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The Effect of Methanolic Leaf Extract of *Boerhavia diffusa* Linn. (Nictaginaceae) on the Activities of Antidiabetic, Anti-inflammatory and Antioxidant Enzymes in Experimental Diabetes

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diabetic therapeutic potentiality of methanol extract of stem leaves of *Boerhavia diffusa* was investigated following i*n-vivo* study models in streptozotocin-induced diabetic rat. Methanol extract of stem leaves of *Boerhavia diffusa* exerted the glucose lowering effect an increase in serum insulin level on 28st day of postadministration. In addition to a higher expression of insulin receptor A. The extract treatment or glibenclamide for 28 days significantly ($p<0.05$) reduced HbA1c.

Boerhavia diffusa L. or glibenclamide for 28 days show no damaging effect on red blood count and hemoglobin when compared to the control group. Significant ($p<0.05$) increase in platelet count and white blood cell count was observed in groups treated with *Boerhavia diffusa* L. and glibenclamide when compared to control group.

Boerhavia diffusa L. and glibenclamide showed significant ($P<0.05$) decrease in total cholesterol (TC), triglyceride (TG) low density Lipoprotein (LDL), Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP) and Gamma glutamyl transferase (GGT). Methanol extract of 600 mg/kg b.w had more lowering effect ($p<0.05$) on TC and TG as opposed to the untreated group.

Methanol extract or glibenclamide also modulated significantly ($P<0.05$) the activities of carbohydrate-metabolising enzymes and Hepatic glycogen content. *Boerhavia diffusa* or glibenclamide administration up-regulated mRNA expression of Glucose Transporter-2 (Glut2)

Boerhavia diffusa or glibenclamide also corrected antioxidant status of diabetic animals in liver. The lipid peroxidation inhibition activity of extracts from *Boerhavia diffusa* is stronger when compared to the reference antioxidant ascorbic acid.

These clearly showed that methanol extract from *Boerhavia diffusa* has the inhibitory activities of the xanthine oxidase, lipoxygenase and acetylcholinesterase enzyme.

Keywords: Boerhavia diffusa Linn; streptozotocin; diabetes mellitus; anti-inflammatory activity; antioxidant enzymes.

1. INTRODUCTION

Diabetes mellitus is a type of metabolic disorder that is characterised by hyperglycemia and alterations in carbohydrate, fat and protein metabolisms associated with absolute or relative deficiencies in insulin secretion and/ or insulin action [1]. Diabetes is characterised by a high incidence of cardiovascular disease [2]. There has been increasing evidence recently that postprandial diabetes and hyperglycemia are important contributory factors in atherosclerosis [2]. In diabetes, the postprandial phase is characterised by a massive rapid increase in blood glucose levels where the alteration in the sensitivity or reactivity of vascular smooth muscle to neurotransmitters and circulating hormones may cause or contribute to diabetic vessel complications [2,3]. The search for appropriate hypoglycemic agents has recently been focused on plants and many herbal medicines have been recommended for the treatment of diabetes [4]. Herbal drugs are frequently considered to be less toxic than their synthetic counterparts and they have fewer side effects [5]. A number of studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species.

The leaf of *Boerhavia diffusa* Linn. (Nyctaginaceae) is traditionally used in Benin and other countries to manage control and treat diabetes. The plant is known to possess anti-inflammatory [6,7], anticonvulsant [8], diuretic [9], hepatoprotective [10,11] and immunomodulatory [12,13] activities. It has also been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers and nephritic syndrome [14,15].

The *Boerhaavia diffusa* plant contains a large number of such compounds as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Phytochemical screening of the roots from garden-grown in vivo plants of B. *diffusa* of different ages revealed that the maximum alkaloid content (2%) accumulated in the roots of 3- year old mature plants. The following are few important chemical constituents present in plant: Alkaloid - Punarnavine [16,17,18], Rotenoid - boeravinone A1, B1, C2, D, E, F [19,20,21], Hypoxanthine 9-L-arabinofuranoside [22], Punarnavoside [23], Ursolic acid [24], E-sitosterol, Lignans-Liirodendrin [25] and syringaresinol mono-E-D-glucoside [26].

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 [27]. 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 oxidised intermediates and act as primary antioxidant substances [27].

Nevertheless, the details of the relationship between the beneficial effects of antioxidant acivity of methanol extract of the leafy stem powder of *Boerhaavia diffusa* L. on the activities of antidiabetic, anti-inflammatory and antioxidant enzymes in normal and streptozotocin induced diabetic rats have not yet been fully elucidated. The purpose of our work was to evaluate the effect of methanol extract of *Boerhaavia diffusa* L. on carbohydrate metabolism, antioxidant enzymes, in hepatic tissues of experimental diabetic rats and the mechanisms involved, lipid peroxidation, anti-inflammatory and anti-acetylcholinesterase activities.

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.

2.2 Plant Material

The stem leaves of *Boerhavia 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 authentification 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 *Boerhavia diffusa* L.

Two hundred and fifty grams (250 g) of dry powder of the barks of *Boerhavia 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 Acute Toxicity Studies

The tests were performed in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD) for the testing of chemicals substances through method 423 (OCDE, 2001). The methanol extract of this plant was dissolved in distilled water and administered to the rats at a ratio of 1 ml/100 g of body weight. Control rats were instead given distilled water. The rats were marked for individual identification. The rats were divided into two batches of six rats after blood tests to ensure homogeneity of batches. Control rats (six) did not receive extract but distilled water while the experimental animals (six) received 5000 mg/kg of an methanol extract of *Boerhavia diffusa* L. The animals were observed individually at least once during the first 30 min and at least twice during the first 24 h after treatment.

2.5 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 [28]. 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 but less than 350 mg/dL were included for this investigation.

2.6 Grouping of Animals

The experimental design consisted of 28 rats, twenty four were rendered diabetic, and four

were normoglycaemic (positive control) rats. The diabetic untreated rats (negative control) were administered 10 ml/kg bodyweight of normal saline. The animals were grouped into seven as shown below:

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 300 mg/kg bodyweight of methanol extract
Group 5: Diabetic treated with 600mg/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.

2.7 Treatment with Extracts

Effect of various extract was checked on blood glucose, and serum biomarkers 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).

2.8 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. Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density Lipoprotein (LDL) levels were measured in serum samples by using enzymatic method kits (Roche Diagnostics). The determination of insulin was performed in samples that were stored at -80°C. Serum insulin was determined using an ELISA kit (LINCO Research Inc, St. Charles, MO, USA), according to the manufacturer's instructions. Glycated haemoglobin (HbA1c) was estimated by kit based on the ion exchange method of Nathan [29]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) were measured by using commercially available kits (Agappe Diagnostics, Ernakulam, India).

The fasting serum glucose measured by using commercially available kits (Agappe Diagnostics, Ernakulam, India). Activity of glycolytic enzymes was assayed: hexokinase was estimated by the method of Crane and Sols [30]; pyruvate kinase was estimated by the method of Bucher and Pfleiderer [31]. Hepatic glycogen content was estimated by the method of Carroll [32]. Activity of Gluconeogenic enzyme activities in the liver were assayed using the following procedures: glucose-6-phosphatase was estimated by the method described by Koide and Oda [33], fructose-1,6-diphosphatase was estimated by the method of Pontremoli [34], and the activity of glycogen phosphorylase was assayed by the procedure described by Singh [35].

Other parts from the liver tissues were also frozen in on liquid nitrogen used for molecular analysis. Hepatic homogenate protein concentration was measured using Trizol reagent (Invitrogen Life Technologies, Groningen, The Netherlands) according to the manufacturer's instructions. The integrity of RNA was electrophoretically checked by ethidium bromide staining and by the OD absorption ratio OD_{260nm}/OD_{280nm}. One microgram of total RNA was reverse transcribed with Superscript II RNAse H-reverse transcriptase using oligo (dT) according to the manufacturer's instructions (Invitrogen Life Technologies, France).

Real-time PCR was performed on an iCycler iQ real-time detection system (Bio-Rad, Hercules, CA, USA), and amplification was done by using SYBR Green I detection (SYBR Green JumpStart, Taq ReadyMix for Quantitative PCR, Sigma-Aldrich, St. Louis, MO, USA). Oligonucleotide primers, used for mRNA analysis, were based on the sequences of rat gene in the GeneBank database. Forward and reverse primers used to amplify beta-actin message in the rat were as follows: forward: 5'-GGCACCACACCTTCTACAATGAGC -3'; reverse: 5'- CGACCAGAGGCATACAGGGACAG -3'. The primers for PK, Glut2, Insulin Receptor A were as follows: (PK) forward: 5'-ATTGCTGTGACTGGATCTGC-3'; reverse: 5'-CCCGCATGATGTTGGTATAG-3'; (Glut2) forward: 5'-AAGGATCAAAGCCATGTTGG-3'; reverse: 5'-GGAGACCTTCTGCTCAGTGG-3'; (Insulin Receptor A) forward: 5'-TTCATTCAGGAAGACCTTCGA-3'; reverse: 5'-AGGCCAGAGATGACAAGTGAC-3'.

The amplification was carried out in a total volume of 25 µl containing 12.5 µl SYBR Green Taq Ready Mix, 0.3 µM of each primer and diluted cDNA. Cycling conditions consisted of an initial denaturation step of 95°C for 3 min as a hot start followed by 40 cycles of 95°C for 30 sec or at 60°C for 30 sec with a single fluorescence detection point at the end of the relevant annealing or extension segment. At the end of the PCR, the temperature was increased from 60 to 95°C for 15 sec and at 58±2°C for 60 sec, and the fluorescence was measured every 15 sec to draw the melting curve. The standard curves were generated for each protein or ß-actin using serial dilutions of positive control template in order to establish PCR efficiencies. All determinations were performed, at least, in duplicates using two dilutions of each assay to achieve reproducibility. Results were evaluated by iCycler iQ software including standard curves, amplification efficiency (E) and threshold cycle (Ct). Relative quantitation of mRNA expression was determined using the ΔΔCt in which ΔΔCt = ΔCt of gene of interest–ΔCt of ß-actin. ΔCt = Ct of interest group - Ct of control group. Relative quantity (RQ) was calculated as follows: RQ = $(1 + E)^{(-\Delta\Delta Ct)}$. The electrophoretic picture was visualised and analysed by gel documentation system (Bio Doc Analyze, Biometra, Göttingen, Germany).

2.9 Hematological Indices

Portions of the blood are taken from all rats by retro-orbital puncture 14 days after the extract administration, for hematological examinations. Blood collection was done on live animals (without anesthesia), kept fasting for 16 h by puncturing the retro orbital sinus using a pasteur pipette previously rinsed with EDTA anticoagulant to 0.01%. The volume of collected blood was 0.5 to 2 ml. The full blood count includes; total red blood cell (RBC), hemoglobin concentration (HGB), white blood cell count (WBC), platelet count (PLT) and other hematological parameters were determined using Swelab Auto Hematology Analyzer.

2.10 Animal Sacrifice and Collection of Organs

After overnight fasting, rats in each group were anaesthetised with pentobarbital (60 mg/kg body weight). The abdominal cavity was opened, and whole blood was drawn from the abdominal aorta. The blood samples were also collected, at different time intervals, by bleeding the tail end. Serum was obtained by low-speed centrifugation (1000 g×20 min). Different organs were removed, washed with cold saline solution (0.9%) and immediately frozen in liquid nitrogen and stored at -80°C.

