changing in serum Na^+, K^+, Mg^{++}, and Ca^{++} and renal Na^+ - K^+ ATPase activity in extract or metformin treated non-diabetic and diabetic rats.

GROUPS	SERUM SODIUM (m eq/L)	SERUM POTASSIUM (m eq/L)	SERUM MAGNESIUM (m eq/L)	SERUM CALCIUM (mg/dl)	RENAL Na+K+ATPase (nM of Pi/ min/ mg protein)
C	129.33±0.88	6.1±0.115	2.6±0.057	12.2±0.17	32±0.123
C + Ex	120±6.358	5.433±0.176	2.53±0.088	11.86±0.29	37±1.21
D	132.33±2.02 [c]	7.6±0.115 [c]	2.13±0.08 [c]	12.4±0.23	23±2.11 [c]
D + Ex	131.66±3.18	5.666±0.033 [e]	2.466±0.03	11.8±0.26*	33±0.88 [e]
D + Mt	126.333±3.76 [e]	5.933±0.033 [e]	2.3±0.173	12±0.578	28±0.9

Data are expressed as Mean±SE
[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, • p< 0.01, [e] p< 0.001 when compared to Diabetic Control

Table 5: Changes in renal protein and antioxidant levels and lipid peroxidation in extract or metformin treated non-diabetic and diabetic rats.

GROUPS	PROTEIN (mg/100mg tissue)	LPO (nM of MDA /100g tissue)	GSH (mg of GSH /min/100g tissue)	GPx (µgof GSH/min/mg protein)	CATALASE (µM of H2O2 decomposed/mg protein/min)	SOD (U/mg protein)
C	10.37±0.43	47.84±2.39	24.39±0.67	2.27±0.15	26.72±1.13	6.42±0.39
C + Ex	12.55±0.16 [a]	39.05±1.22 [b]	30.84±1.19 [b]	4.43±0.68 [b]	29.20±1.47	6.89±0.04
D	8.36±0.31	71.07±0.98 [c]	13.12±0.28 [c]	1.23±0.11	12.47±1.37 [c]	4.79±0.14[c]
D + Ex	13.55±0.90 [e]	32.42±0.97 [e]	24.29±1.99 [e]	3.23±0.30•	26.87±1.07 [e]	6.54±0.46 [e]
D + Mt	11.70±0.20 [e]	28.03±1.29 [e]	23.78±0.99 [e]	2.66±0.22	24.60±1.87 [e]	5.22±0.55•

Data are expressed as Mean±SE
[a] p<0.05, [b] p<0.01, [c] p< 0.001 when compared with Normal * p< 0.05, • p< 0.01, [e] p< 0.001 when compared to Diabetic Control

DISCUSSION

Herbal remedies are re-emerging as preferred choice of treatment for various disorders including diabetes. The traditional Indian system of medicine is replete with recorded evidences for many plants possessing curative/ameliorative properties against diverse manifestations. There is therefore need to explore the remedial properties of various plants on diabetic manifestations essentially to develop an ideal combination treatment as, diabetes clearly forms a multifaceted metabolic and endocrine disorder. The present study in this context is an attempt to explore the renoprotective potentials of *Boerhaavia diffusa* L. as compared to the commonly used drug Metformin.

Alloxan induced diabetes as seen in the present study is characterized by severe loss of body weight along with symptoms of polydipsia and polyphagia (Odetola *et al*., 2006; Oi *et al.*, 1997). The loss in body weight attributed mainly to negative nitrogen balance and depletion of tissue proteins (Rajkumar *et al.*, 1991) stand validated by the presently observed significant decrease in kidney protein content. Supplementation with BD extract was effective in reversing this trend and increase body weight and renal protein content, as effective as metformin. The efficacy of BD extract in countering the diabetic symptoms of increased water and food intake and renal hypertrophy stands substantiated by the herein observed decreased food and water intake and decreased renal weight in D+E rats. Enlargement of the lining of kidney cells is a feature reported in alloxan induced diabetes (Evan *et al.,*1984) and apparently, extract treatment prevents this deleterious consequence of diabetes.

