



**PROTECTIVE EFFECTS OF BOERHAVIA DIFFUSA LINN. (NICTAGINACEAE) ON
LIVER OF STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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ABSTRACT

The aim of this study was to evaluate the hepatoprotective of *Boerhavia diffusa* Linn. used in Benin and other countries to manage control and treat diabetes. Streptozotocin (STZ) is selectively toxic to cells in the pancreatic islets. It is well known that STZ causes specific death of B cells and induces diabetes mellitus. Rats were kept under eighteen hours fasting and then subjected to diabetic by intraperitoneal (i.p.) injection of streptozotocin (40 mg/kg body weight). The duration of experiment was 28 days. Blood glucose levels, the relative percentage of liver weight and liver histology were studied. There were significant increase in relative liver weight, blood glucose levels in diabetic untreated comparing to normoglycemic (control) ($p < 0.05$). The results showed several morphological and histological alterations in liver tissues. These pathological changes were ameliorated in the *Boerhavia diffusa* Linn. extract and glibenclamide treated rats. *Boerhavia diffusa* Linn. extract has a protective effect on tissue damage probably due to its antioxidant activity and possess the ability to regenerate β -cell in STZ-induced diabetic rats.

KEYWORDS: *Boerhavia Diffusa* Linn. (Nyctaginaceae), Diabetes Mellitus, Glibenclamide, Liver, Rats, Streptozotocin.

1. INTRODUCTION

Chronic hyperglycemia in the course of diabetes contributes to the oxidative stress, causing the redox balance of the body to change with excessive production of reactive oxygen species.^[1] Oxidative stress leads to an increase in protein, lipid, carbohydrate and DNA oxidation. Accordingly, it causes tissue and organ damage.^[2] It is emphasized that this condition causes micro- and macrovascular complications of diabetes.^[3] Oxidative stress is observed to be increased in many organs, particularly in the liver, due to diabetes.^[4] Since the liver has a central and critical role in the regulation of carbohydrate metabolism^[5], various structural and functional disorders affecting glycogen and lipid metabolism are formed in the liver as a result of diabetes.^[6] Similarly, the oxidative stress which is caused by hyperglycemia is effective in the pathogenesis of

diabetic kidney complications.^[7] In addition, damage observed in β cells of pancreas, which is known to be one of the most sensitive structures to oxidative stress, is thought to be caused due to toxic effects of hyperglycemia.^[8] Glycation-mediated free radical production leads to beta cell apop-tosis and decreases the gene transcription of insulin.^[9] Glutathione peroxidase (GPx), which is one of the natural antioxidant defense systems against free radicals, prevents lipid peroxidation by catalyzing the reduction of hydrogen peroxide (H_2O_2) or organic hydroperoxides to water or alcohols by using reduced glutathione^[10], and it protects cell membranes against oxidative damage.^[11] GPx1 is expressed in most tissues, and it is an intracellular selenoprotein with high expression levels in erythrocytes, liver, and kidneys.^[12] In patients with diabetes, oxidative stress is important in the pathogenesis of chronic complications of diabetes

due to the reduction of antioxidant parameters such as vitamins C and E, glutathione, superoxide dismutase (SOD), catalase (CAT) and GPx.^[13] In several studies, polyphenolic compounds are displayed to be potential antioxidants in the treatment and prevention of oxidative stress-related diabetic complications in rats induced by using streptozotocin (STZ).^[14,15]

Our results from studies have demonstrated that the methanol extract of the leafy stem powder of *Boerhaavia diffusa* L. gave a positive result for all groups secondary metabolites investigate. The highest content of total phenolics, flavonoids and tannins were detected in methanol extract followed respectively by ethanol, dichloromethane and ethyl acetate. The lowest total phenolics were obtained in n- Hexane.^[16]

Moreover, the results obtained in studies indicate that methanol extract of *Boerhaavia diffusa* Linn. have a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species, reduce the oxidized intermediates and act as primary antioxidant substances.^[16]

Nevertheless, the details of the relationship between hepatoprotective effects and antioxidant activity of methanol extract of the leafy stem powder of *Boerhaavia diffusa* L. in normal and streptozotocin induced diabetic rats have not yet been fully elucidated. Because of the importance of the liver in carbohydrate metabolism, the present study is to investigate the hepatoprotective of *Boerhaavia diffusa* Linn. in diabetic mice.