2.11 Determination of Superoxide Dismutase, Catalase Activities and Glutathione Peroxidase Activities

The specific activity of superoxide dismutase was determined following the method of Marklund [36] that involves the inhibition of autooxidation of pyrogallol at pH 8.0. A single unit of enzyme was defined as the quantity of superoxide dismutase required to produce 50% inhibition of autooxidation. The cytosolic fraction was treated with Triton X-100 (1%) and kept at 4 °C for 30 min then added to the assay mixture that contained 0.05 M sodium phosphate buffer (pH 8.0), 0.1 mM EDTA and 0.27 mM pyrogallol. The absorbance was recorded at 420 nm for 5 min. The specific activity of catalase was determined according to the method of Aebi [37]. In the ultraviolet range, H_2O_2 shows a continual increase in absorption with a decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in the absorbance at 240 nm. The difference in absorbance (ΔA240) per unit time is a measure of catalase activity. The absorbance was observed for approximately 30 sec. The catalase activity is defined in specific units/milligram hemoglobin. One unit of catalase corresponds to the amount of enzyme needed to decompose H_2O_2 in phosphate buffer, at pH 7.0, in 1 sec of reaction. The specific activity of glutathione peroxidase was determined by the method of Paglia and Valentine [38]. The reaction mixture consisted of cytosolic fraction, 50 mM sodium phosphate buffer (pH 7.0) containing EDTA, 0.24 U/ml yeast glutathione reductase, 0.3 mM reduced glutathione, 0.2 mM NADPH, 1.5 mM H_2O_2 and cytosolic. The reaction was initiated by the addition of NADPH and decrease in the absorbance was monitored at 340 nm for 5 min. One unit of enzyme activity has been defined as nmoles of NADPH consumed/min/mg protein based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.12 Inhibition of Lipid Peroxidation in Rat Liver Homogenate

The inhibition activity of extracts or fractions on lipid peroxidation was determined according to the thiobarbituric acid method. $FeCl_2$–H_2O_2 was

used to induce the liver homogenate peroxidation to the method of Su [39] with slightly modification. In this method, 0.2 mL of extract at the concentration of (0.0625–1.000 mg.mL^{-1}) was mixed with 1.0 mL of 1% liver homogenate (each 100 mL homogenate solution contains 1.0 g rat liver), then 50 µL of $FeCl_2$ (0.5 mM) and 50 µL of H_2O_2 (0.5 mM) was added. The mixture was incubated at 37°C for 60 min, then 1.0 mL of trichloroacetic acid (15%) and 1.0 mL of 2-thiobarbituric acid (0.67%) was added and the mixture was heated up in boiled water for 15 min. The absorbance was recorded at 532 nm. Ascorbic acid was used as the positive control. The percentage of inhibition effect was calculated according to following equation:

$$\text{Inhibition \%} = [1-(A_1-A_2)/A_0] \times 100$$

Where, A_0 is the absorbance of the control (without extract), A_1 is the absorbance of the extract addition and A_2 is the absorbance without liver homogenate.

2.13 Determination of Anti-inflammatory Activity

Xanthine Oxidase Inhibitory: The Xanthine oxidase inhibitory activity was measured as previously reported [40]. The substrate and the enzyme solutions were prepared immediately before use. The reaction mixture contains 80 mM sodium pyrophosphate buffer (pH = 8.5), 0.120 mM xanthine and 0.1 unit of XO. The absorption at 295 nm, indicating the formation of uric acid at 25ºC, was monitored and the initial rate was calculated. The dried extract, initially dissolved and diluted in the buffer, was incorporated in the enzyme assay to assess its inhibitory activity. All assays were triplicated; thus inhibition percentages are the mean of 3 observations. A negative control (blank; 0% XO inhibition activity) was prepared containing the assay mixture without the extract. Allopurinol, a known inhibitor of XO, was used as a positive control in the assay mixture. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of Xanthine oxidase in the above assay mixture system, calculated as follows:

$$\text{\% of Inhbition} = 1-(T_I/B_I)$$

Where Test inclination (T_I) is the linear change in the absorbance of test material per minute, and Blank Inclination (B_I) is the linear change in the absorbance of blank per minute.

Lipoxygenase-inhibitory Assay: The inhibition of lipoxygenase activity was determined by a spectrophotometric method [41].

The reaction mixture, containing test compound solution (inhibition solution), lipoxygenase solution in 0.1 M phosphate buffer (pH 8.0) was incubated for 10 min at 25 ˚C. Then, the reaction was initiated by addition of a solution substrate. After 6 min, absorbans value was measured at 234 nm. Ascorbic acid was used as standard inhibitor. The percent inhibition of lipoxygenase activity was calculated as:

$$\text{Inhibition (\%)} = (1-A/B) \times 100$$

Where A is the enzyme activity without inhibitor, B is the activity in presence of inhibitor.

2.14 Acetylcholinesterase Inhibitory Activity

The inhibitory effect of methanol extract from *Boerhavia diffusa* L. on acetylcholinesterase (AChE) activity was evaluated according to the method of Eldeen [42]. Into a 96-well plate was placed: 25 µl of 15 mM ATCI in water, 125 µl of 3 mM DTNB in Buffer C (50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M MgCl2.6H2O), 50 µl of Buffer B (50 mM, pH 8, containing 0.1% bovine serum albumin) and 25 µl of plant extract (0.0625, 0.125, 0.25, 0.50 or 1 mg/ml). Absorbance was measured spectrophotometrically (Labsystems Multiscan EX type 355 plate reader) at 405 nm every 45 s, three times consecutively. Thereafter, AChE (0.2 U/ml) was added to the wells and the absorbance measured five times consecutively every 45 s. Galanthamine served as the positive control. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the absorbance before adding the enzyme from the absorbance after adding the enzyme. The percentage inhibition was calculated using the equation:

$$\text{Inhibition(\%)} = 1-(\text{Asample/Acontrol}) \times 100$$

where Asample is the absorbance of the sample extracts and Acontrol is the absorbance of the blank [methanol in Buffer A (50 mM Tris–HCl, pH 8)].

2.15 Statistical Analysis

The statistical analysis of the data was carried out in Predictive Analytics SoftWare Statistics for

Windows version 18 (IBM SPSS Statistics, Endicott, New York, USA). One-way analysis of variance was used to determine the statistical differences between groups followed by Duncan's multiple range test to analyse the inter-grouping homogeneity. Data were presented as mean ± standard deviation. $P<0.05$ was considered statistically significant.23

3. RESULTS

3.1 Serum Glucose, Insulin and Glycated Hemoglobin (HbA1c)

The development of diabetes in rats was confirmed after an intraperitoneal (i.p.) injection of streptozotocin 40 mg/kg. There was a significant elevation in fasting blood glucose (350.20±25.01 mg/dL) in untreated diabetic animals when compared with non-diabetic control rats. STZ-induced diabetic rats treated with methanol extract at doses 300, 600 mg/kg body weight or glibenclamide for 28 days resulted in a significant lowering of fasting blood glucose level ($p<0.05$) (Fig. 1).

The plasma insulin level decreased significantly in the diabetic group (1±0.08 ng/ mL) when compared with other groups and it was improved by methanol extract of stem leaves of *Boerhavia diffuse* or glibenclamide for 28 days (Fig. 2 (a)). The methanol extract of stem leaves of *Boerhavia diffus* action was dose dependent where the highest effects observed in rats treated with a dose of 600 mg/kg bwt.

The STZ induced diabetic rats had significant decrease in the mRNA expression of hepatic insulin. Methanol extract of stem leaves of *Boerhavia diffus* or glibenclamide for 28 days showed a increase the hepatic IRA relative gene expression when compared with the diabetic rats (Fig. 2 (b)). The methanol extract of stem leaves of *Boerhavia diffus* action was dose dependent where the highest effects observed in rats treated with a dose of 600 mg/kg bwt, and less effect with a low dose 300 mg/kg bwt.

Higher value of glycated hemoglobin level was found in the untreated diabetic group (10±2%) when compared with the control group. The data presented in Fig. 3 indicated the effect of *Boerhavia diffusa* extract and glibenclamide for 28 days on HbA1c. The extract treatment or glibenclamide for 28 days significantly ($p<0.05$) reduced HbA1c. Methanol extract of stem leaves of *Boerhavia diffus* or glibenclamide treatment to the diabetic rat for 28 days resulted in a significant recovery of this parameter. The methanol extract of stem leaves of *Boerhavia diffus* action was dose dependent.

3.2 Clinical Signs Observed

No obvious clinical signs (tremor, breathing rate, paralysis) were observed although quantitative assessments were no carried out.

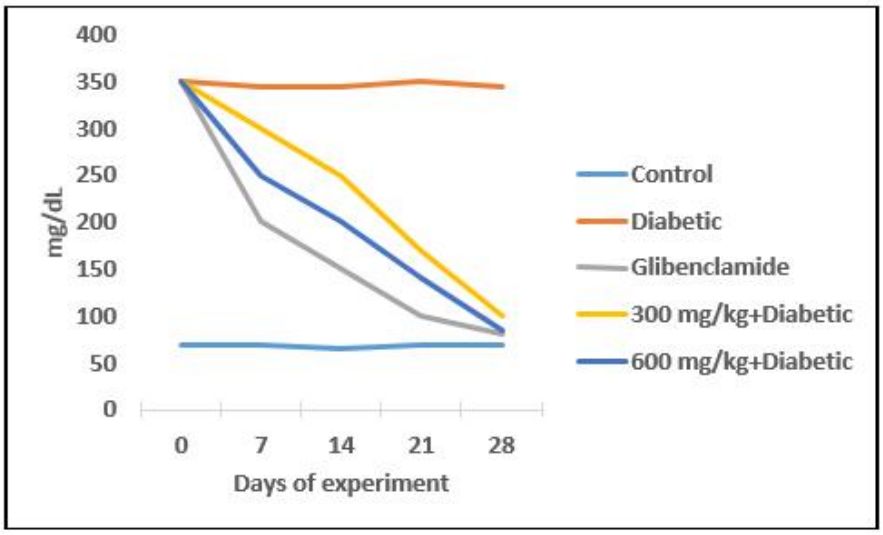

Fig. 1. Fasting blood glucose levels in control and diabetic rats, treated or not with of methanol fraction of stem leaves of *Boerhavia diffus* for 4 weeks. Each value represents the mean± SEM (n=6)

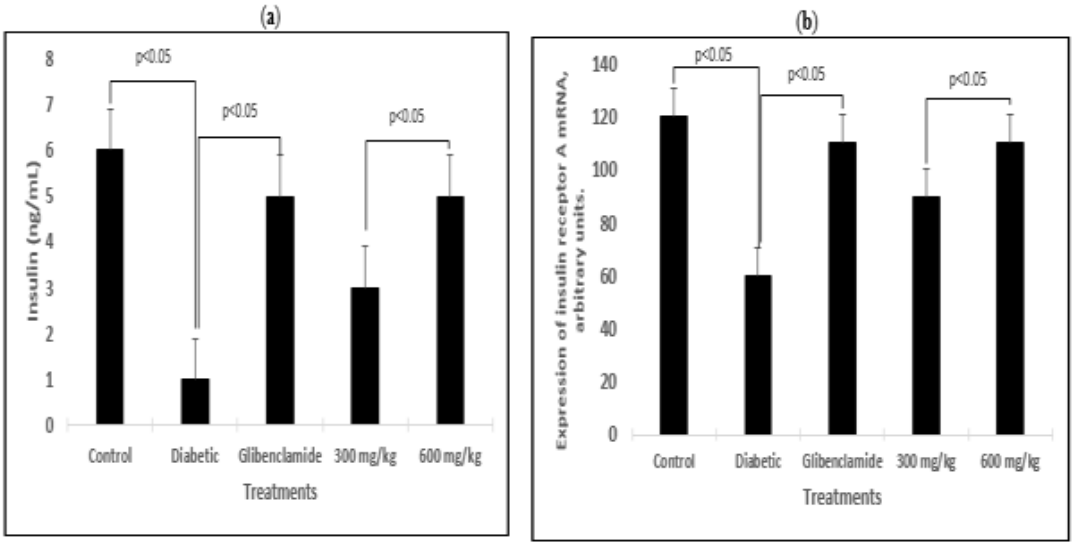

Fig. 2. Serum insulin levels (a), expression of insulin receptor A mRNA (b) in control and diabetic rats, treated or not with of methanol fraction of stem leaves of *Boerhavia diffus* for 4 weeks.. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05)

