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Oral Administration of Root Extract of *Boerhaavia diffusa* Mitigates Diabetes-induced Kidney Damage in the Golden Hamster *Mesocricetus auratus*

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Abstract

Diabetes, a common metabolic disorder, is affecting people irrespective of age group and/or gender. The chronic hyperglycemia during diabetes leads to heart-, kidney-, eye- and nerve damages. *Boerhaavia diffusa,* commonly known as Punarnava, is one of the traditional medicines described in Ayurveda for the treatment of a number of diseases. *B. diffusa* has been reported to exhibit antidiabetic, diuretic, anti-inflammatory, hepatoprotective, and immunomodulatory properties. The present study was carried out to evaluate the anti-hyperglycemic potential of ethanolic extract of root of *B. diffusa* and its effect on diabetes-induced kidney damage in hamster model. Treatment of ethanolic extract of *B. diffusa* resulted in significant reduction in the serum glucose level and increased insulin concentration with a simultaneous increment in the levels of muscle and liver glycogen. The oral supplementation of *B. diffusa* root extract improved lipid profile by reducing the cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and by increasing high density lipoprotein cholesterol (HDL-C). The activity of antioxidant enzymes Superoxide Dismutase (SOD) and Catalase (CAT) in the kidney significantly improved following oral administration of *B. diffusa* while a significant decrease in Lipid Peroxidation (LPO) level was noted in the kidney after the treatment. Ethanolic root extract of *B. diffusa* decreased the level of serum creatinine, urea and Alkaline Phosphatase (ALP) significantly. *B. diffusa* administration demonstrated marked improvement in renal histology as evident by the regenerative changes in glomerulus and Bowman's capsule. Thus, the use of ethanolic extract of *Boerhaavia diffusa* may prove therapeutically effective in prevention as well as inhibition of the progression of diabetes and associated kidney damages.

Keywords: *Boerhaavia diffusa*, Diabetes, Diabetic Nephropathy, Hamsters, Punarnava

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1. Introduction

Diabetes, a metabolic lifestyle disorder, is spreading around the globe like an epidemic, and is affecting people irrespectively of their age group and gender. The long-term effects of diabetes lead to heart-, kidney-, eye- and nerve-damage. The hypoglycaemic agents used in the treatment of diabetes lead to various side effects and ailments[1]. On the contrary the natural medicines involving plant products are relatively safe and free from side effects. One of the plants of high repute in the field of herbal medicines is *Boerhaavia diffusa,* popularly known as Punarnava. *B. diffusa,* belonging to family Nyctaginaceae, is one of the traditional medicines described in Ayurveda for the treatment of a number of diseases[2]. *B. diffusa* has been reported to exhibit antidiabetic, diuretic, anti-inflammatory, anticonvulsant, antibacterial, hepatoprotective, and immunomodulatory effects[2–4]. The antidiabetic properties in root, leaf and whole plant extracts of *B. diffusa* have been demonstrated by various research groups[2-7].

The nephroprotective potential of *B. diffusa* in drug-induced nephrotoxicity is well established[8-12]. Pareta *et al.*,[8] found that aqueous extract of *B. diffusa* roots improves ethylene glycol-induced hyperoxaluric oxidative stress and renal cell injury in kidney of rats. Sawardekar and Patel[9] demonstrated that aqueous extract of *B. diffusa* protects against structural and functional damages induced by gentamicin. Leaf extract of *B. diffusa* was reported to ameliorate drug-induced renal toxicity and reduce the toxicity induced by mercuric chloride[10]. In a similar study pre-treatment of *B. diffusa* potentially prevented acetaminophen-induced nephrotoxicity in rats[11]. Oburai *et al*[12] carried out a clinical comparison of *B. diffusa* root extract with modern drug Enalapril in Chronic Renal Failure (CRF) in dogs. The study revealed that *B. diffusa* effectively improved several clinical parameters along with less mortality in canines when compared to Enalpril.

Although *B. diffusa* has been utilized as a traditional medicine for kidney diseases, yet there are only a few preliminary reports[13–15] that suggest the use of *B. diffusa* in diabetes-induced kidney damage. Therefore, the present study has been carried out to evaluate the anti-hyperglycemic activity of this plant and explore its effect in diabetes-induced kidney damage in the golden hamster, *Mesocricetus auratus*.