Significant hypoglycaemic effect seen in diabetic rats treated with BD extract finds corroboration in the reported glucose lowering effect of this plant (Pari *et al.*, 2004). The increase in serum insulin level recorded in the present study suggests its role as an insulin secretogogue. This secretogogue action of BD appears to be a specific effect as seen by its insulin elevating influence even in non –diabetic animals. This effect of DB finds justification in the reported ability of many hypoglycemic medicinal plants to increase insulin levels even in normoglycemic animals (Lamela *et al*., 1985). Significant decrement in diabetic glycemic status induced by BD extract, even better than that shown by the reference drug, metformin, is clearly indicative of the presence of active hypoglycemic principles in this plant. Apparently, the dosage of 500mg/kg body weight seems more effective in preventing diabetic glucose elevation as seen by the 45% lesser level in BD treated rats as against 36% by metformin (herein) and 38% by 200mg/kg body weight of the extract (Rao *et al*., 2004). In the absence of any study on insulin level in BD extract supplemented diabetic rats, different mechanisms of action like stimulation of residual pancreatic function and/or extra pancreatic mechanisms (Chude *et al*., 2001), and lowering of cortisol level

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(Gholap and Kar, 2004) have been suggested as the possible modes of action. The present observation of near normal insulin level in extract supplemented diabetic rats clearly suggests probable insulin mediated hypoglycemic effect of BD. Apparently, apart from stimulated secretion from residual β cells, an actual increase in β cell population by proliferation and/or regeneration can also be inferred. In this respect, the extract seems more effective than even metformin as seen by the relative insulin titres.

Supplementation with BD extract not only ameliorates glucose and insulin levels but also improves glucose tolerance and insulin sensitivity, as seen by the GTT and IRT curves and the area under the curve, providing ample evidence for its multipronged action in combating diabetic hyperglycemia.

The disturbances in carbohydrate, lipid and protein metabolisms along with oxidative stress are likely to affect renal functions in severe diabetic condition. It is known that, diabetic hyperglycaemia induced elevation in the levels of urea and creatinine, are important markers of renal dysfunction reflecting decreased glomerular filtration rate (Mauer *et al.,*1981). Apart from the decreased rate of filtration, enhanced tissue proteolysis and decreased protein synthesis can also contribute equally to the increased levels of both the markers, portending impaired renal functions in diabetic rats as evidenced in the present study (Jensen *et al*., 1981). Treatment of diabetic rats with an ethanolic extract of *Boerhaavia diffusa* L. in the present study minimizes the elevation in serum levels of both the markers of renal dysfunction, strongly suggestive of the renoprotective potentials of this plant and, in fact, this is the only study that provides evidence for the renoprotective effect of BD extract.

The role of sodium, potassium, calcium and magnesium in blood pressure regulation, particularly in DM, is well established (Bloomgarden, 2001). In diabetic hypertension, regulation of these serum ions is very crucial, as both these conditions are independent risk factors for development of diabetic nephropathy, a serious secondary complication associated with DM. Abnormalities of sodium metabolism at all levels (whole body, renal and cellular), are characteristic of diabetes-associated hypertension. Both hyper and hyponatrinemia have been reported during Type I Diabetes (Khardori and soler, 1984; Arieff and carroll, 1992; Tzamaloukas *et al.,* 2008). Reporting in UpToDate, an evidence based peer-reviewed information resources available via the Web, desktop/laptop computer and mobile device, Burton (2000) has elaborated that, glucosuria-induced osmotic diuresis tends to counteract the direct effect of hyperglycaemia mediated diuretic loss of water in excess of sodium, which tends to raise the plasma sodium concentration and plasma osmolality. A compensatory increase in water intake however results in hyponatrinemia. Apparently, the currently recorded higher plasma sodium content in diabetic rats tends to suggest a tilt more towards diuretic water loss, probably an initial or early consequence of

hyperglycemia. Hyperkalemia is a recorded manifestation in hyperglycemia as seen in the present study, an effect related with osmotic dieresis with or without renal dysfunction (Rohrscheib *et al*., 2003; Tzamaloukas *et al*., 2005, 2008). Magnesium is the second most common intracellular cation implicated as a facilitator cofactor for a multitude of metabolic, biochemical and energy reactions. In this context, maintenance of optimal serum magnesium concentration is of pivotal importance and, decrease in its level is likely to cause subtle effects on various processes. Varied functions like glucose transport mechanisms at the cell surface, functioning of various enzymes of carbohydrate oxidation, insulin secretion, binding and action or cellular glucose utilization due to relevance to protein kinases seem compromised by sub-optimal Mg^{++} (Reinhart, 1988; Paolisso and Barbagallo, 1997; Resnick *et al*., 1999; Djurhuus *et al*., 2000; Hans *et al*., 2002; Shahid *et al*., 2005). The presently observed decrease in serum Mg^{++} level in diabetic rats is easily relatable with such compromised functions. However, BD supplementation tends to restore serum ionic homeostasis and afford protection against disturbance in ionic balance and progression to diabetic nephropathy. This effect of BD extract may however be related with its ability to normalize insulin level as, insulin therapy has been shown to reverse the ionic changes in most of the above works.