2. MATERIALS AND METHODS

2.1 Experimental animals

Adult male wistar rats, 2–3 months old and weighing 250-300 g, were used in the study. The rats are acclimatized in the Laboratory of Physiopathologie Moléculaire et Toxicologie (Faculty of Science and Technology of the University of Abomey-Calavi) for two weeks before the beginning of the experiment at a constant temperature of 22 ± 1 °C with a cycle of 12h of light and 12 h of darkness. They are fed with granulated feed and ad libitum water without discontinuity in feeding bottles.

The experimental protocol was approved by the Scientific Ethics Committee of the Doctoral School (Life Sciences) of the Faculty of Science and Technology (FAST) at the University of Abomey Calavi (UAC) under the number (UAC/FAST/EDSV/353600).

2.2 Plant material

The stem leaves of *Boerhaavia diffusa* Linn. were used in this study. Fresh stem leaves of *Boerhaavia diffusa* were collected from Calavi, Department of Atlantic, South Bénin. The samples of *Boerhaavia diffusa* were submitted in Abomey-Calavi University Herbarium, Department of Botany and voucher specimen deposited for authentication under the reference AA 6716/ HNB.

The collected material was dried for two weeks in laboratory (22°C) and ground to a fine powder using an electric grinder (Excella mixer grinder).

2.3 Preparation of methanol extract of stem leaves of *Boerhaavia diffusa* L.

Two hundred and fifty grams (250 g) of dry powder of the stem leaves of *Boerhaavia diffusa* were successively extracted by maceration with methanol for 72 h stirring. Extract were dried by evaporating using rotary evaporator. This methanol extract stored at 4°C till ready for use.

2.4 Induction of Diabetic with streptozotocin

Initially normoglycaemic (fasting blood glucose level 70-80 mg/dL) rats were selected for this study. Rats were kept under eighteen hours fasting and then subjected to diabetic by intraperitoneal (i.p.) injection of streptozotocin (40 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5. In control group, 6 rats were injected with citrate buffer alone.^[17] Diabetic condition was confirmed by estimation of fasting blood glucose level after 24 hrs interval and then on the 7th day after day of injection to investigate the stability of the diabetic condition. The rats with fasting blood glucose more than 250 mg/dl were included for this investigation.

2.5 Grouping of Animals

In this experiment 24 rats (6 control and 18 STZ diabetic existing rats) were used. They were separated into four groups of 6 rats each.

Group 1: Normoglycemic (control) received a single intramuscular injection of citrate buffer (0.1 mL/100 g body weight/rat).

Group 2: Diabetic untreated (Negative control) was made diabetic by a single intramuscular injection of STZ at a dose of 40 mg/kg body weight.

Group 3: Diabetic treated with standard drug–glibenclamide (Positive control)

Group 4: Diabetic treated with 600 mg/kg bodyweight of methanol extract.

The duration of experiment was 28 days. Initial body weight of all rats were recorded and divided into following four equal groups.

2.6 Treatment with Extracts

Effect of extract was checked on blood glucose, and liver histopathological changes of experimental rats. The methanol extract was dissolved in distilled water and administered to the rats at a ratio of 1 ml/100 g of body weight and glibenclamide (standard drug) were dissolved in 10 ml normal saline (0.9% NaCl).

2.7 Biochemical assays

On the last day of study, a complete blood sample was collected from the abdominal aorta after deep anaesthesia

and the plasma was isolated by centrifugation at 2500 rpm for 5 min at 4°C. Blood glucose levels were measured by the glucose-oxidase method using an Accu-chek blood glucose meter.

2.8 The measurement of body weight and relative percentage of liver weight

The measurement of body weight (g) for all rats was recorded on the 1st, 5th, 10th, 20th, and 30th day of. At sacrifice, the measurement of liver relative to weight was determined for all rats according to the equation:

The relative percentage of liver weight = [liver weight / body weight] × 100

The experimental protocol was approved by the Scientific Ethics Committee of the Doctoral School (Life Sciences) of the Faculty of Science and Technology (FAST) at the University of Abomey Calavi (UAC) under the number (UAC/FAST/EDSV/353600).

