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Assessment of Hepatoprotective and Antidyslipidemic activities of Phenolic leaf extract of *Vitex doniana* on Alloxan-Induced Diabetic Rats

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Abstract

This study investigated the hepatoprotective and antidyslipidaemic activities of phenolic leaf extract of V*itex doniana* (PEVD) on alloxan-induced diabetic rats. Five non-alloxan exposed rats were labelled Normal control and 25 alloxan-induced diabetic rats were divided into five groups (D.C, D-100, D-200, D-400 and D-std) of five rats each. All the groups were administered orally for 14 days the following: Normal control and Diabetic control (D.C) rats received 1.0 ml/kg body weight (b.wt) normal saline; D-100, D-200 and D-400 received 100 mg/kg, 200 mg/kg and 400 mg/kg b.wt PEVD respectively, and D-std received 0.50 mg/kg b.wt glibenclamide. The results showed that induction of diabetes caused significant elevation in serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and negative fluctuations in the concentrations of serum protein and bilirubin, elevation in serum concentrations of total cholesterol, triacylglycerides, low density lipoproteins, and reduction in high density lipoproteins indicating significant elevation of atherogenic index. However, treatment with PEVD presented significant reduction in activities of these enzymes and amelioration of serum proteins and bilirubin concentrations near to normal. Furthermore, the results showed dose dependent amelioration of altered lipid profile and atherogenic and cardiac indices. These results indicate that phenolic leaf extract of *Vitex doniana* possess hepatoprotective and anti-dyslipidaemic capacity, to maintain the livers' functional integrity and ameliorate diabetic-induced dyslipidaemic conditions respectively, justifying its use in traditional medicine to manage diabetes and its related diseases.

Keywords:*Vitex doniana*, phenols, diabetes, liver enzymes, dyslipidemia, atherogenic index

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1. Introduction

Diabetes mellitus is a prevalent disease of public health importance with 80% occurrence in low- and middle-income countries [1]. The human population living with diabetes is continuously increasing globally due to the diverse changes in diet in all cultures [2]. Diabetes mellitus is ranked seventh among the leading cause of death and third in diseases with life threatening complications, affecting various body organs [3]. The liver, an organ responsible for carbohydrate homeostasis is threatened by diabetic complications, which include the loss of hepatic membrane integrity, leading to extrusion of marker enzymes into the plasma [4,5]. Elevated liver enzymes are predictors of biochemical abnormalities and future risk of cardiovascular disease in some poorly controlled diabetic conditions. These biochemical disturbances and hepatomegaly are found to be reversible with good glycemic control [6]. The insulin deficiency that characterizes diabetes mellitus leads to accumulation of lipids- total cholesterol, triacylglycerides etc among others in diabetic patients. Diabetic alteration of serum lipid levels is termed dyslipidemia. Dyslipidemia is a metabolic disorder that constitutes a crucial risk factor of atherosclerosis and cardiovascular disease (CVD) [7]. Hyperglycaemia and dyslipideamia are common occurrences in diabetes mellitus [8,9], and they are characterized by altered metabolism of carbohydrate, lipid and protein. Dyslipidemia (hypercholesterolemia and hypertriglyceridemia) [10,11], is a major predisposing factor for coronary heart disease

(CHD) [12], such as cardiovascular disease, a leading cause of morbidity and mortality [13]. Elevated levels of serum total cholesterol, and especially LDL-cholesterol are important cardiovascular risk factors [14,15]. Elevated level of serum LDL-cholesterol is induced by insulin deficiency which causes increased mobilization of free fatty acid from adipose tissues [16]. There is also reduced HDL-cholesterol, and elevated triacylglyceride levels [17]. Each of these dyslipidemic features is associated with an increased risk of cardiovascular disease. Increased hepatic secretion of large triglyceride rich VLDL and impaired clearance of VLDL appears to be of central importance in the pathophysiology of dyslipidemia [17]. These biochemical disturbances and hepatomegaly are found to be reversible with good glycemic control [6].The search for substances with good glycemic control [6], that can effectively manage diabetic complications with minimal and/or no side effects is renewed. Herbal medicine has used the leaves of *Vitex doniana* plant for treatment of various human disease conditions due to the rich content of phytochemicals. The leaves of *Vitex doniana* is rich in bioactive compounds such as flavonoids, tannins, saponins, anthraquinones, balsam, carbohydrates and resin etc. [18,19]. This study was conceived due to the need to find compounds within the reach of the low- and middle-income populations to effectively manage and/or treat diabetic conditions with readily available and affordable medicinal plants. Therefore, this study assessed hepatoprotective and antidyslipidaemic activities of phenolic leaf extract of V*itex doniana* on alloxan-induced diabetic rats.

2. Materials and methods

2.1. Chemicals reagents, kits and their sources

The chemicals and reagents used in this study include; Aminotranferase kits:ALT, AST, and Alkaline phosphatase test kits (Randox), Bilirubin test kit Randox), Total Protein (Randox), Albumin (Randox), Total Cholesterol (Randox), HDL- Cholesterol test kit (Randox, Triglyceride test kit (Biosystem), LDL- Cholesterol test kit (Randox), Sodium dodecylsulfate (Fluka Biochemika), Sodium phosphate anhydrous (BDH, English), Alloxan monohydrate (Randox laboratories Ltd, Germany), ethylacetate (Sigma, Germany), methanols (Sigma, Germany). All chemicals and reagents used were of analytical grade.