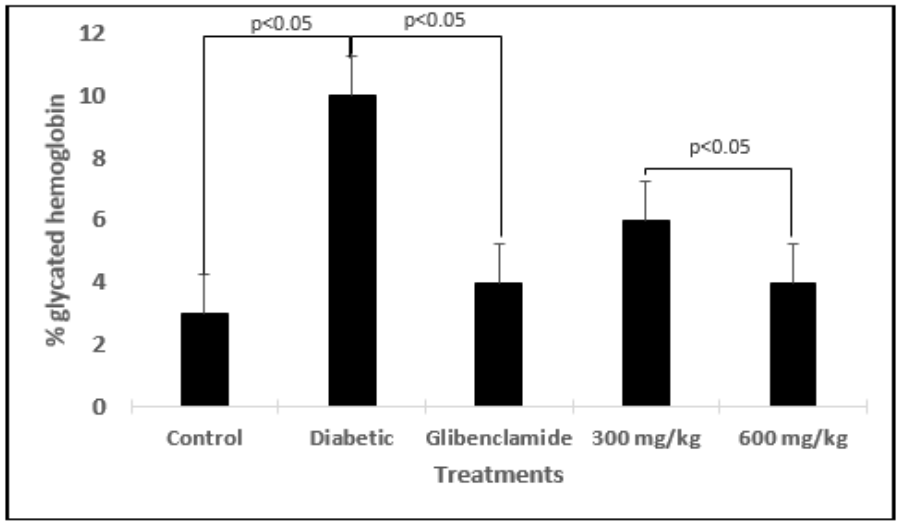

Fig. 3. Glycated hemoglobin levels in control and diabetic rats, treated or not with of methanol fraction of stem leaves of *Boerhavia diffus* for 4 weeks. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05)

3.3 Hematological Indices

There was significant decrease (p<0.05) in red blood count, packed cell volume and hemoglobin levels of diabetic untreated group (Table 1). Methanol extract of stem leaves of *Boerhavia diffusa* Linn or glibenclamide for 28 days show no damaging effect on red blood count and hemoglobin when compared to control group. Diabetic untreated rats indicated a significant (P<0.05) reduction in platelet and white blood count when compared to control group. Significant (p<0.05) increase in platelet count and white blood cell count was observed in groups treated with 300 mg and 600 mg of methanol extract and glibenclamide when compared to control group.

Table 1. Hematological indices of the control group, and rats treaded and untreated rats

Blood hematological parameters	Control rats	Diabetic untreated	Glibenclamide	300 mg/kg b.w	600 mg/kg b.w
RBC(10^{12}/l)	4.9±0.15	1.70±0.13	4.70±0.28*	4.85±0.43*	4.77±0.24*
MCV (f l)	55.63±1.10	54.73±0.78	55.90±0.73*	54.96±1.39*	55.40±1.02*
HCT (%)	28.95±1.92	14.70±0.90	27.30±1.80*	26.40±2.30*	26.60±1.60*
PLT (109/L)	170.14±11	71.00±2.40	169.40±2.40**	140.33±11.36**	141.90±11.22**
WBC (109/L)	5.71±1.1	2.62±1.33	5.92±0.71**	4.96±1.76**	5.95±1.50**
HGB(g/dl)	12.10±1.72	5.70±0.30	11.06±0.70*	11.85±0.80*	11.98±0.60*
MCH(pg)	20.00±0.39	19.60±0.18	19.80±0.14*	20.25±0.33*	20.40±0.22*
MCHC(g/dl)	37.26±0.44	37.83±0.31	37.07±0.25*	38.15±0.47*	37.20±0.40*

**: Insignificant statistical difference (p>0.05), **: Significant statistical difference between rats treaded and control rats for the parameters considered (p<0.05), M ± esm = mean ± standard error on average, n = 3.*
RBC: red blood count, MCV: Mean cell volume, HCT: Hematocrit, PLT: platelet, WBC: White blood coun, HGB: hemoglobin, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration

3.4 Plasma Lipid Profiles

Fig. 4 showed a significant ($p<0.05$) decrease in total cholesterol (TC), triglyceride (TG) and low density Lipoprotein (LDL) levels in all diabetes treated groups when compared with diabetic untreated group. Administration of methanol fraction of 600 mg/kg b.w had more lowering effect ($p<0.05$) on TC and TG whereas the diabetic rats treated with 300 mg/kg b.w. But varying the dose of this methanol extract or glibenclamide for 28 days increased HDL ($p<0.05$) (Fig. 4) compared to control group, glibenclamide and treated groups.

3.5 Liver Function Tests

The activities of ALT, AST, ALP, and GGT were significantly altered in the Diabetic group, indicating damage to hepatocytes. Treatment with methanol extract of stem leaves of *Boerhaavia diffusa* L or glibenclamide significantly ($p<0.05$) lowered these enzyme activities in standard drug treated group, 300 and 600 mg/kg b.w of methanol extract compared to control group (Fig. 5). There was no statistically significant difference ($p>0.05$) in most of the liver toxicity markers between the groups treated with the extract at doses 300 and 600 mg/kg body weight.

3.6 Effect of Methanol Fraction of Stem Leaves of *Boerhavia diffusa* on Glycolytic Enzymes

The activities of hexokinase (Fig. 6) and pyruvate kinase Fig. 7) were significantly diminished ($p<0.05$) in STZ-induced diabetic rats as compared with normal control animals. However, methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide for 28 days treatment significantly increased ($p<0.05$) the activities of hexokinase and pyruvate kinase in liver tissues of diabetic rats. The STZ induced diabetic rats had significant decrease in the levels of pyruvate kinase relative gene expression compared with control rats ($p<0.05$). While animals administered with methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide stimulated the expression of hepatic pyruvate kinase at protein and transcript levels when compared with the STZ-induced diabetic rats (Fig. 7a and b).

The expression of Glut2 tested in liver tissue of the STZ induced diabetic rats and *Boerhavia diffusa* supplemented diabetic rats. We observed that *Boerhavia diffusa* or glibenclamide stimulated the expression of Glut2 both at protein and transcript levels (Fig. 8).

Hepatic glycogen content in diabetic rats was found to be significantly reduced ($p<0.05$) compared with the normal control. All treated groups showed significant ($p<0.05$) increase in hepatic glycogen when compared with the diabetic rats. The methanol extract of stem leaves of *Boerhavia diffusa* action was dose dependent where the highest effects observed in rats treated with a dose of 600 mg/kg bwt, and less effect with a low dose 300 mg/kg bwt in comparison with the glibenclamide (Fig. 9).

3.7 Effect of Methanol Fraction of Stem Leaves of *Boerhavia diffusa* on Gluconeogenic Enzyme Activities in the Liver

There was an increase in the activities of glucose-6-phosphatase (Fig. 10) and fructose-

1,6-diphosphatase (Fig. 11) in diabetic rats as compared with the normal rats. Supplementation of *Boerhavia diffusa* showed restoration of glucose-6-phosphatase and fructose-1,6-diphosphatase and ($p<0.05$), as well as the standard drug glibenclamide as compared with control rats. STZ administration significantly ($p<0.05$) elevated the activity of glycogen phosphorylase in diabetic control rats as compared with the normal animals. Altered activity of the enzyme is reverted to near normal levels by extract administration and standard drug glibenclamide in diabetic rats (Fig. 12).

3.8 Effect of Methanol Fraction of Stem Leaves of *Boerhavia diffusa* on Superoxide Dismutase, Catalase and Glutathione Peroxidase Activities

Superoxide dismutase, catalase and glutathione peroxidase activities was determined in the liver during aging in response to *Boerhaavia diffusa* L treatment.

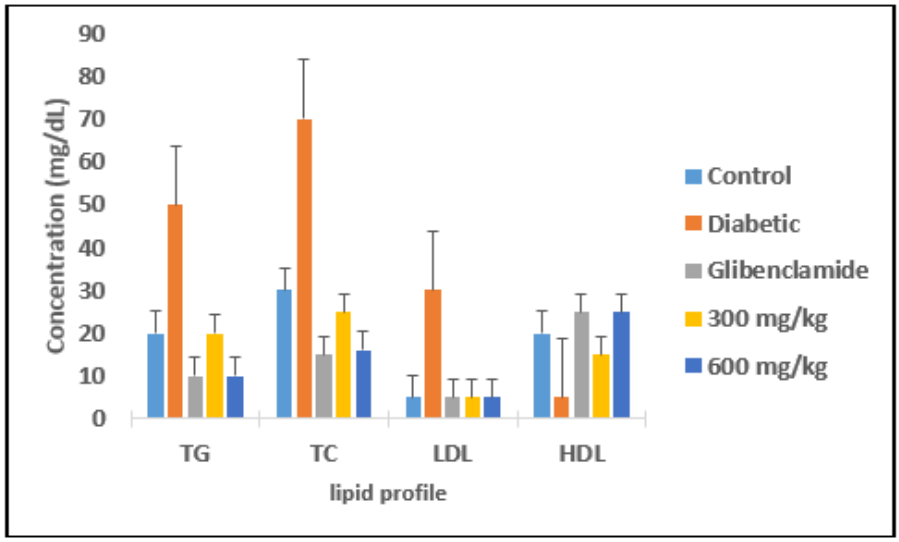

Fig. 4. Anti-hyperlipidemic activities of stem leaves of *Boerhavia diffus* for 4 weeks on strepto-zotocin-induced diabetics Wistar rats. Each value represents the mean ± SEM (n=6)

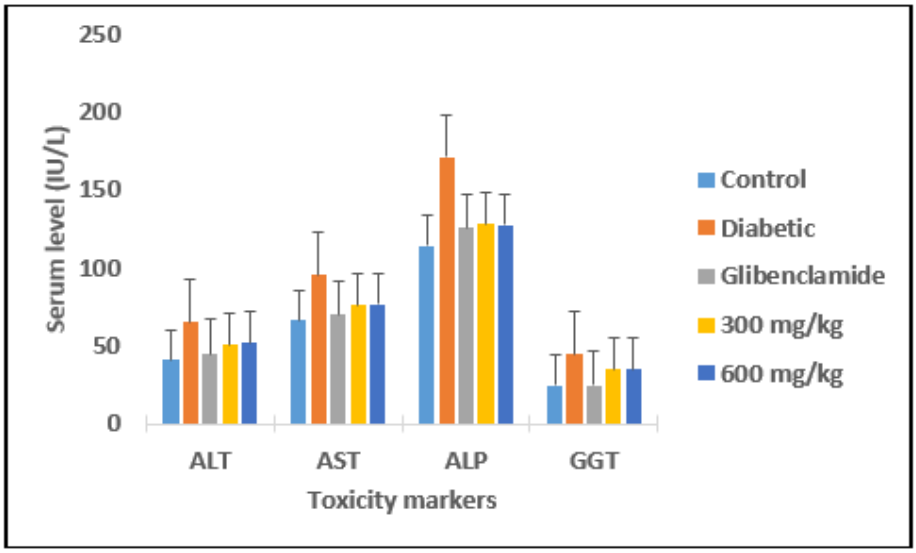

Fig. 5. Effect of methanol fraction of stem leaves of *Boerhavia diffus* on some liver enzymes for 4 weeks. Each value represents the mean ± SEM (n=6)