2.9 Histological Studies

At the end of the experimental period, the tested animals were sacrificed using Ketamine as an aesthetic. Liver specimens were rapidly removed, fixed in 10% formal saline for 72 hours, dehydrated through graded alcohols and cleared using two changes of xylene and embedded in paraffin wax. Serial transverse sections of 4-5 micron thickness were prepared using the microtome, stained Haematoxylin and eosin (H & E), others stained by periodic Acid-Schiff stain (PAS) with salivary amylase application.

2.10 Statistical analysis

Data analyses were performed using statistic software IBM SPSS Statistics 21 (IBM Corporation, NY, USA). Data were expressed as mean ± standard deviation (SD). The results were considered as significant at P value less than 0.05. Student *t*-test were used to compare the data within the group. Additionally, one way analysis of variance (ANOVA) was used to compare the data between experimental groups, followed by Tukey's post hoc test for multiple groups' comparison.

3. RESULTS

3.1 Effects of methanolic leaf extract of *Boerhavia Diffusa* Linn. (Nictaginaceae) on serum glucose

Anti-hyperglycemic effects of *Boerhavia Diffusa* Linn. (Nictaginaceae) on control and STZ-induced diabetic rats which were measured every week over the entire experimental time are reported in Figure 1. Fasting blood glucose levels of diabetic group were increased after the induction by streptozotocin and remained over 300 g/dl up to the end of study. Treatment of diabetic rats with glibenclamide and *Boerhavia Diffusa* Linn. extract has reduced the elevated levels of fasting blood glucose throughout the study period. In glibenclamide group, the mean fasting blood glucose slightly increased on day 7, then decreased on days 14 and 28. The reduction of fasting glucose level in glibenclamide group was statistically significant as compared to diabetic group ($p < 0.05$).

In *Boerhavia Diffusa* Linn. group, the rats showed a slight elevation in the mean fasting glucose level on the 7th day of study period. After 2 week of treatment, blood glucose concentration was reduced by 50%, and at the end of experiment blood glucose concentration was diminished by 80 % from the initial concentrations.

3.2 Effects of methanolic leaf extract of *Boerhavia Diffusa* Linn. (Nictaginaceae) on Mean body weight

The effect of methanolic leaf extract of *Boerhavia Diffusa* Linn. on the body weight of control and STZ-induced diabetic Wistar rats, recorded for 28 days are shown in Figure 2. Body weight of control rats was increased during the period of experiment, on the contrary, with diabetic group which showed decreasing in body weight from the second week of experiment. In control groups, the rats maintained a steady increase in body weight and reached to about 300 ± 8.65 g on day 28 compared to 200 ± 5.85 g on the first day of the study (Figure 2). The body weight of other groups treated with methanolic leaf extract of *Boerhavia Diffusa* Linn. and glibenclamide was ascended. The ameliorative effect of glibenclamide and *Boerhavia Diffusa* on body weight was statistically significant compared to diabetic group ($p < 0.05$).

3.3 Effects of methanolic leaf extract of *Boerhavia diffusa* Linn. (Nictaginaceae) on absolute liver weight and relative liver weight of experimental groups

The absolute liver weight was nearly similar in all groups. However, a significant increase in the relative percentage of liver weight has been shown in this study (Figure 3). The relative weight of liver with respect to body weight was higher in diabetic groups than in control groups (Figure 3).

The relative weight of liver in control groups was approximately 3 ± 0.35 g. Diabetic group was presented with a relative liver weight of 5.10 ± 0.30 g, which is significantly higher than that in control group ($p < 0.05$). The increase in liver relative weight in diabetic rats was ameliorated in methanolic leaf extract of *Boerhavia diffusa* Linn. and glibenclamide groups to be 4.20 ± 0.35 g and 3.10 ± 0.40 g respectively.

3.4 Effects of methanolic leaf extract of *Boerhavia diffusa* Linn. (Nictaginaceae) on liver histopathological changes:

Control rat liver section showed normal liver architecture consisting of central vein and anastomose network of healthy hepatocytes arranged in strands, some of them showed double nuclei as a result of regeneration, strands of hepatocytes separated from each other by blood sinusoids, Kupffer cells were abundant in the sinusoids (Fig. 4. A).