2. Materials and Methods

2.1 Ethical Consideration

The experiments conducted on the hamsters were in accordance with the prescriptions of the Institutional Animal Ethics Committee (IAEC) under the framework of CPCSEA (Committee for Purpose of Control and Supervision of Experiments on Animals), Government of India (2001).

2.2 Animals

Golden hamster *Mesocricetus auratus* were procured from CDRI, Lucknow, UP, India, and kept in polypropylene cages. The hamsters were maintained in a well-ventilated room with ambient conditions (25±2°C, with gentle ventilation). They were fed the commercial feed and water *ad libitum*. Hamsters weighing 100±10 g were selected for the experiments.

2.3 Preparation of the Root Extract

The root extract of *Boerhaavia diffusa* was prepared at the Department of Dravyagun, Institute of Medical Science, Banaras Hindu University, Varanasi, UP, following the procedure of Akhter *et al*[13]. The shade-dried roots of *B. diffusa* were processed mechanically to obtain a course granular powder. About 250g of dried powder was extracted with 90% ethanol by continuous hot percolation, using Soxhlet apparatus. The resulting dark–brown extract was concentrated up to 100 m Lusing Rota vapor under reduced pressure. The concentrated crude extracts were lyophilized into powder and was used as aqueous suspension for the study.

2.4 Induction of Diabetes

The randomly selected animals were fasted overnight followed by a single dose of streptozotocin (STZ) the next day. Streptozotocin (Sigma-Aldrich, USA) dissolved in citrate buffer (pH=4), was injected through *intra-peritoneal* route (*ip*) at the dose of 50 mg/kg bw[16]. The animals were given 20% glucose solution for twenty-four hours to avoid STZ-induced initial mortality. The Fasting Blood Glucose (FBG) of the hamsters was checked after 72 hour of STZ injection (AccuChek, USA). The hamsters depicting FBG greater than 200 mg/dL were considered diabetic.

2.5 Experimental Design

The hamsters were randomly divided into three groups of six animals each.
Group I: Control (normal, untreated)
Gropu II: Diabetic control (STZ treated)
Group III: Diabetic; *B. diffusa*-treated (STZ-treated, and administered aqueous suspension of *B. diffusa;* 200 mg/kg body weight for 4 weeks)[2-6].

2.6 Sample Collection

The diabetic animals received *B. diffusa* through an oral gavage for 4 weeks. After completion of the experiment the animals were fasted overnight, weighed and sacrificed. The serum was separated from the blood which was directly collected from the heart, and was frozen at -80° C which was later used for the ELISA for insulin determination (DIAMETRA, Lot No. DKO076), and biochemical estimations of serum glucose, Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), and Low-Density Lipoprotein Cholesterol (LDL-C), circulating levels of creatinine, urea and alkaline phosphatase. Kidney, liver and muscle were dissected out on ice, blotted and cleared of extra tissue. The right kidney was used for assays of Lipid Peroxidation (LPO), Superoxide Dismutase (SOD) and catalase (CAT). Liver and muscles were used for biochemical estimation of glycogen. Left kidney was fixed in neutral buffered formalin for histological studies.

2.7 Biochemical Estimations

Manufacturer's protocol (BioLab Diagnostics, India) was used to determine glycogen level in liver and muscle, serum glucose, creatinine, urea and alkaline phosphatase (ALP), Total Cholesterol (TC), High density lipoprotein Cholesterol (HDL-C), and LowdensityLipoprotein Cholesterol (LDL-C). Serum insulin level was measured using the ELISA kit (Diametra, DKO076). The antioxidant status in kidney was evaluated by measuring the activity of antioxidant enzymes *viz.* Super Oxide Dismutase (SOD) activity using the method of Das *et al*[17]. The protocol described by Sinha[18] was followed to determine catalase (CAT) activity whereas Lipid Peroxidation (LPO) was evaluated using the method of Okhawa *et al*[19].

2.8 Histological Studies

After fixation in neutral buffered formalin, the kidney was processed for routine histological procedure. Some 6-µm sections were deparaffinized, and stained using Ehrlich's hematoxylin and eosin. The stained sections of the tissues were observed under microscope (Leica MPV-3, Germany) and documented.

3. Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 8 (USA). The data were analyzed using Student's t-test (For two groups). One-way Analysis of Variance (ANOVA) followed by Tukey's multiple-range test for multiple comparisons were conducted. All the data were expressed as the means ± Standard Error of Means (SEM). Values of $p<0.05$ were considered as statistically significant.

4. Results

B. diffusa treatment reduced the serum glucose level and elevated insulin concentration significantly (Figure 1.1 & 1.2). The oral supplementation of *B. diffusa* improved lipid profile by reducing the cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and increasing high density lipoprotein cholesterol (HDL-C) (Figure 1.3). Significant increase in glycogen level was found in both muscle and liver following *B. diffusa* treatment (Figure 1.4). There was a significant decrease in Lipid Peroxidation (LPO) in the kidney following *B. diffusa* treatment (Figure 1.5). The level of super oxide dismutase SOD (Figure 1.6) and catalase (Figure 1.7) in kidney was found to be significantly elevated after the oral administration of *B. diffusa*. The root extract of *B. diffusa* decreased the level serum creatinine (Figure 1.8), serum urea (Figure 1.9) and Alkaline Phosphatase (ALP) significantly (Figure 1.10). *B.diffusa* treatment demonstrated marked improvement in renal histology as evident in the regenerative changes of glomerular tufts in glomerulus and decreasing Bowman's space in Bowman's capsule (Figure 1.11).

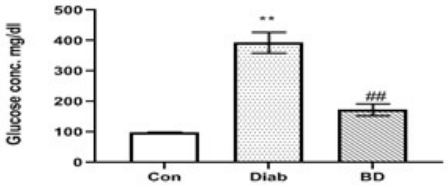

Figure 1.1 Bar graphs depicting significant reduction of serum glucose following BD treatment

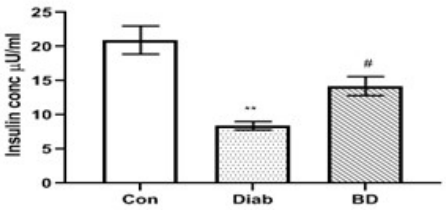

Figure 1.2 Bar graphs depicting significant increase in serum insulin following BD treatment

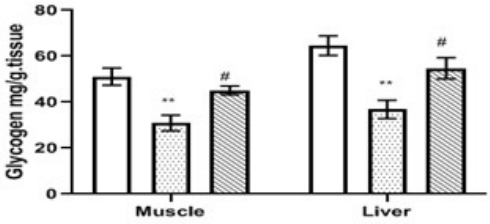

Figure 1.3 Bar graphs depicting significant elevation in muscle and liver glycogen following BD treatment

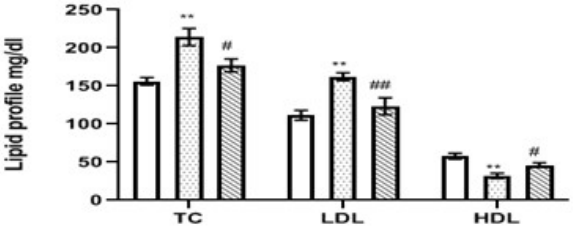

Figure 1.4 Bar graphs showing significant reduction in TC, LDL-C, and increase in HDL-C following BD treatment

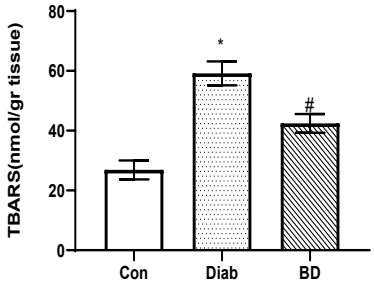

Figure 1.5 Bar graphs depicting significant reduction in Lipid Per-Oxidase (LPO) level in kidney after BD treatment.

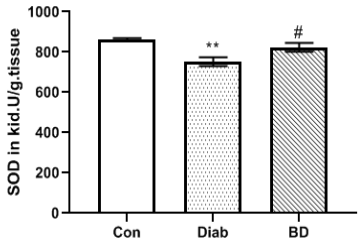

Figure 1.6 Bar graphs showing significant increase in activity of SOD, in kidney after BD treatment.