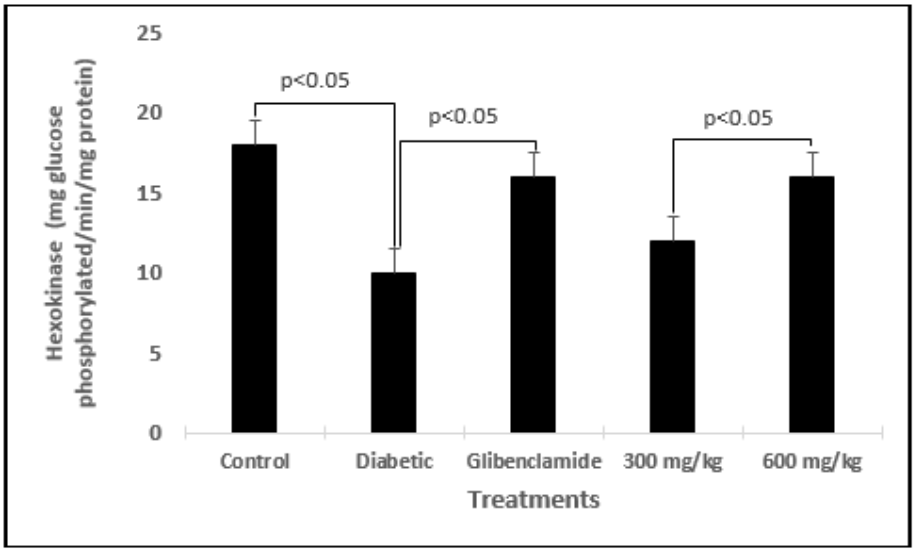

Fig. 6. Effect of methanol fraction of stem leaves of *Boerhavia diffusa* on hepatic hexokinase activity in streptozotocin-diabetic rats. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05)

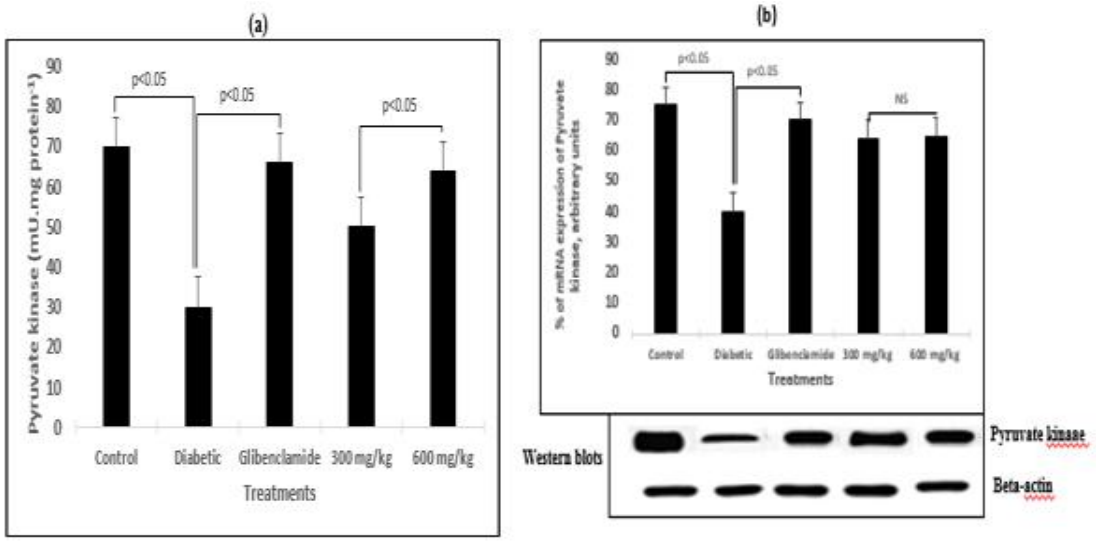

Fig. 7. The activity of hepatic Pyruvate kinase (a) and relative gene expression (relative to beta-actin gene expression) (b) of Pyruvate kinase in streptozotocin-diabetic rats. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05). NS=insignificant differences

We observed that the superoxide dismutase activity in liver was decreased in diabetic animals (p<0.05) (Fig. 13). The animal groups treated with Glibenclamide, methanol extract of stem leaves of *Boerhavia diffusa* with 300 mg/kg b.w and 600 mg/kg b.w) showed augmentation in the specific activity of superoxide dismutase by 1.75 fold ($p < 0.05$), 1.25 fold ($p < 0.05$) and 1.62 fold ($p < 0.05$), respectively as compared to control group.

Diabetes increased catalase activity in liver. Methanol extract of stem leaves of *Boerhavia diffusa* extract significantly decreased the

catalase activity in diabetic animals ($p<0.05$) (Fig. 14). Similarly, methanol extract of stem leaves of *Boerhavia diffusa* extracts and glibenclamide decreased the activity of glutathione peroxidase which was increased in diabetic animals (Fig. 15). The methanol extract of stem leaves of *Boerhavia diffusa* action was dose dependent where the highest effects observed in rats treated with a dose of 600 mg/kg bwt, and less effect with the low dose 300 mg/ kg bwt in comparison with the standard drug glibenclamide.

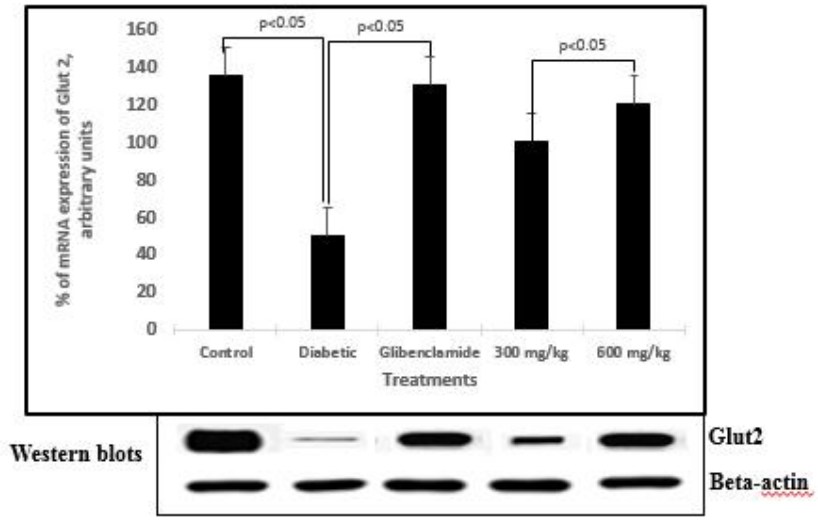

Fig. 8. RT-PCR analysis of Glut2 expression after oral administration of *Boerhavia diffusa* for 28 days to the STZ induced diabetic group wistar rats as described in materials and methods. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out for Glut2 genes. Densitometric analysis was carried for 6 different rats. Each value represents the mean ± SEM (n=6). Significant differences ($p<0.05$)

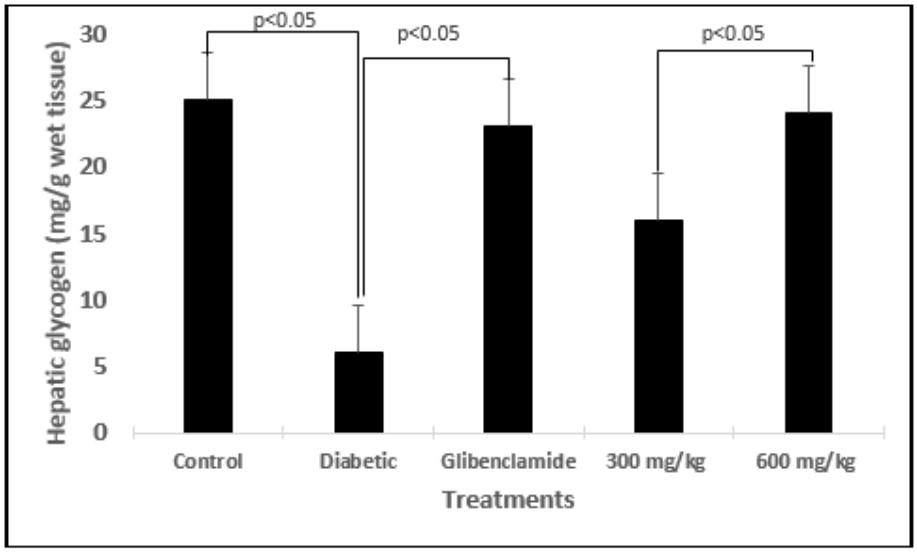

Fig. 9. The activity of Hepatic glycogen in streptozotocin-diabetic rats. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences ($p<0.05$). NS=insignificant differences

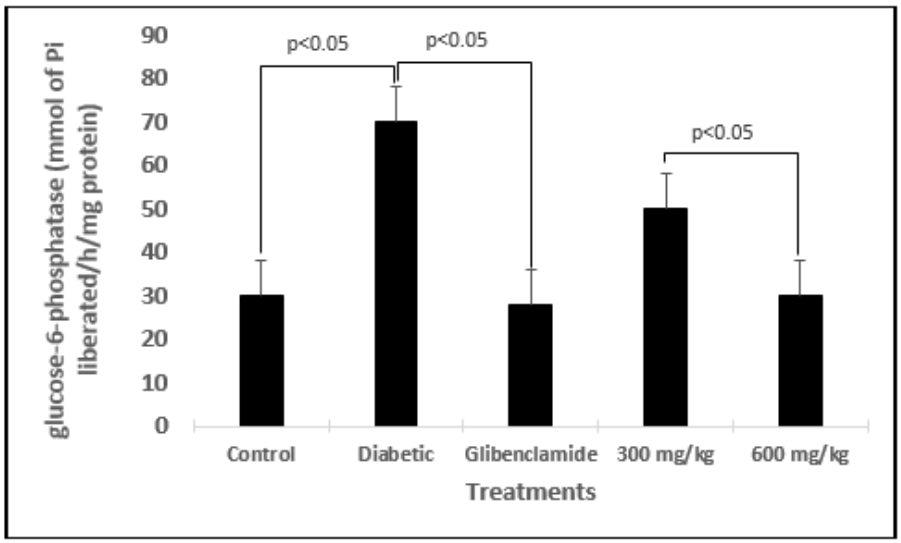

Fig. 10. Effect of methanol fraction of stem leaves of *Boerhavia diffusa* on glucose-6-phosphatase activity in streptozotocin-diabetic rats. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05)

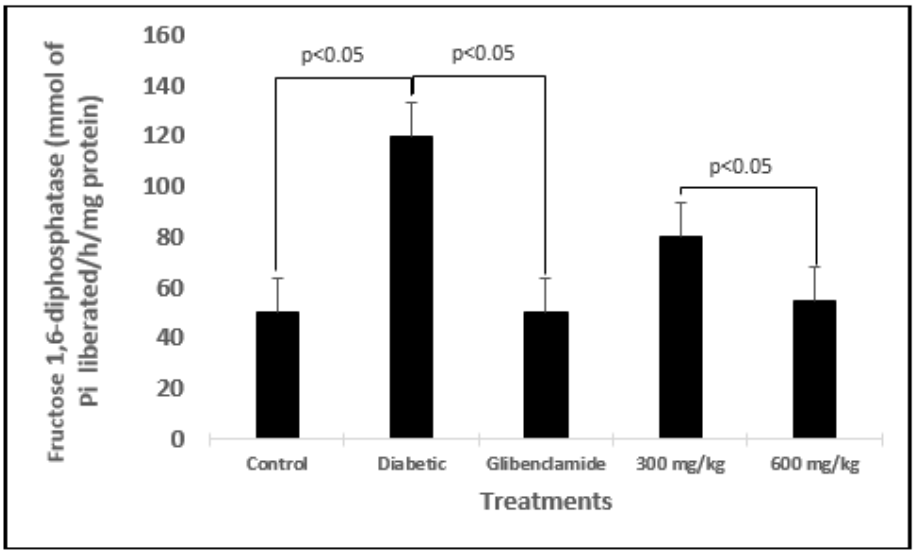

Fig. 11. Effect of methanol fraction of stem leaves of *Boerhavia diffusa* on Fructose 1,6-diphosphatase activity in streptozotocin-diabetic rats. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05)

3.9 Inhibition Peroxidative Damage by Methanol Fraction of Stem Leaves of *Boerhavia diffusa*

Peroxidation was initiated by the Fenton reagent and determined in terms of TBARS formation. Methanol extract of stem leaves of *Boerhavia diffusa* demonstrated a strong anti-lipid peroxidative effect (94±2%). Inhibitory effect peroxidative damage, in a dose dependent manner where the highest effects observed in rats treated with a dose of 1.000 mg/ml, and less effect with the low dose 0.0625 mg/ml (Fig. 16). The lipid peroxidation inhibition activity of extracts from *Boerhavia diffusa* is stronger when compared to the reference antioxidant ascorbic acid (Fig. 16).