Na^+ - K^+ ATPase is an enzyme that ensures the maintenance of transmembrane gradient of sodium and potassium. Herein, induction of Type I Diabetes has brought about a significant reduction in renal Na^+ - K^+ ATPase activity. Interestingly, Totan and greaby (2002) also reported a decrement in erythrocyte Na^+ - K^+ ATPase activity in their study on diabetes. Based on the observation, they suggested a possible implication of reduced renal Na^+ - K^+ ATPase activity with many attendant complications of DM. The presently observed decrease in renal Na^+ - K^+ ATPase activity in this behest stands a reasonable chance of finding implication in diabetic nephropathy and even renal hypertension. The reported association between neuropathy and hypertension with compromised erythrocyte and neural Na^+ - K^+ ATPase activity respectively (Sanson and O`Neil, 1981; Muto *et al*., 1987; Weidmann and Ferrari, 1991; Issautier *et al*., 1994; Raocah *et al*., 1996; Nakayama et al., 1998; Biwititi *et al*., 2000; Ustundag *et al*., 2000; Shahid *et al*., 2005) and, the presently observed increase in serum markers of renal dysfunction adequately support the above inference. In short, the presently observed alterations in serum electrolytes and renal Na^+ - K^+ ATPase activity portend the possibility of diabetic complications leading to nephropathy and hypertension. Interestingly, all these alterations find redressal on supplementation with BD extract, even bettering the effect of metformin. The positive effect of DB is co-relatable with its insulin secretomimetic action as already noted.

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Oxidative stress has a cause and effect relation with diabetes. Present study clearly suggests augmented oxidative stress as marked by increased renal LPO and decreased levels of nonenzymatic and enzymatic antioxidants. Much evidence indicates severe oxidative stress in diabetic patients and, uncontrolled diabetic hyperglycaemia not only engenders free radicals but also impairs the antioxidant defense system (Saxena *et al*., 1993; Bonnefont – Rousselot *et al*., 2000; Maritim *et al*., 2003). There are a couple of reports that show increased LPO levels and decreased endogenous antioxidant system in the tissues of diabetic animals as seen in the present study and the efficacy of BD *extract in reversing these changes (Prince and Menon, 2001; Satheesh and* Pari,2004). Several plant extracts have proved to be very effective in combating oxidative stress as has been shown and reviewed in one of our recent studies (Singh *et al.,* 2010 a). Many plants have relatively better glucoregulatory effect or antioxidant activity; however, the present BD extract seems to have equally potent glucoregulatory effect as well as powerful antioxidant effect. Since the extract could exert protective effect against all parameters under study, along with restoration of near normal insulin levels, it is difficult to decipher as to whether the antioxidant effect of the extract is direct and independent of insulin. Nevertheless, the decrease in LPO and increase in antioxidants in the renal tissue seen with extract supplementation of non-diabetic animals tend to suggest some insulin independent antioxidant role as well. The recovery in antioxidant status and lesser LPO of renal tissue seen in diabetic animals suggest primary insulin induced effect as well as, secondary direct action of extract. It is also inferable from the present study that BD extract is even more potent than metformin with respect to restoration of enzymatic antioxidants.

In conclusion, the present study clearly demonstrates potent reno-protective potentials of *Boerhaavia diffusa* L. on alloxan induced diabetes. Effective glucoregulation, maintenance of serum ionic status and renal Na^+ - K^+ ATPase activity and antioxidant status, all point towards a plurality of action of DB extract. The present study provides compelling scientific evidence for *Boerhaavia diffusa* L. as a potent agent against possible nephropathy and diabetic manifestations.

REFERENCES

Aftab, K., Usmani, S.B., Ahmad, S.I., and Usmanghani, K. 1996. Naturally occurring calcium channel blockers-II. Hamdard Medicus 39:44–54.

Antonios, H., Tzamaloukas, M.D., Pratap, S. ,Avasthi, M.D. 1987. Serum Potassium Concentration in Hyperglycemia of Diabetes Mellitus With Long-term Dialysis. West J Med. 146(5), 571–575.