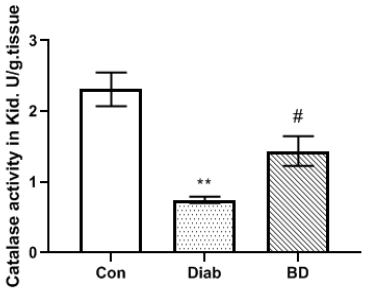

Figure 1.7 Bar graphs showing significant increase in activity of CAT in kidney after BD treatment.

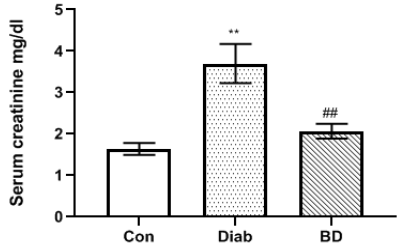

Figure 1.8 Bar graphs representing significant reduction in serum creatinine following BD treatment.

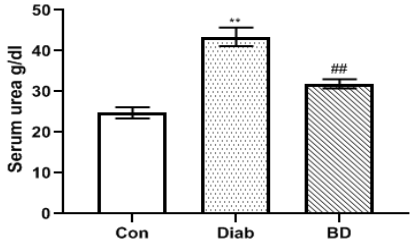

Figure 1.9 Bar graphs representing significant reduction in serum urea following BD treatment.

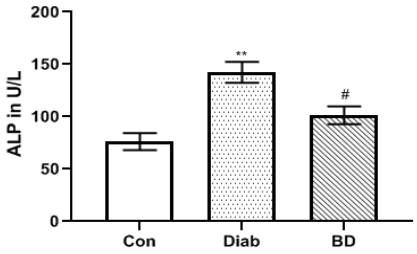

Figure 1.10 Bar graphs representing significant reduction in serum ALP following BD treatment.

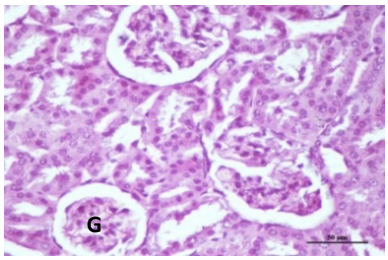

Figure 1.11 A. (Control)

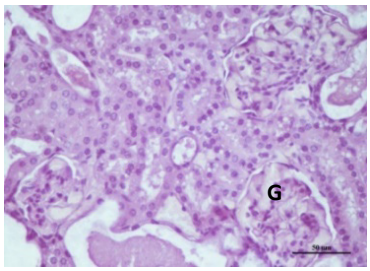

Figure 1.11 B. (Diabetic)

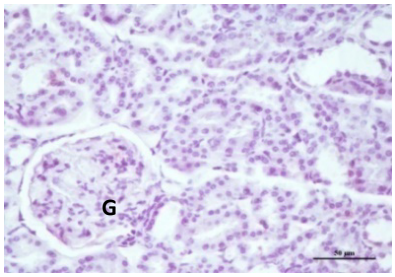

Figure 1.11 C. (*B. diffusa)*

Figure 1.11 Photomicrographs of H & E-stained kidney sections from control hamster **(A),** diabetic hamster **(B),** Punarnava-treated (C). G: Glomerulus. Microscopic examination of kidney of control animals (A) showed normal histoarchitecture of the tissue, glomeruli (G) appeared as dense tufts of capillaries enclosed in the outer layer of Bowman capsules.On the other hand, the kidney of the diabetic hamsters demonstrated severe degeneration in glomerulus (B). Punarnava treatment demonstrated marked improvement in renal histology as evident by the regenerative changes in glomerulus and Bowman's capsule (C).

5. Discussion

The present study was aimed at evaluating the efficacy of *Boerhaavia diffusa* against the hyperglycemia-induced kidney damages. *B. diffusa*, popularly known as Punarnava, is used in Ayurveda and Unani medicines and is commonly consumed as a green leafy vegetable by South Asian population in view of its nutraceutical properties. It is known for its anti-inflammatory, anti-stress, anti-diabetic, hepatoprotective and immunomodulatory properties[1,3-7]. Despite its traditional use as a diuretic and kidney tonic there are no reports to-date that could support the utility of *B. diffusa* in chronic hyperglycemia-induced kidney damages. In the present study ethanolic root extract of *B. diffusa* was administered orally to hyperglycemic hamsters for 4 weeks. The effects of root extract administration were assessed on glucose, insulin, glycogen, creatinine, urea and ALP concentrations, lipid profile, oxidative stress markers and histology of kidney.