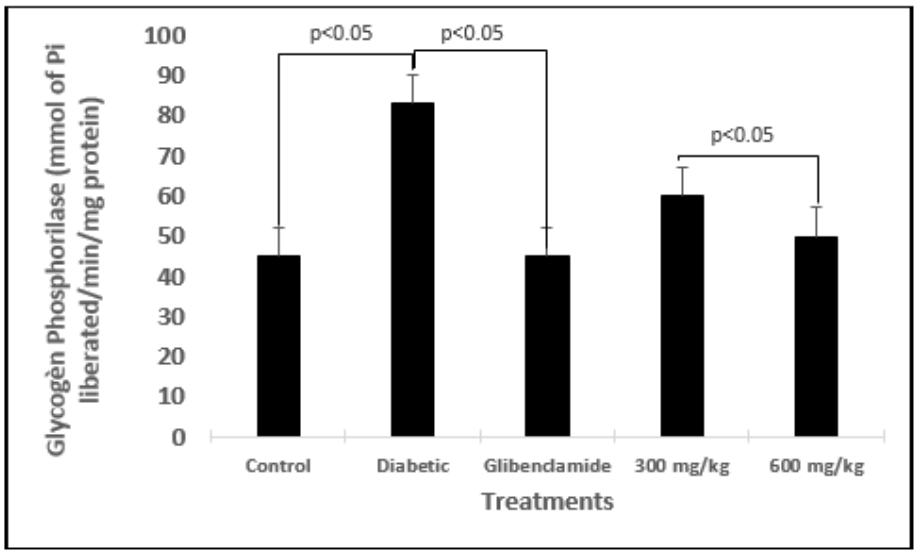

Fig. 12. Effect of methanol fraction of stem leaves of *Boerhavia diffusa* on glycogen Phosphorilase activity in streptozotocin-diabetic rats. Each value is the mean of six determinations. Each value represents the mean ± SEM (n=6). Significant differences (p<0.05)

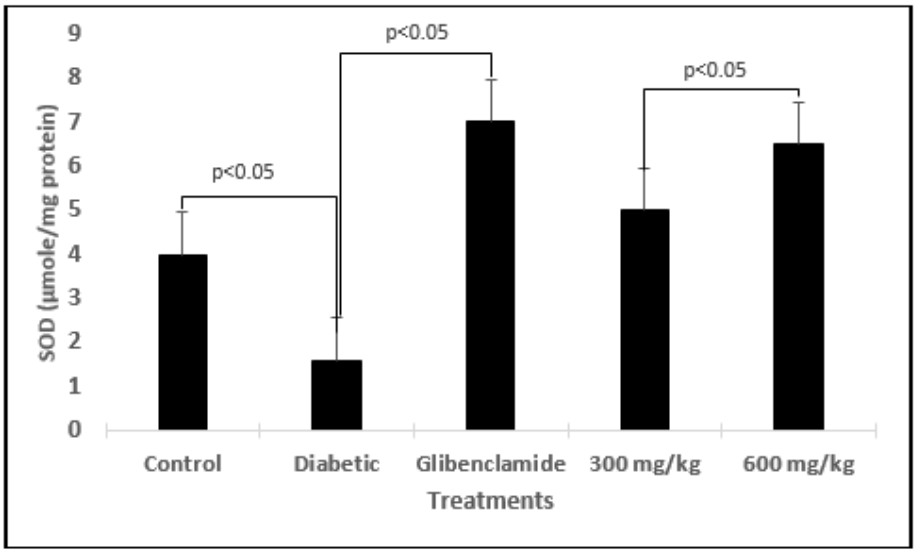

Fig. 13. Activities of superoxide dismutase in liver (c) day 28 in diabetic and control rats, treated or not with extracts of *Boerhaavia diffusa* L. The determination of activities of superoxide dismutase were performed as described in the Materials and Methods section. Each value represents the mean±SEM (n=6). Abbreviations: SOD superoxide dismutase

3.10 Anti-inflammatory Activity of Stem Leaves of *Boerhavia diffusa*

Fig. 17 (a) shows the inhibitory effects of the extracts and ascorbic acid of *Boerhavia diffusa* on Xanthine Oxidase (XO) activities. These clearly showed that methanol extract from *Boerhavia diffusa* has the inhibitory activities of the xanthine oxidase enzyme in a dose dependent manner. A better inhibitory activity (75±0.66%) of the methanol extract of *Boerhavia diffusa* on the xanthine oxidase at a concentration of 1.00 mg.mL^{-1}.

The 5-LOX pathway generates an important class of inflammatory mediators, such as leukotrienes. Fig. 17 (b) also shows the inhibitory effects of the extracts on lipoxygenase activity in a dose dependent manner, enzyme involved in generating free radicals. Methanol extract of *Boerhavia diffusa* showed stronger inhibitory activity towards lipoxygenase at a concentration of 1.00 mg.mL^{-1} comparatively of the reference ascorbic acid.

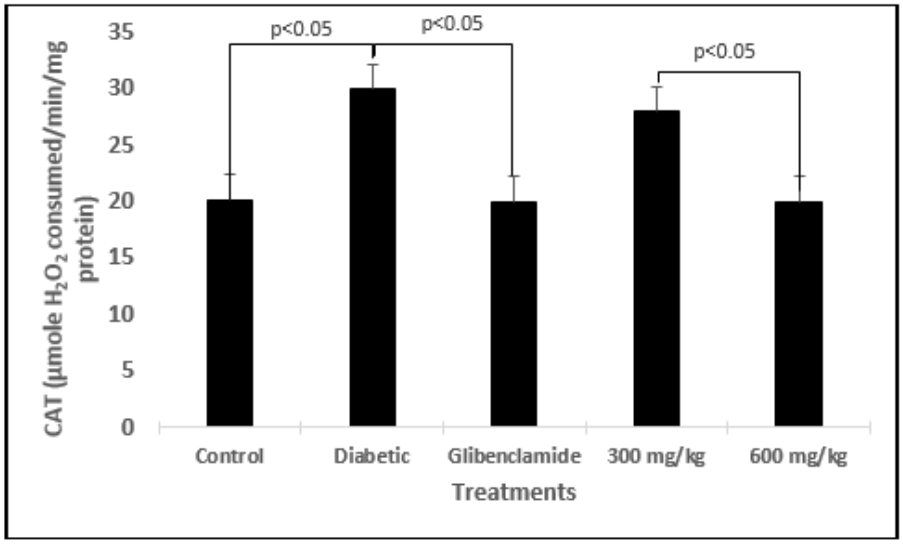

Fig. 14. Activities of Catalase in liver day 28 in diabetic and control rats, treated or not with extracts of *Boerhaavia diffusa* L. The determination of activities of catalase were performed as described in the materials and methods section. Each value represents the mean±SEM (n=6). Abbreviations: CAT catalase

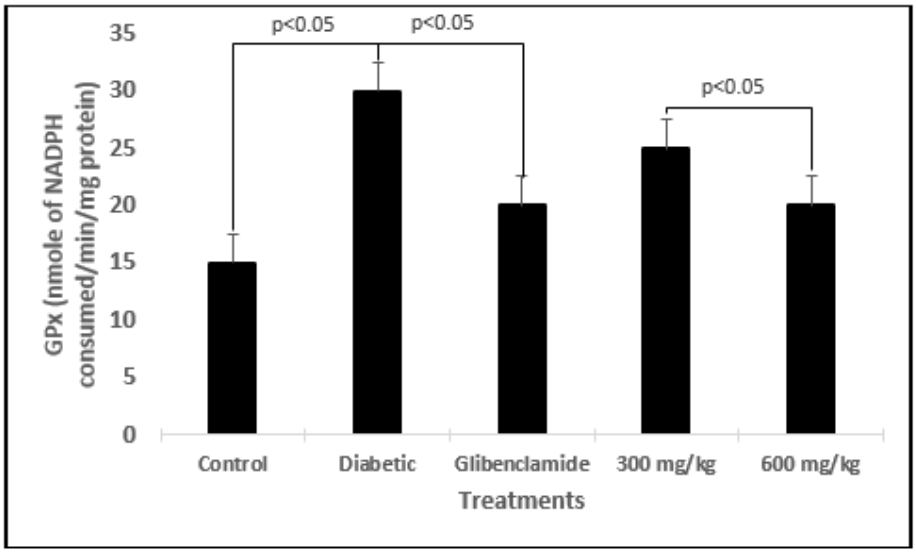

Fig. 15. Activities of Glutathione peroxidase in liver day 28 in diabetic and control rats, treated or not with extracts of *Boerhaavia diffusa* L. The determination of activities of Glutathione peroxidase were performed as described in the Materials and Methods section. Each value represents the mean±SEM (n=6). Abbreviations: GPx glutathione peroxidas

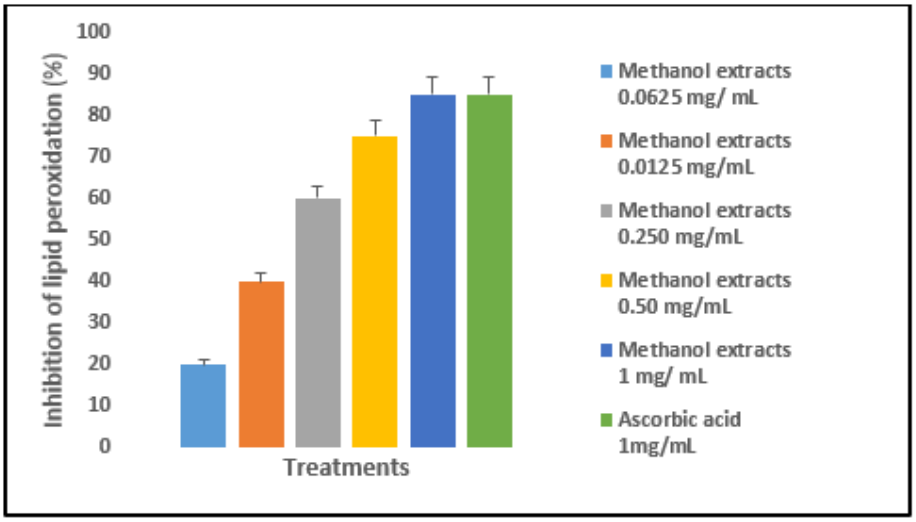

Fig. 16. Anti-lipid peroxidation activity. The percent inhibition of lipid peroxidation was quantified by measuring the reduction of thiobarbituric acid (TBARS) production with respective controls in the presence of extracts. Ascorbic acid was used as standard antioxidant. Data are Mean±SEM (n = 6)