Arieff, A.I. and Carroll, H.J. 1972. Nonketotic hyperosmolar coma with hyperglycemia: Clinical features, pathophysiology, renal function, acid-base balance, plasma-cerebrospinal fluid equilibria and the effects of therapy in 37 cases. Medicine (Baltimore). 51, 73.

Basu, N.K., Lal, S.B., and Sharma, S.N. 1947. Investigations on Indian medicinal plants. Quarterly Journal of Pharmacy and Pharmacology 20, 38–42.

Beuge, J.A. and Aust, S.V. 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302-310.

Beutler, E., Duron, O. , Kelly, B.M. 1963. Improved method for the determination of blood glutathione. J. Lab. and Clinical Med. 61, 883- 887.

Bhalla, T.N., Gupta, M.B., Sheth, P.K. ,Bhargava, K.P. 1968. Antiinflammatory activity of Boerhaavia diffusa. Indian J. of Physiology and Pharmacology. 12, 37.

Biwititi, P., Musabayane, C.T., Nhachi, C.F. 2000. Effects of opuntia megacantha on blood glucose and kidney function in streptozotocin diabetic rats. J. Ethnopharmacol. 69, 247-252.

Bloomgarden, Z.T. 2001. Perspectives on the News: Diabetes and Hypertension. Diabetes Care. 24, 1679-1684.

Bonnefont-Rousselot D, Bastard, J.P., Delattre, J. 2000. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab. 26, 163–76.

Burton, D. R. 2000. Plasma sodium in uncontrolled diabetes mellitus UpToDate (an evidence-based, peer-reviewed information resource available via the Web, desktop/laptop computer and mobile device).

Chandan, B.K., Sharma, A.K., Anand, K.K. 1991. Boerhaavia diffusa: A study of its hepatoprotective activity. J. of Ethnopharmacol. 31(3), 299–307.

Chopra G.L. 1969. Angiosperms, Systemics and life cycles. S. Nagin & Co., Jalandhar, Punjab, India, 361-365.

Chude, M.A., Orisakawe, O.E., Afonne, O.J., Gamanial, K.S., Vongtau, O.H., Obi. E. 2001. Hypoglycemic effect of the aqueous extract of Boerrhavia diffusa leaves. Indian J Pharmacol. 33, 215-216.

Djurhuus, M.S., Skott, P., Vagg, A., Hother-Nielsen, O., Anderson, P., Parving, H.H. 2000. Hyperglycemia enhances renal magnesium excretion in type 1 diabetic patients. Scand. J. Clin. Lab. Inves. 60, 403-409.

DOI: 10.2202/1553-3840.1533

Elliol, M.D., Kapoor, A., Parker, M.A. 2001. Improvement in hypertension in patients with diabetes mellitus after kidney/pancreas transplantation. Circulation. 104, 563-569.

Evan, A.P., Mong, S.A., Connors, B.A., Aronoff, G.R., Luft, F.C. 1984. The effect of alloxan, and alloxan-induced diabetes on the kidney. Anatom. Rec. 208, 33–47.

Fiske, C.H., Subbarow, Y. 1925. The colorimetric detection of phosphorus. J. Biol Chem. 66, 375–400.

Floreani, M., Bonetti, A.C., Carpenedo, F. 1981. Increase of Na+K+ATPase activity in intact brain synaptosomes after their interactionwith phosphatidylserine vesicles. Biochem Biophys Res Commun. 101, 1337–44.

Gholap, S., Kar, A. 2004. Hypoglycaemic effects of some plant extracts are possibly mediated through inhibition in corticosteroid concentration. Pharmazie. 59, 876-8.

Hans, C.P., Sialy, R., Bansal, D.D. 2002. Hypomagnesemia in Diabetic Patients: Correlation with Oxidative Stress. Int J Diab Dev Ctries. 22, 122-31

Issautier, T., Kovacic, H., Gallice, P., Raccah, D., Vague, P. , Crevat, A. 1994. Modulation defect of sodium pump evidenced in diabetic patients by a microcalorimetric study. Clin. Chem. Acta. 228, 161-170.

Jensen, P.K., Chrisiansen, J.S., Steven, K., Parving, H.H. 1981. Renal function in streptozotocin diabetic rats. Diabetologia. 21, 409–14.

Kar, A., Choudhary, B.K., Bandyopadhyay, N.G. 2003. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J. of Ethnopharmacol. 84, 105-108.