Histopathological examination of diabetic rat liver shows severe pathological alterations manifested by dilated and congested portal vein. Hepatocytes suffered from severe cytoplasmic degeneration was observed in STZ induced diabetic rat liver (Figure 4. B) when compared to normal

rats (Figure 4. A). Diabetic rats treated with *Boerhavia diffusa* group indicated improved histopathology of the liver as compared to the diabetic control group rats (Figure 5. C). Glibenclamide treated group rats showed liver histopathology similar to the normal control rats (Figure 5. D).

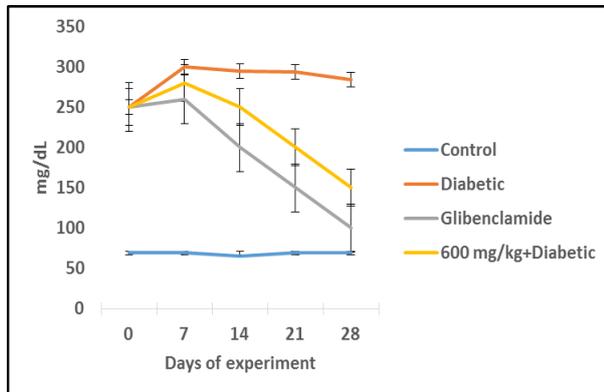


Fig. 1. Fasting blood glucose levels of all groups during different days, treated with methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide for 4 weeks. Each value represents the mean \pm SEM (n=6).

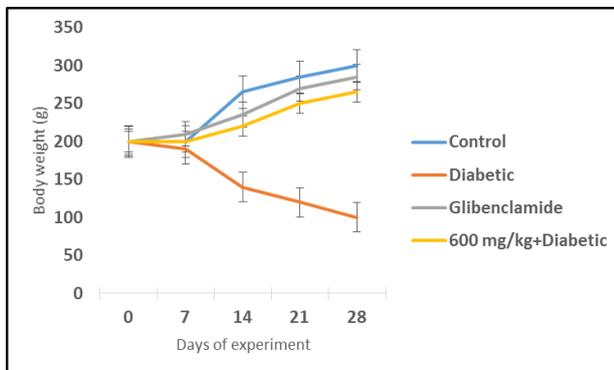


Fig. 2. Body weight of all groups during different days, treated with methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide for 4 weeks. Each value represents the mean \pm SEM (n=6).

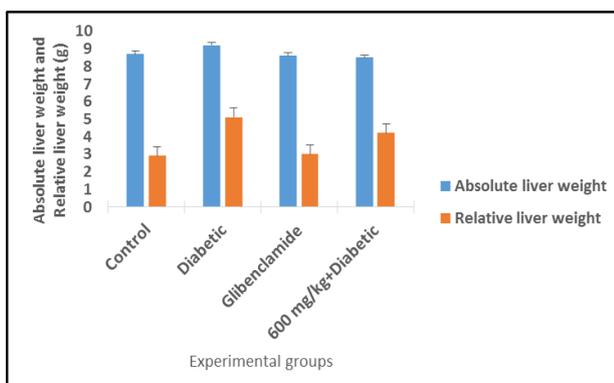


Fig. 3. Absolute liver weight and relative liver weight of experimental groups, treated with methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide for 4 weeks. Each value represents the mean \pm SEM (n=6).

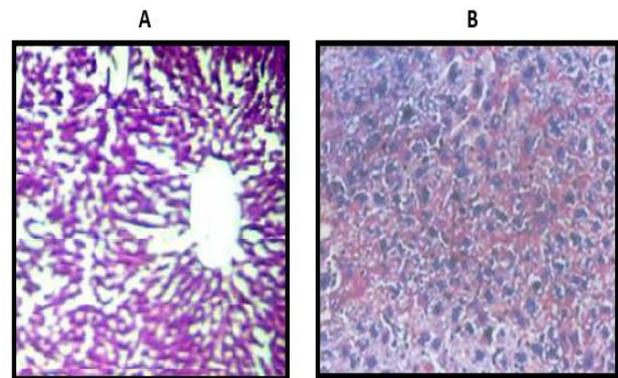


Fig. 4. A. normal control rats showing normal histological structure, regular distinct hepatocytes with sinusoidal spaces arranged radially around the central vein. B. STZ diabetic rats showing degeneration of hepatocytes, vacuolization of cytoplasm, and cloudy swelling, congested central vein and loss of glycogen granule and disarranged hepatocytes of liver parenchyma.