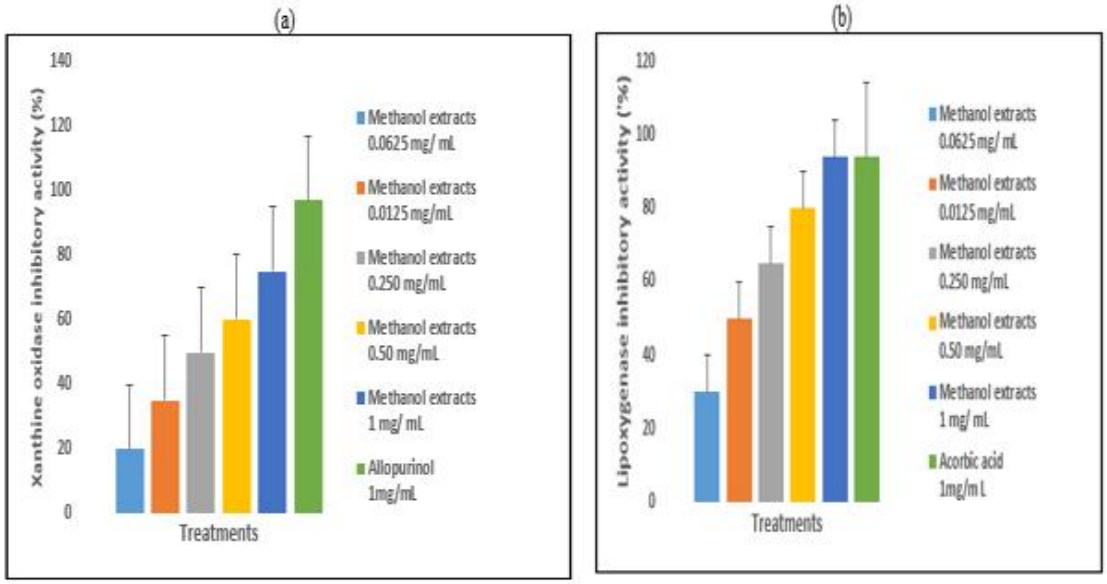

Fig. 17. Xanthine oxidase (a) and lipoxygenase (b) inhibitory activity. Allopurinol, a known inhibitor of XO, Ascorbic acid were used as positive controls, in a final concentration of 100 mg.mL-1 in the reaction mixture. Data are Mean±SEM (n = 6)

3.11 Anti-Acetylcholinesterase Activity of Stem Leaves of *Boerhavia diffusa*

Methanol extract of *Boerhavia diffusa* exerted an inhibitory effect on acetylcholinesterase (Fig. 18). All the doses showed inhibitory effects.The *Boerhavia diffusa*-induced inhibitory effects were not statistically different from 0.50 mg/mL and 1 mg/mL. 0.50 mg/mL showed strong inhibition of acetylcholinesterase with a percentage value of 70.±1.55%. Methanol extracts of *Boerhavia diffusa* 0.0625 mg/ mL showed the weak inhibition of acetylcholinesterase with a percentage value of 30±2%.

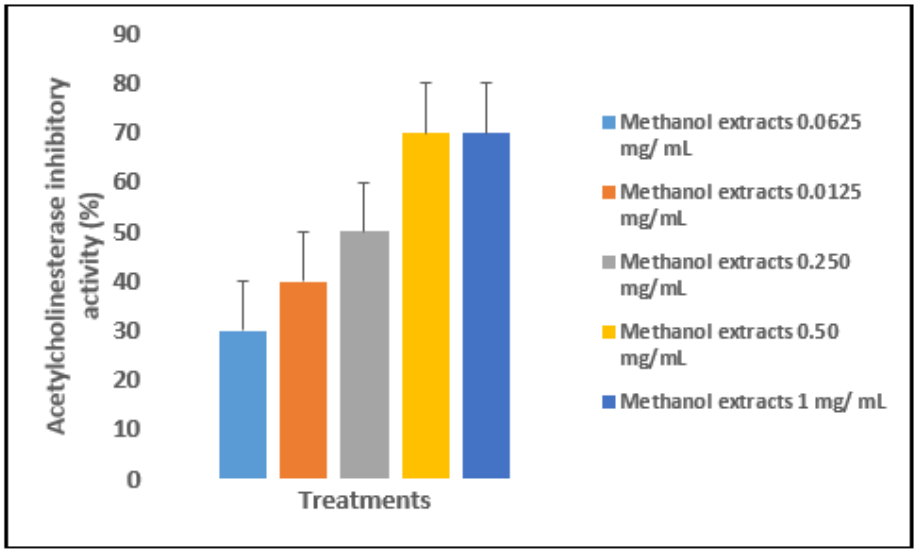

Fig. 18. The inhibitory effect of extracts from *Boerhaavia diffusa* L. on acetylcholinesterase (AChE) activity. The determination of activities of acetylcholinesterase were performed as described in the Materials and Methods section. Each value represents the mean±SEM (n=6)

4. DISCUSSION

The increasing prevalence of diabetes in both developed and developing countries has challenged scientists to further conduct research in sourcing for potent therapeutic agents from natural sources for more efficient usage in the treatment and management of diabetes [43]. Evidence has shown that tight and optimal blood glucose control eliminates diabetic complications [44].

The rapid discovery of various medicinal plants and natural products with anti-diabetic potentials has provided a remarkable intervention in the history of many diseases including diabetes [45].

The basis for the use of a number of plants as novel remedies for diabetic complications cannot be overemphasised [46,47].

Diabetic rats injected with STZ showed elevated plasma glucose levels, which is indicative of hyperglycemia, an observation also reported by other authors [48,49]. 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 [50].

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 [51,52]. 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 [53]. Consequently, ROS are formed during this process and a cascade of reactions occur leading to increased levels of superoxide radicals, hydrogen peroxide, and hydroxyl radicals with potential damaging effects on cell macromolecules in the animals [54,55].

In streptozotocin induced diabetic rat, the elevation in levels of fasting blood glucose and glycated hemoglobin along with diminution in liver levels due to low levels of serum insulin as per present findings and in parallel with our previous reports [56,57].

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 [58] and inducing the sensitivity of cell receptors to insulin, or reduced glucose absorption from the gastrointestinal tract. In the entire cases, methanol extract of stem leaves of

Boerhavia diffus shows its potency, which may be due to flavonoids, and phenol or phenolic compound present in it. Polyphenols has major antioxidant activity with redox properties [59] adsorb and neutralises free radical, extinguish singlet and triplet oxygen, and scavenges peroxides.

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 [60].

This appears through the induction of expression of the insulin gene in pancreatic cells and IRA in hepatic cells, and increasing the serum insulin levels consequently increased glucose uptake through induction of Glut2 gene expression. The STZ induces a selective destruction of pancreatic-cells leading to poor glucose utilisation 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 through increasing the level of gene expression, and so increasing its level in the blood, which can improve glucose metabolism.

Insulin receptors are expressed with different ranges in all tissues that are sensitive to insulin [61]. This enforce our results, which showed high hepatic IRA gene expression levels in the groups that were administrated doses of *Boerhavia diffusa*. Hepatic glucose utilisation was induced possibly due to the induction of gene expression of the Glut2 gene.

The latter is a membrane bound glucose transporter present mainly in the liver, and not dependent on insulin.

The level of HbAlc is monitored as a reliable index of glycemic control in diabetes. Elevated HbAlc was observed in the diabetic group which indicates poor glycemic control. Uncontrolled and long-term diabetes was often accompanied with high glycosylated hemoglobin which is responsible for the development of late diabetic complications namely vascular dysfunction, neuropathy, and diabetic nephropathy. In case of diabetic rats treated with the *Boerhavia diffusa* extract, the HbAlc levels were brought down from elevated level to almost normal.

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 [62].

Hematological complications consist mainly of abnormalities in the functions, morphology and metabolism of erythrocytes, leukocytes and platelets [63].

The primary reasons for assessing the red blood cell (RBC) is to check anemia and to evaluate normal hematopoiesis [64]. There was significant decrease ($p<0.05$) in RBC, HGB levels of diabetic untreated group (Table 1). These may be as a result of anemia or the onset of glycosylation process because the reactive oxygen species (ROS) generated during STZ metabolism has been implicated in red cell damage [65]. Anemia has also been identified as a common complication of chronic kidney disease, affecting over half of all patients and the most common cause of chronic kidney disease in about two-third (2/3) of cases is diabetes mellitus [66]. Both fractions show no damaging effect on RBC and HGB when compared to normoglycemic. Platelets are fragment of cells that participates in blood clotting, and initiate repair of blood vessels, and are also considered as acute phase reactant to infection or inflammation. Platelet count (PLT) showcases the precise method of determining the degree of acute blood loss while white blood cell count (WBC) measures the total number of white blood cells which defend the blood against opportunistic infection. Diabetic untreated rats indicated a significant ($P<0.05$) reduction in PLT and increase in WBC when compared to normoglycemic. This is in line with the studies carried out by Edet [67] that STZ diabetogenesis may cause perturbation in the bone marrow stem cells. Significant ($p<0.05$) increase in platelet count and white blood cell count was observed in groups treated with 300 mg and 600 mg of methanol extract of stem leaves of *Boerhavia diffusa*) when compared to normoglycemic and other treated groups (glibenclamide and methanol extract of stem leaves of *Boerhavia diffusa*). Generally, a reactive thrombocytosis due to abnormal increase in platelet is associated with an increased thrombotic risk when it is accompanied with overproduced red blood cells and white blood cells to some degree [68]. Mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular

hemoglobin concentration (MCHC) in all groups were normal.

Diabetes induces dyslipidemia due to Insulin deficiency or insulin resistance because insulin has an inhibitory action on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-coA) reductase, a key role rate-limiting enzyme responsible for the metabolism of cholesterol rich low density lipid particles [69]. Acute insulin deficiency initially causes an increase in free fatty acid mobilisation from adipose tissue. High density lipoprotien (HDL) is an anti-atherogenic lipoprotein. Fig. 4 showed a significant ($p<0.05$) decrease in low density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG) levels in all diabetes treated groups when compared with diabetic untreated group. This might be due to the reduced hepatictriglyceride synthesis and/ or reduced lipolysis as a result of oral administration of the methanol extract of stem leaves of *Boerhavia diffusa* of 600 mg/kg b.w had more lowering effect ($p<0.05$) on TG and TC. Oral administration of the methanol extract of stem leaves of *Boerhavia diffusa* increased HDL, thus indicating a reversed atherogenic risk. But 600 mg/kg b.w of methanol extract of stem leaves of *Boerhavia diffusa*, glibenclamide revealed significant ($p<0.05$) increase compared to control groupe. The diabetic untreated group depicted a significant $p<0.05$ decrease in HDL levels.

ALT and AST are determined predominantly for hepatocellular damage. High level of AST indicates that the liver is damage due to toxicant effect during cardiac infection and muscle injury. ALT is however more specific to the liver for detecting hepatocellular damage [70].

STZ-induced diabetic rats showed marked hepatocellular damage in the form of inflammation, sinusoidal dilation, fatty changes, and extensive vacuolisation with the disappearance of nuclei. Serum ALT, AST, ALP, and GGT levels were substantially higher in STZ-induced diabetic rats, but it was restored to near normal levels after the treatment with *Boerhavia diffusa*. Thus analysis showed the protective effect of methanol extract of stem leaves of *Boerhavia diffusa* in experimental diabetes.

Increase in the serum level of ALP is due to increased synthesis in presence of increasing biliary pressure [71]. Generally there was significant decrease ($p<0.05$) in ALP, ALT, AST and GGT level in all diabetic treated group which indicate that the methanol extract of stem leaves of *Boerhavia diffusa* has hepatoprotective potentials. ALT was restored to near normal levels after the treatment with *Boerhavia diffusa* and glibenclamide was due to gradual decrease of diabetic complications.

According to previous reports, Diabetes mellitus was presented with alterations in glucose homeostasis that contribute to persistent hyperglycemia and liver plays a major role in the regulation of glucose metabolism [72]. The activity of enzymes like hexokinase, pyruvate kinase, glucose-6-phosphatase, and fructose-1,6-diphosphatase was markedly altered, resulting in hyperglycemia, which leads to the pathogenesis of diabetic complications [73]. The altered the activity of hexokinase and pyruvate kinase, key enzymes in the catabolism of glucose, diminishing the metabolism of glucose and ATP production in diabetic conditions. The reduction in the activities of these enzymes in the liver tissues of diabetic rats is an indication of reduced glycolysis and amplified gluconeogenesis signifying that these two pathways are distorted in diabetes. In agreement with the above reports, the activities of hexokinase and pyruvate kinase were significantly decreased in the STZ-induced Diabetes mellitus group.