Khardori, R., soler, M.G. 1984. Hyperosmolar hyperglycemic nonketotic syndrome. Report of 22 cases and brief review. Am J Med. 77, 899.

Lamela, M., Cadavid, I., Gato, A., Calleja, J.M. 1985. Effects of Lythrum saricaria in normoglycaemic rats. J Ethnopharmacol. 41, 83-91.

Lami, N., Kadota, S., Tezuka, Y., and Kikuchi, T. Constituents of the roots of *Boerhaavia diffusa* Linn. II. 1990. Structure and stereochemistry of a new rotenoid boeravinone C2. Chemical and Pharmaceutical Journal 38(6):1558–1562.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.G. 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem. 193, 265–275.

Mauer, S.M., Steffes, M.W., Brown, D.M. 1981. The kidney in diabetes. Am. Med., 70, 603-612.

Maritim, A.C., Sanders, R.A., Watkins, J.B. 2003. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 17, 24-38.

Marklund, S., Marklund, G. 1974. Involvement of the superoxyde anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxyde dismutase. Eur. J. Biochem. 47, 469-474.

Marles, R.J. and Farnsworth, N.R. 1995. Antidiabetic plants and their active constituents. Phytomedicine. 2, 137–189.

Mishra, A.N. and Tiwari, H.P. 1971. Constituents of the roots of *Boerhaavia diffusa.* Phytochemistry 10:3318.

Mohan, V., Sandeep, S., Deepa, R., Shah, B. and Varghese, C. 2007. Epidemiology of type 2 diabetes: Indian scenario. Indian J. Med. Res. 125, 217–230.

Muto, S.G., Giebish and Sansolu, S. 1987. Effects of adrenalectomy on CCD: Evidence for differential of two cell types. Am. J. Physio. 253, 742.

Nakayama, T., Izumi, Y., Soma, M. and Kanmatsuse, K. 1998. Adrenal rennin angiotensin aldosterone system in streptozotocin diabetic rats. Horm. Metab. Res. 30, 12-15.

Narendhirakannan, R.T., Subramanian, S. and Kandaswamy, M. 2006. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin induced diabetes in experimental rats. Clinical and Experimental Pharmacology and Physiology. 33, 1150 –1157.

Odetola, A.A., Akinloye, O., Egunjobi, C., Adekunle, W.A. and Ayoola, A.O. 2006. Possiblem antidiabetic and antihyperlipidaemic effect of fermented Parkia biglobosa (JACQ) extract in alloxan-induced diabetic rats. Clin. Exp. Pharmacol. Physiol. 33, 808–812.

Oi, K., Komori, H. and Kajinuma, H. 1997. Changes in plasma glucose, insulin, glucagon, catecholamine, and glycogen contents in tissues during development of alloxan diabetes mellitus in rats. Biochem. Mol. Med. 62, 70–75.

Paolisso, G. and Barbagallo, M. 1997. Hypertension, diabetes mellitus and insulin resistance; the role of intracellular magnesium. Am. J. Hypertens. 10, 346-355.

Pari, L. and Amarnath Satheesh, M. 2004. Antidiabetic activity of Boerhaavia diffusa L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol. 91(1), 109-13.

Prince,S.M.P. and Menon,V. P. 2001. Antioxidant action of Tinospora cordifolia roots extract in alloxan diabetic rats. Phytother Res. 15, 213–218.

Rajkumar, L., Srinivasan, N., balasubramanian, K. and Govindarajulu, P. 1991. Increased degradation of dermal collagen in diabetic rats. Indian J. Exp. Biol. 29, 1081–1083.

Ramachandran, A. 2007. Socio-economic burden of diabetes in India. J. Assoc. Phys. India. 55, 9–12.

DOI: 10.2202/1553-3840.1533

Rao, K., Nalamolu, Krishna, M., Boini and Srinivas nammi. 2004. Effect of chronic administration of Boerhaavia diffusa Linn. leaf extract on experimental diabetes in rats. Tropical J. of Pharmaceutical Research. 3(1), 305-309.

Raocah, D.A., Cloudie and Azulay, J.P. 1996. Erythrocyte Na-k-ATPase activity, metabolic control and neuropathy in IDDM patients. Diabetes Care. 19, 564-568.

Reinhart, R.A. 1988. Magnesium Metabolism: a review with special reference to the relationship between intracellular content and serum levels. Arch. Intern. Med. 148, 2415-2420.