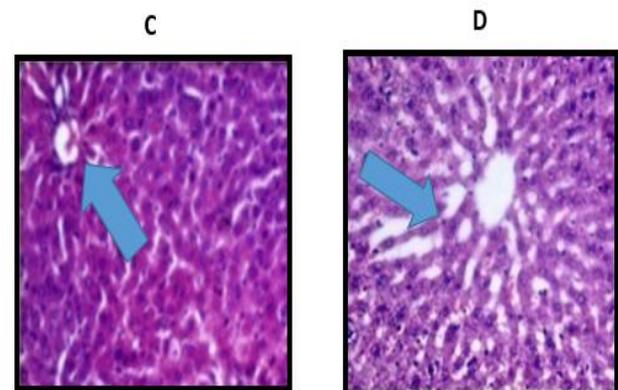


Fig. 5. (C) Diabetic rats treated with *Boerhavia diffusa* extract and (D) glibenclamide showing restoration of liver architecture and normal glycogen granule contents in the hepatocytes.

4. DISCUSSION

Diabetes mellitus (DM) could be a bunch of metabolic disorders portrayed via hyperglycemia following imperfections in insulin emission, insulin activity, or both. The chronic hyperglycemia is identified with stretched pathology and damage, which distress multiple organs. It additionally incorporates a bigger chance of getting dyslipidemia, high blood pressure, and obesity.^[18]

Streptozotocin induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia.

In this study, STZ (40 mg/kg b.w.) was utilized as diabetogenic operator to prompt the diabetes in albino Wistar rats. Heavy loss in body weight was observed in STZ induced diabetic rats. Minimum dosage of STZ was normally utilized to incite type I diabetes.^[19] STZ partly destroys the beta cells bringing about inadequate insulin

discharge, creating type 2 diabetes.^[20] It is the generally used animal model for the diabetic study.^[21]

Diabetic rats STZ-induced registered significant decrease in weights and significant increase in liver weights at variable time intervals. Moreover, the body weight of diabetic rat was significantly ($p < 0.05$) lowered than that of the nondiabetic group and relative weights of liver were significantly higher in diabetic mice compared to nondiabetic control rat.

Diminishing body weight in diabetic rats plainly affirms a corruption of basic proteins because of diabetes.^[22]

Certain herbal extracts have shown the ability to treat diabetes and prevent the development of its longterm complications without causing adverse effects. *Boerhavia diffusa* is a rich source of lignans, which has antidiabetic and antioxidant properties.^[23, 24] Considering these properties, this study was conducted to evaluate the hepatoprotective effect of *Boerhavia diffusa* extract in a model of STZ-diabetic rats. Changes in different parameters, including body weight, liver relative weight, blood glucose level and histopathological picture were determined in the diabetic rats treated with *Boerhavia diffusa* extract, and compared with those receiving a commercially available drug, glibenclamide.

Diabetic rats injected with STZ showed elevated plasma glucose levels, which is indicative of hyperglycemia, an observation also reported by other authors.^[25, 26] Promotion of excessive oxidative stress in the vascular and cellular milieu results in endothelial cell dysfunction, which is one of the earliest and most pivotal metabolic consequences of chronic hyperglycemia.^[27]

Hyperglycemia-induced oxidative stress has been shown to be actively involved in the onset and progression of diabetes, leading to various complications such as cardiovascular diseases, nephropathy, amputation of limbs and blindness.^[28, 29] The mechanism of STZ as a toxicant used to induce hyperglycemia in experimental animals involves its toxic effect on the beta cells of the pancreatic islet.^[30]

The STZ induces a selective destruction of pancreatic-cells leading to poor glucose utilization inducing hyperglycemia, but leaving many of the surviving beta cells, which can be regenerated. Such regeneration is enhanced by the administration of *Boerhavia diffusa*, and results in stimulating insulin release and so increasing its level in the blood, which can improve glucose metabolism.