Administration of methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide to diabetic rats significantly elevated these enzyme activities in liver. The activities of regulatory enzymes in gluconeogenesis, like glucose-6-phosphatase and fructose-1,6-diphosphatase, are elevated in Diabetes mellitus [74] and increased activities of these enzymes in STZ- induced diabetic rats may be due to insulin insufficiency [75]. Glucose-6-phosphatase and fructose-1,6-diphosphatase are dephosphorylating enzymes which impair hepatic glucose utilisation. Our results showed that the activities of glucose-6-phosphatase and fructose-1,6-diphosphatase were significantly decreased by the administration of methanol extract of stem leaves of *Boerhavia diffusa* or glibenclamide.

Glycogen is the primary intracellular storage form of glucose and its quantity in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition [76]. The reduced glycogen store in diabetic rats has been attributed to the loss of glycogen synthase-activating system and/or the increased activity of glycogen phosphorylase [77]. In the present study, there was a decrease in the hepatic

glycogen content of diabetic rats which suggests the increased glucose output during insulin deficiency. Here diabetic animals showed increased glycogen phosphorylase activity when compared with control animals. Treatment with *Boerhavia diffusa* or glibenclamide restored the levels of glycogen, probably by means of decreasing the activity of glycogen phosphorylase.

Modulatory effect of *Boerhavia diffusa* was also studied on the free radical metabolising enzymes which render the protection against oxidative stress, in addition to the status of the oxidative damage in the liver of rat.

Antioxidant enzymes (Superoxide dismutase, catalase and glutathione peroxidase) delay or prevent the oxidation of substrates and prevent ROS-induced oxidative stress [78]. The synergistic relationship between superoxide dismutase and Catalase against ROS accumulation inactivates peroxyl radicals and superoxide anions, converting them to water and oxygen [79]. Glutathione peroxidase detoxifies H_2O_2 and lipid peroxides using GSH as substrate.

As a preventive measure against oxidative stress, organisms have evolved endogenous defence. Superoxide dismutase, one of the enzymes of defence system dismutates O^{2-} to H_2O_2 is subsequently removed by catalase and glutathione peroxidase by reducing it to H_2O [80].

Boerhavia diffusa L.modulaed the specific activities of superoxide dismutase, catalase, GPx significantly, as consequence O^{2-} likely to be dismuted and H_2O_2 thus formed be reduced to H_2O resulting into protection against oxidative stress.

In a cell, the one electron reduction of H_2O_2 catalysed by transition metals generates $HO^{\cdot}$, the most reactive oxygen species which interacts with biomolecules by abstracting the hydrogen and subsequently breaking the chemical bond hemolytically [81]. As mentioned earlier, $HO^{\cdot}$ initiates free radical chain reaction in the form of peroxidation. The protective action exhibited by *Boerhavia diffusa* L.against oxidative damage in the present study confirm their ability to scavenge the free radicals and in turn their antioxidant activity.

Boerhavia diffusa downregulated the activity of catalase and glutathione peroxidase that was very high in diabetes rats [82]. It is possible that polyphenols, present in these extracts may be responsible for these beneficial effects. It has been shown that *Boerhavia diffusa* exhibited free radical-scavenging activity, significant blood sugar reduction capacity, and reduced the levels of oxidative stress markers like catalase in animal model [83].

The role of peroxidative processes in disease is a subject of intense research interest. Lipid peroxidation of cell membranes is associated with various pathological events such as atherosclerosis, inflammation and liver injury [84]. Decrease in lipid peroxidation by extracts from stem leaves of *Boerhavia diffusa* may be a result of it scavenging OH produced by $FeCl_2$-H_2O_2 and H_2O_2 in the reaction system [85].

Our extracts reduced lipid peroxidation in liver, the primary target organ of drug metabolism. These results suggest that the methanol extract of stem leaves of *Boerhavia diffusa* may not cause hepatotoxicity; but acts as protective agent by preventing oxidative damage.

These findings could be explained by the production of pre-oxidised myoglobin that is susceptible to further oxidation [86] or by cooking's decreased ferric ion reducing capacity, but increases non-heme iron of meat, resulting in comparatively lower ferric ion reducing capacity in cooked than raw meat.

Xanthine oxidase (XO) inhibitors are known to be therapeutically useful for the treatment of gout, hepatitis and brain tumor [87]. The inhibition of XO activity has been attributed to various compounds such as polyphenols and flavonoids. Our results are confirmed by those obtained by Boumerfeg et al. [88], Wu et al. [89], Baghiani et al. [90], Baghiani et al. on Carthamus caurulis [91]. They found that the richest extracts phenolic compounds are the most active XO.

The 5-LOX pathway generates an important class of inflammatory mediators, such as leukotrienes (LTs), which plays a major part in the inflammatory process [92]. ROS have been implicated in the process of inflammation [93]. Antioxidants (such as polyphenolics, flavonoids) are known to inhibit plant lipoxygenase. Polyphenols are widely distributed in nature and some studies have revealed that polyphenols constitute rich inhibitors of LOX product synthesis [94]. The role of antioxidants in the inhibition of inflammatory enzymes such as LOX enzymes [95,96]. The antiinflammatory (anti-

lipoxygenase) activities of plants extracts could be explained by the potent inhibitory effects of their phenolic compounds on arachidonic acid metabolism through the lipoxygenase pathway. The results suggest that methanol extract of stem leaves of *Boerhavia diffusa* have potentially high anti-inflammatory effect (antilipoxygenase activity), which might be related to polyphenolic content and other antioxidant substances. Phenolic compounds and antioxidants such as flavonoids, saponin etc may block the arachidonic acid pathway by inhibiting LOX activity and thus may serve as scavengers of ROS which are produced during arachidonic acid metabolism.

5. CONCLUSION

Our study shows that oral administration of methanol extract of stem leaves of *Boerhavia diffusa* exerted antidiabetic mediated through the regulation of carbohydrate metabolic enzyme activities, modulate the activities of anti-inflammatory and antioxidant enzymes in experimental diabetes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shen SC, Cheng FC, Wu NJ. Effect of Guava (*Psidiumguajava* Linn.) leaf soluble solids on glucose metabolism in type 2 diabetic rats. Phytotherapy Research. 2008;22:1458-1464.
2. Ceriello A. Postprandial hyperglycemia and dia-betes complications. Is it time to treat? Diabetes. 2005;54:1-7.
3. Abe A, Kawasoe C, Kondo Y, Sato K. Enhancement of norepinephrine-induced transient con-traction in aortic smooth muscle of diabetic mice. Acta Medica Okayama. 2003;57:45-48.
4. Marles RJ, Farnsworth N. Antidiabetic plants and their active constituens. Phytomedicine. 1995;2:137-189.
5. Pari L, Umamaheswari J. Antihyperglycae-mic activity of Musa Sapientum flowers: Effect on lipid peroxidation in alloxan diabetic rats. Phytotherapy Re-search. 2000;14:1-3.
6. Bhalla TN, Gupta MB, Bhargava KP. Antiinflammatory activity of *Boerhaavia diffusa.* J Res Ind Med. 1971;6:11-15.
7. Hiruma-Lima CA, Gracioso JS, Bighetti EJ, Germonsen RL, Souja BAR. The juice of fresh leaves of *Boerhaavia diffusa* L.(Nyctaginaceae) markedly reduces pain in mice. J Ethnopharmacol. 2000;71:267-274.
8. Mudgal V. Studies on medicinal properties of*Convolvulus pluricaulis* and *Boerhaavia diffusa*. Planta Med. 1975;28:62-68.
9. Singh RP, Shokala KP, Pandey BL, Singh, RG, Usha, Singh R. Recent approach in clinical and experimental evaluation of diuretic action of Purnarnava (*Boerhaavia diffusa)* withspecial effect to nephrotic syndrome. J Ind Med Res. 1992;11:29-36.
10. Chakraborty KK, Handa SS. Antihepatotoxic investigations of *Boerhaavia diffusa L*. Ind Drugs. 1989;27: 161-166.
11. Rawat AKS, Mehrotra S, Tripathi SC, Shome U. Hepatoprotective activity of *Boerhaavia diffusa* L. roots – A popular Indian ethnomedicine. J Ethnopharmacol. 1997;56:61-66.
12. Mehrotra S, Mishra KP, Maurya R, Srimal RC, Singh VK. Immunomodulation by ethanolic extract of *Boerhaavia diffusa* roots. Int Immunopharmacol. 2002;2:987-996.
13. Mehrotra S, Singh VK, Agarwal SS, Maurya R, Srimal RC. Anti-lymphoproliferative activity of ethanolic extract of *Boerhaavia diffusa* roots. Exptl Mol Pathol. 2002;72:236-242.
14. Mishra J, Singh R. The effect of indigenous drug *Boerhaavia diffusa* on kidney regeneration. Ind J Pharmacol. 1980;12:59-64.
15. Singh RH, Udupa KN. Studies on the Indian indigenous drug Punarnava (*Boerhaavia diffusa* L.) Part IV. Preliminary controlled clinical trial in nephrotic syndrome. J Res Ind Med. 1972;7:28-33.

16. Agarwal RR, Dutt SS. Chemical examination of punarnava or *Boerhaavia diffusa* Linn. II. Isolation of an alkaloid punarnavine. Chemical Abstract. 1936;30: 3585.
17. Basu NK, Lal SB, Sharma SN. Investigations on Indian medicinal plants. Quarterly Journal of Pharmacy and Pharmacology. 1947;20:38-42.
18. Surange SR, Pendse GS. Pharmacognostic study of roots of *Boerhaavia diffusa* Willd. (punarnava). Journal of Research in Indian Medicine. 1972;7:1.
19. Kadota SN, Kikuchi T. Constituents of the roots of *Boerhaavia diffusa* Linn. I. Examination of sterols and structures of new rotenoids (boeravinones A and B). Chemical and Pharmaceutical Bulletin. 1989;37(12):3214-3220.
20. Lami N, Kadota S, Tezuka Y, Kikuchi T. Constituents of the roots of *Boerhaavia diffusa* Linn. IV. Isolation and structure determination of boeravinones D, E and F. Chemical and Pharmaceutical Bulletin. 1992;39(7):1863-1865.
21. Lami N, Kadota S, Tezuka Y, Kikuchi T. Constituents of the roots of *Boerhaavia diffusa* Linn. II. Structure and stereochemistry of a new rotenoid boeravinone C2. Chemical and Pharmaceutical Journal. 1990;38(6):1558-1562.
22. Ahmad KA. Isolation, synthesis and biological action of hypoxanthine-9-Larabinofuranoside. Journal of Agricultural and Biological Sciences. 1968;11:41.
23. Jain GK, Khanna NM. Punarnavoside: A new antifibrinolytic agent from *Boerhaavia diffusa* Linn. Indian Journal of Chemistry. 1989;28(B):163-166.
24. Mishra AN, Tiwari HP. Constituents of the roots of *Boerhaavia diffusa*. Phytochemistry. 1971;10:3318.
25. Aftab K, Usmani SB, Ahmad SI, Usmanghani K. Naturally occurring calcium channel blockers-II. Hamdard Medicus. 1996;39:44-54.
26. Lami N, Kadota S, Kikuchi T, Momose Y. Constituents of the roots of *Boerhaavia diffusa* L. III. Identification of a Ca^{2+} channel antagonistic compound from the methanol extract. Chemical and Pharmacological Bulletin. 1991;39(6): 1551-1555.
27. Mama SAI, Attakpa SE, Béhanzin GJ, Amoussa AM, Lagnika L, Guinnin F, Akotègnon R, Yédomonhan H, Sezan A, Baba-Moussa F, Baba- Moussa L. Antioxidant and free radical scavenging activity of various extracts of *Boerhavia diffusa* Linn. (*Nictaginaceae*). Pharmaceutical and Chemical Journal. 2018; 5(2):62-71.
28. Jaiswal D, Rai PK, Mehta S, Chatterji S, Shukla S, Rai DK, Sharma G, Sharma B, Watal G. Role of *Moringa oleifera* in regulation of diabetes-induced oxidative stress. Asian Pac. J. Trop. Med. 2013;6: 426–432.
29. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med. 1984;310:341–6.
30. Crane RK, Sols A. The association of hexokinase with particulate fractions of brain and other tissue homogenates. J Biol Chem. 1953;203:273–92.
31. Bucher T, Pfleiderer G. Pyruvate kinase from muscle. Methods Enzymol. 1955;1: 435–40.
32. Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem. 1956;220:583–93.
33. Koide H, Oda T. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. Clin Chim Acta. 1959;4:554–61.
34. Pontremoli S. Fructose-1,6-diphosphatase: I. Rabbit liver (crystalline). Methods Enzymol. 1966; 9:625–31.
35. Singh VN, Venkatasubramanian TA, Viswanathan R. The glycolytic enzymes of guinea pig lung in experimental bagassosis. Biochem J. 1989;78:728–732.
36. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469–74.
37. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121–6.
38. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1): 158–69.
39. Su XY, Wang ZY, Liu JR. *In vitro* and *in vivo* antioxidant activity of *Pinus koraiensis* seed extract containing phenolic compounds. Food Chem. 2009;117:681-686.
40. Filha FZS, Vitolo IF, Fietto LG, Lombardi JA, Saude-Guimaraes DA. Xanthine