Administration of *B. diffusa* to diabetic hamsters lowered glucose level and elevated insulin concentration. Insulin is the most important factor in lowering the blood glucose level by enhancing glycogenesis in the liver and muscle. Hence, glycogen level is an important marker for the study of insulin activity[20]. The induction of T2D in the hamsters significantly reduced the glycogen level in both liver and muscle tissues. Previous studies by Shajeela *et al.*,[21] and Soliman *et al.*,[22], suggested the disrupted glucose homeostasis while in the present study it caused derangements in the lipid profile as manifested by increased Total Cholesterol (TC), LDL cholesterol (LDL-C) and decreased HDL cholesterol (HDL-C). This marked hyperlipidemia characterizes the diabetic state and the insulin deficiency can be related to the excess lipolysis, increased influx of free fatty acids to the liver and deranged lipoprotein metabolism during diabetes[23]. Clinically, estimation of insulin secretion is highly relevant in diabetes, but C-peptide test is also one of the reliable methods for estimating the activity of beta cells of pancreas since c-peptide and insulin are equivalently generated following proinsulin cleavage. Further, c-peptide is degraded slowly in the body as compared to insulin[24]. However, we have not performed the c-peptide test to extend further the discussion.

Hyperglycemia promotes production of excessive reactive oxygen species (ROS). Lipid Peroxidation (LPO) is regarded as one of the important attributes of diabetes. The streptozotocin treatment results in formation of hydroxyl radical that would damage the cell membranes and thus increases the levels of LPO. In the current experiment the level of LPO was found to be significantly elevated in the kidney of diabetic hamsters. The increased level of ROS resulted in oxidative stress condition in the kidney of diabetic hamsters that was exhibited by its enzymatic markers i.e., Super Oxide Dismutase (SOD), and Catalase (CAT). SODs are a family of metallo-enzymes that catalyze the degradation of superoxide into oxygen and hydrogen peroxide[25]. Catalase (CAT) is also an important enzyme in the supportive team of defense

against ROS; it catalyzes the reduction of hydrogen peroxide[26].

Diabetic nephropathy is a major complication associated with T2D and is considered to be the main cause of end stage renal disease[27]. Diabetes leads to a significant change in renal architecture like reduction of glomerular tufts and increasing Bowman's space[28]. The diabetes led changes were also noted in the histology of the kidney of diabetic hamsters in the present study. Depletion of renal tissue in diabetic model can mainly be attributed to the excessive production of ROS which also impairs other renal functions such as serum creatinine, urea and serum levels of alkaline phosphatase[29]. According to Sharma *et al*.,[30] creatinine is a very sensitive and specific test of renal function, and serum creatinine is considered useful in diagnosing renal failure. The current investigation revealed that induction of diabetes resulted in elevation of serum creatinine indicating disturbance in kidney functions. The next marker to be tested for kidney function was the level of serum urea. Urea is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extra cellular fluid. Serum urea concentration is the most frequently determined clinical indices for estimating renal functions[31]. Significant elevation (depicting abnormal renal function) was noted in the level of serum urea in the diabetic hamsters in present study. Alibawi *et al*.,[32] explain that alkaline phosphatase is produced from the enzymes that cause loss of the phosphorus group, which is effective in several tissues (kidney, liver, bone, *etc*). The increment in the level of alkaline phosphatase might be the result of diabetes-induced damage to the renal tissues[33].

In the present study the diabetic hamsters showed marked improvement in all the parameters following ethanolic *B. diffusa* treatment.

6. Conclusion

In conclusion, the STZ-induced diabetes caused adverse effects on glucose, insulin, glycogen, creatinine, urea and ALP concentrations, lipid profile, and oxidative stress markers. The oral administration of *B. diffusa* for 4 weeks affected the biochemical parameters to variable degrees. The antioxidant parameters considered in the present study were improved following ethanolic *B. diffusa* treatment. The histological study of the kidney also depicted marked improvement following the Punarnava treatment.

The use of Punarnava, *Boerhaavia diffusa,* may prove therapeutically effective in the prevention and inhibition of the progression of diabetes and associated kidney damages. However, further studies are needed to explore the use of *Boerhaavia diffusa* in the treatment of diabetes and nephropathy.

7. Acknowledgments

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