Resnick, L.M., Gupta, R.K., Bhargava, K.K., Gruenspanlt Alderman, M.H. and Laragh, J.H. 1999. Cellular ions in hypertension, diabetes and obesity: a nuclear magnetic resonance spectroscopic study. Hypertension. 17, 951-957.

Rohrscheib, M., Tzamaloukas, A.H., ing, T.S., Siamopoulos, K.C., Elisaf, M.S. and Murata, H.G. 2005. Serum potassium concentration in hyperglycemia of chronic dialysis. Adv Perit Dial. 2, 102-5.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G. and Selenium. 1973. Biochemical role as a component of glutathione peroxidase. Science. 179, 588-590.

Sansom, S.C. and O'Neil, R.G. 1981. Mineralocorticoid requirement of apical cell membrane of sodium and potassium transport of cortical collecting ducts. Am. J. Physiol. 248, F858.

Saxena, A.K., Srivastava, P., Kale, R.K. and Baquer, N.Z. 1993. Impaired antioxidant status in diabetic rat liver.Effect of vanadate. Biochem Pharmacol. 45(3), 539–542.

Shahid,S.M., Rafique,R. and Mahboob,T. 2005. Electrolytes and sodium transport mechanism In diabetes mellitus. Pakistan J. of Pharmaceutical Sciences. 18(2), 6-10.

Singh, P.K., Baxi, D.B., Mukherjee, R. and Ramachandran, A.V. (A). 2010. Evaluation on the efficacy of a Poly herbal supplement along with exercise in alleviating Dyslipidemia, Oxidative stress and hepatic and renal toxicity associated with Type-1 diabetes. Journal of Herbal Medicine and Toxicol. 4(1), 207-215.

Singh, P.K., Baxi, D.B., Mukherjee, R., Selvaraj, J. and Ramachandran, A.V. (B). 2010. Diabetic amelioration by poly herbal supplement and exercise: studies on type – i diabetic rat model. J. of Herbal Medicine and Toxicology. 4 (1), 217-226.

Sinha, A. 1972. Catalase - An extra ordinary enzyme. Sci. 210, 71-82.

Surange, S.R. and Pendse, G.S. 1972. Pharmacognostic study of roots of *Boerhaavia diffusa* Willd. (*punarnava*). Journal of Research in Indian Medicine 7:1.

Totan, A.R. and Greaby, M. 2002. Effect of chronic hyperglycemia and vanadate treatment on erythrocyte Na+- K+-ATPase and Mg++- ATPase in streptozotocin in diabetic rats. Acta. Pol. Pharm. 59, 307-311.

Trinder, P. 1969. Glucose estimation by glucose oxidase method using phenol and 4-aminophenazone. Ann.Clin.Biochem. 6, 24-31.

Turner, R.C., Millns and Noil, A.W. 1998. Risk factors for coronary artery diseases in non-insulin dependent diabetes mellitus. Uniterd Kingdom Prospective Diabetes Study (UKPDS: 23) B M J, 316, 823-828.

Tzamaloukas, A.H., ING, T.S., Siamopoulos, K.C., Rohrscheib, M., Elisaf, M.S., Raj, D.S. and murata, G.H. 2008. Body fluid abnormalities in severe hyperglycemia in patients on chronic dialysis: review of published reports. J Diabetes Complications. 22(1), 29-37.

Tzamaloukas, A.H., Rohrscheib, M., ING, T.S., Siamopoulos, K.C., Qualls, C., ELISAF, M.S., Vanderjagt, D.J. and Spalding, C.T. 2005. Serum potassium and acid-base parameters in severe dialysis-associated hyperglycemia treated with insulin therapy. Int J Artif Organs. 28(3), 229-36.

Ustundag, B., Canatan, H., Cinkiline, N., Halifoeglu, I. and Bacecioglu, I.H. 2000. Angiotensin converting enzyme's activity in insulin dependent diabetes mellitus and effect of ACE levels on diabetic patients with nephropathy. Cell Biochem. Func, 18, 23-28.

Wahab,A.N., Natalia, Y., Benjamin, S., Weston, T. R., Xin, J., Brinkman,H. and Roger, M. 2001. Role of connective tissue growth factor in the pathogenesis ofdiabetic nephropathy. Biochem. J. 359, 77-87.

Weidmann, P. and FerrarI, P. 1991. Central role of sodium in hypertension in diabetic subjects. Diabetes care. 14, 220-232.

DOI: 10.2202/1553-3840.1533