oxidase inhibitory activity of Lychnophora species from Brazil (Arnica). J. Ethnopharmacol. 2006;107:79-82.
41. Malterud KE, Rydland KM. Inhibitors of 15-lipoxygenase from orange peel. J. Agric. Food Chem. 2000;48:5576-5580.
42. Eldeen IMS, Eldeen EE, Elgorashi J, Van S. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. Journal of Ethnopharmacology. 2005;102:457-464.
43. Gupta R, Mathur M, Bajaj VK, Katariya P, Yadav, S, Kamal R, Gupta RS. Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes. J. Diabetes. 2012;4:164–171.
44. Thirumurugan K, Ankita BJ, Shihabuden MS. Screening of fifteen ayurvedic plants for alpha glucosidase inhibitory activity and enzyme kinetics. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3:267-274.
45. Koul B, Chase NJ. *Moringa oleifera* Lam: Panacea to several maladies. J. Chem. Pharm. Res. 2015;7:687–707.
46. Nishikawa T, Araki E. Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications. Antioxid. Redox Signal. 2007;9:343–353.
47. Sharma K, Ziyadeh FN. Hyperglycemia and diabetic kidney disease: The case for transforming growth factor-β as a key mediator. Diabetes. 1995;44:1139–1146.
48. Yassa HD, Tohamy AF. Extract of *Moringa oleifera* leaves ameliorates streptozotocin-induced diabetes mellitus in adult rats. Acta Histochem. 2014;116:844–854.
49. Toma A, Makonnen E, Debella A, Tesfaye B. Antihyperglycemic effect on chronic administration of Butanol fraction of ethanol extract of *Moringa stenopetala* leaves in alloxan induced diabetic mice. Asian Pac. J. Trop. Biomed. 2012;2:1606–1610.
50. Ayeleso A, Brooks N, Oguntibeju OO. Modulation of antioxidant status in streptozotocin-induced diabetic male Wistar rats following intake of red palm oil and/or rooibos. Asian Pac. J. Trop. Med. 2014;7:536–544.
51. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. Front. Pharmacol. 2012;3:24.
52. Akbarzadeh A, Norouzian D, Mehrabi, MR, Jamshidi S, Farhangi A, Verdi AA. Induction of diabetes by streptozotocin in rats. Indian J. Clin. Biochem. 2007;22:60–64.
53. Novío S, Nfflçez MJ, Amigo G, Freire-garabal M. Effects of fuoxetine on the oxidative status of peripheral blood leucocytes of restraint-stressed mice. Basic Clin. Pharmacol. Toxicol. 2011;109: 365–371.
54. Fahey JW. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees Life J. 2005;1:1–5.
55. Ayepola OR, Chegou NN, Brooks NL, Oguntibeju OO. A Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. BMC Complement. Altern. Med. 2013;13: 363–372.
56. Chatterjee K, Ali KM, De D, Mallick Ghosh D. Antihyperglycaemic, antioxidative activities of a formulated polyherbal drug MTEC (modified) in streptozotocin-induced diabetic rat. J. Med. Plants Res. 2009;3: 468-480.
57. Mandal S, Barik B, Mallick C, De D, Ghosh D. Therapeutic effect of ferulic acid, an ethereal fraction of ethanolic extract of seed of *Syzygium cumini* against streptozotocin- induced diabetes in male rat, Method Find. Exp. Clin. Pharmacol. 2008;30:121-128.
58. Metz E, Houghton A. Insulin receptor substrate regulation of phosphoinositide 3-kinase. Clin Cancer Res. 2011;17:206-211.
59. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J. Agri. Food Chem. 2001;49:5165-5170.
60. Hii CS, Howell SL. Effects of flavonoids on insulin secretion and Ca^{2+} handling in rat islets of Langerhans. J Endocrinol. 1985; 107:1–8.
61. Yadav JP, Kalia AN, Dangi AS. Hypoglycaemic activity of extracts of *Salvadora oleoides* in normal and alloxan induced diabetic rats. Journal of Indian Pharmacology. 2008;40:23-27.
62. Kasetti RB, Rajasekhar MD, Kondeti VK. Antihyperglycemic and antihyperlipidemic activities of methanol: Water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin

induced diabetic rats. Food Chem Toxicol. 2010; 48:1078–84.

63. Comazzi S, Spagnolo V, Bonfanti U. Erythrocyte changes in canine diabetes mellitus: *in vitro* effects of hyperglycaemia and ketoacidosis. Journal of Comparative Clinical Pathology. 2004;12:199-205.
64. Asanga E, Edet EEP, Eseyin AO. Hematological parameters of alloxaninduced diabetic rats treated with ethanol extracts and fractions of *Naucleala filoia* leaf. European Scientific Journal. 2014;9(27):203-210.
65. Rao G, Kamath U, Raghothama C, Pradeep KS, Rao P. Maternal and fetal indicators of oxidative stress in various obstetric complications. Indian Journal on Clinical Biochemistry. 2003;18:80-86.
66. Jeremy MB, John LT, Lubert S. Textbook of Biochemistry. 5th edition. Integrated Media. W.H. Freeman and Company. New York; 2002.
67. Edet EE, Akpanabiatu MI, Uboh FE, Edet TE, Eno AE, Itam EH, Umoh IB. *Gongronema latifolium* crude leaf extract reverses alterations in hematological indices and weight loss in diabetic rats. Journal of Pharmacology and Toxicology. 2011;6:174-181.
68. Patel SS, Shah RS, Goyal RK. Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a poly herbal Ayurvedic formulation in streptozotocin induced diabetic rats. Indian Journal of Experimental Biology. 2009;47:564-570.
69. Cryer PE. Hypoglycaemia: The limiting factor in the glycaemic management of type I and type II diabetes. Diabetologia. 2002;45:937-948.
70. Thomas HA, David JD, John EH, Thomas EL, Davis RM, Gregory M, David BY. In: Pocket companion to Guyton & Hall Textbook of Medical Physiology. Eleventh Edition. Saundres Elsevier. 2006;604-610.
71. Uma M, Shalimol A, Arumugasamy K, Udhayasankar MR, Punitha D. Effect of methanolic extract of Smilax Wightii A. Dc. on serum protein profile in streptozotocin induced diabetic rats. International Journal of PharmTech Research. 2014;6(5):1746-1750.
72. Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Biosci Rep. 2001;21:19–24.
73. Bhavapriya V, Govidasamy S. Biochemical studies on the hypoglycemic effect of *Aegle marmelos* (Linn). Correa Ex. RoxB. In streptozotocin induced diabetic rats. Indian Drugs. 2000; 37:474–477.
74. Grover JK, Vats V, Rathi SS. Anti-hyperglycaemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol. 2000;73:461–70.
75. Baquer NZ, Gupta D, Raju J. Regulation of metabolic pathways in liver and kidney during experimental diabetes. Indian J Clin Biochem. 1998;13:63–80.
76. Pederson BA, Schroeder JM, Parker GE. Glucose metabolism in mice lacking muscle glycogen synthase. Diabetes. 2005;54:3466–73.
77. Golden S, Wals PA, Okajima F. Glycogen synthesis by hepatocytes from diabetic rats. Biochem J. 1979;182:727–34.
78. Naugler WE, Karin M. The wolf in sheep's clothing: The role of interleukin-6 in immunity, inflammation and cancer. Trends Mol. Med. 2008;14:109–119.
79. Kukreja RC, Hess ML. The oxygen free radical system: From equations through membrane-protein interactions to cardiovascular injury and protection. Cardiovasc. Res. 1992;26:641–655.
80. Thanh T, Thanh HN, Thi Minh HP, Thi Thu HL, Thi Ly HD, Duc LV. Protective effect of *Tetracera scandens* L. leaf extract against CCl4-induced acute liver injury in rats. Asian Pacific Journal of Tropical Biomedicine. 2015;5(3):221–7.
81. Kale R. Post-irradiation free radical generation: Evidence from the conversion of xanthine dehydrogenase into xanthine oxidase. Indian J Exp Biol. 2003;41(02): 105–11.
82. Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol Cell Biochem. 1995;151:113-119.
83. Montefusco-Pereira CV, de Carvalho ML, de Araujo Boleti AP, Teixeira Ls, Matos HR. Antioxidant, anti-inflammatory, and hypoglycemic effects of the leaf extract from *Passiflora nitida* Kunth. Appl Biochem Biotechnol. 2013;170:1367–1378.
84. Roome TA, Dar S, Ali S. A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts. J. Ethnopharmacol. 2008;118:514-521.

85. Wang H, Gao XD, Zhou GC, Cai L, Yao WB. *In vitro* and *in vivo* antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. Food Chem. 2008;106:888-895.
86. Samouris GI, Bampidis VA, Sossidou E, Zantopoulos N. Lipid oxidation of raw and cooked turkey breast meat during refrigerated storage. Arch fur Geflugelkd. 2007;71:41–4.
87. Song YS, Kim SH, Sa JH, Jin C, Lim CJ, Park EH. Anti-angiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. J. Ethnopharmacol. 2003;88:113-116.
88. Boumerfeg S, Baghiani A, Messaoudi D, Khennouf S, Arrar L. Antioxidant properties and xanthine oxidase inhibitory effects of *Tamus communis* L. Root Extracts. Phytother Res. 2009;23:283-8.
89. Wu N, Zu Y, Fu Y, Kong Y, Zhao J, Li X, Li J. Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. J Agric Food Chem. 2010;58:4737-43.
90. Baghiani A, Boumerfeg S, Belkhiri F, Khennouf S, Charef N, Harzallah D. Antioxidant and radical scavenging properties of *Carthamus caeruleus* L. extracts grow wild in the Algeria flora. Comun Sci. 2010;1:128-36.
91. Widyarini KD, Sukandar EY, Fidrianny I. Xanthine oxidase inhibitory and antihyperuricemic activities of *Anredera cordifolia* (Ten) Steenis, *Sonchus arvensis* L. and its combination. Int J Pharm Pharm Sci. 2015;7(3):87-90.
92. Li RW, Lin GD, Leach DN, Waterman PG, Myers SP. Inhibition of COXs and 5-LOX and activation of PPARs by Australian Clematis species (*Ranunculaceae*). J. Ethnopharmacol. 2006;104:138-143.
93. Trouillas P, Calliste CA, Allais DP, Simon A, Marfak A, Delage C, Duroux JL. Antioxidant, antiinflammatory and antiproliferative properties of sixteen water extracts used in the Limousin countryside as herbal teas. Food Chemistry. 2003;80: 399-407.
94. Werz O. Inhibition of 5-lipoxygenase product synthesis by natural compounds of plant origin. Planta Medica. 2007;73:1331-1357.
95. Middleton E, Kandaswami C, Theoharis C. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacological Reviews. 2000;52:673-751.
96. Arts ICW, Hollman PCH. Polyphenols and disease risk in epidemiologic studies. The American Journal of Clinical Nutrition. 2005;81:317S-325.

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