STZ administration was associated with hepatocellular damage. The increased activities of marker enzymes like TG, TC, LDL, GGT in serum are suggestive of liver injury, which might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream.^[31] There was a significant reduction ($p < 0.05$) in blood

glucose concentration of diabetic rats (shown in Fig. 1) after administration of extract of methanol extract of stem leaves of *Boerhavia diffusa*. The hypoglycaemic activity exhibited by these extract may be due to the ability of the extract to inhibit the endogenous glucose production, inhibit insulinase activity, or increase insulin production from the β cells of the islet of Langerhans^[32], or reduced glucose absorption from the gastrointestinal tract. That possess hypoglycemic, as well as antioxidant properties. Some flavonoids have hypoglycemic properties because they improve altered glucose and oxidative metabolisms of the diabetic states. They also exert a stimulatory effect on insulin secretion by changing Ca^{2++} concentration.^[33]

Treatment with glibenclamide has also decreased the blood glucose levels in diabetic rats, and it was found to be more effective in comparing with *Boerhavia diffusa* extract. STZ-induced diabetic rats showed severe loss of body weight. The deficit in body weight noticed in STZ instigated diabetic control rats may be due to muscle squandering.^[34, 35] Diminishing body weight in diabetic rats plainly affirms a corruption of basic proteins because of diabetes.^[36]

Oral administration of diabetic group and glibenclamide results in weight gain in STZ induced diabetic rats. However, there is an increase in liver weight in proportion to the body weight. Liver hypertrophy during diabetes mellitus might be the result of an increased hepatic accumulation of triglycerides^[31] with the previous studies which reported increased liver relative weight in STZ-induced diabetic rat.^[37, 38, 39]

Treatment with glibenclamide and *Boerhavia diffusa* extract has reversed the progressive changes of body weight and liver weight in diabetic rats. The capability of these treatments to protect the change of body weight and liver weight seems to be due to their ability to reduce hyperglycemia.

In the present study administration of 600 mg/kg of *Boerhavia diffusa* extract for 28 days revealed reduction of hepatotoxicity in diabetic rat STZ-induced manifested by healthy hepatocytes as an evidence of regeneration and to some extent normal central vein, mild steatosis and inflammation whereas, no ballooning cells were registered in histopathological system score. There is increasing evidence that aldehydes generated endogenously during lipid peroxidation contribute to the pathophysiologic effects associated with oxidative stress in cells and tissues. A number of reactive lipid aldehydes, such as 4-hydroxy-2-alkenals and malondialdehyde, have been implicated as causative agents in cytotoxic processes initiated by the exposure of biologic systems to oxidizing agents. Recently, acrolein, a ubiquitous pollutant in the environment, was identified as a product of lipid peroxidation reactions. The identification of acrolein as an endogenous lipid-derived product suggests an examination of the possible role of

this aldehyde as a mediator of oxidative damage in a variety of human diseases. Lipid peroxidation is implicated in the pathogenesis of numerous diseases; including atherosclerosis, diabetes, cancer, and rheumatoid arthritis, as well as in drug-associated toxicity, post is chemic reoxygenation injury, and aging. Lipid peroxidation proceeds by a free-radical chain reaction mechanism and yields lipid hydroperoxides as major initial reaction products. Subsequently, decomposition of lipid hydroperoxides generates a number of degradation products that lead to a wide variety of damaging actions.

Our findings are in good agreement with several previous studies, which reported similar histopathological changes following induction of diabetes using STZ injection.^[37,40,41] However, less pathological changes and improved liver architecture were observed in *Boerhavia diffusa* extract and glibenclamide treated diabetic rats, indicating protective effects of these treatments against the hepatic changes associated with diabetes.

In recent studies, it has been suggested that regeneration of β -cells may be possible after they are damaged severely due to STZ therapy.^[42,43]

The present study aimed to investigate whether *Boerhavia diffusa* extract could reduce hyperglycemia, hepatotoxicity and oxidative stress in the liver. The overall results showed that *Boerhavia diffusa* extract could reduce glucose levels in diabetic mice STZ-induced, moreover, *Boerhavia diffusa* extract could improve injured liver due to diabetes and reduce oxidative stress in liver.

5. CONCLUSIONS

It is concluded that daily treatment with *Boerhavia diffusa* extract improves blood glucose level, and histopathological status of STZ-induced diabetes mellitus. The experimental evidence obtained from this study indicates that *Boerhavia diffusa* extract represents a candidate alternative treatment to control diabetes mellitus and its related hepatopathy.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no personal or financial conflict of interest.

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