2.2. Plant sample collection

Fresh and healthy plants of *Vitex doniana* were harvested from a farm in Abakiliki, Ebonyi State, Nigeria, in March 2016. The plant was identified by a plant taxonomist, in the Department of Crop Science and Technology, Federal University of Technology Owerri (FUTO) Nigeria. Leaf sample of *Vitex doniana* was given a voucher number, IMSUH/467.

2.2. Preparation of extracts

The fresh leaves of *V. doniana* plant were removed from the stalk, air dried at room temperature (28±2^{o}C), milled to powder. Three portions of 200 g each of the powdered samples were soaked in 800 ml absolute methanol for 72hr with agitation at intervals. Afterwards, the mixture was subjected to coarse and fine filtration. The filtrates were pooled together and concentrated with rotary evaporator at 49°C to obtain the methanol crude extracts of *V. doniana*. Furthermore, the crude methanol extract was dispersed in water and partitioned between ethyl acetate and water in a ratio of 2:1 respectively. The setup was separated with separating funnel and concentrated with rotary evaporator at 40°C to obtain the fraction called phenolic extracts of *V. doniana* (PEVD) [21]. Ethyl acetate has a concentrating effect on plant phenolic compound content due to its solvent polarity [22]. The phenol content was determined by the method of [23] and refrigerated until needed.

Figure 1. *Vitex doniana* Plant; African Plants-A photo Guide [20]

2.3. Experimental Design

Forty male Wistar rats weighing 150±20g were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were housed in metal cages at the Animal House of the Department of Biochemistry, FUTO, acclimatized for one week and allowed free access to water and commercial animal pellets (Guinea Feeds Ltd, Delta State, Nigeria). Approval reference number: FUTO/BCH/EC/2016/05 was given by the Ethics committee of the Department of Biochemistry, FUTO, Nigeria. The experiment adhered to guidelines for protection of human subjects and animal welfare [24].

After acclimatization, animals were weighed and their baseline blood glucose concentrations measured using One Touch Basic Glucometer (Code – 12). To induce diabetes, 30 rats received a single dose intraperitoneal injection of 150 mg/kg body weight (b.wt) of Alloxan monohydrate [25]. Three days after alloxan monohydrate administration, the blood sugar levels of the rats were recorded and 25 rats with blood glucose concentrations of 250 mg/dl and above were selected and referred as diabetic rats [26]. Five rats, randomly selected from the ten non-alloxan exposed rats were labelled Normal control. The diabetic rats were divided into five groups (D.C, D-100, D-200, D-400 and D-std) of five rats each. For 14 days, all the rats were administered orally the following: Normal control and Diabetic control (D.C) rats received 1.0 ml/kg b.wt normal saline, D-100, D-200 and D-400 received 100 mg/kg, 200 mg/kg and 400 mg/kg b.wt phenol leaf extract of *Vitex doniana* respectively, and D-std received 0.50 mg/kg b.wt glibenclamide.All the extracts and drugs were dissolved in normal saline for oral administration.

2.4. Collection and preparation of blood samples

At the end of 14 days study period, all the rats were anaesthetized, sacrificed and blood samples collected by cardiac puncture. Blood samples collected in plain tubes were allowed to clot and the serum separated by centrifugation at 3000 rpm for 10 mins, using Labofuge 300 centrifuge.

2.5. Determination of liver function parameters

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST):Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST):were assayed by the method of Reitman and Frankel [27] using Randox assay kits. *Procedure:* Exactly, 0.5ml of reagent 1 (phosphate buffer, L-alanine and α-oxoglutarate) was added into two test tubes, one containing 0.1 ml of serum and the other containing 0.1ml distilled water (blank). The content in each test tube was mixed, incubated at 37ºC for 30 mins. Also, 0.5 ml of reagent 2 (2, 4-dinitrophenylhydrazine) was added to each of the test tubes, mixed and allowed to stand at 25°C for 20 mins. Then 0.5ml of NaOH solution was added to each of the test tubes, the content of each tube was mixed and absorbance read against blank at 540 nm after 5 mins and the activity determined.

Alkaline phosphatase (ALP): Activities of ALP was assayed by method of Haussament [28] using Randox assay kits. *Procedure*: Exactly 1.0 ml of reagent 1 (Diethanolamine buffer, magnesium chloride and substrate (P-nitrophenylphosphate)) was added into test tube containing 0.02 ml serum. This was mixed, initial absorbance was read and timer was started simultaneously, absorbance was read again after 1, 2 and 3 min at 405 nm and activity (U/l) determined. Total protein (TP): Total protein concentration was determined by the method of Fine [29]. *Procedure:* Exactly 1ml of biuret reagent was added to three test tubes labeled as sample, standard and blank, containing 0.02 ml of serum, 0.02 ml of standard and 0.02 ml of distilled water respectively, the content in each of the test tube was mixed, incubated at room temperature for 10 mins, absorbance read at 540nm and total protein concentration calculated. The method of Doumas et al. [30] was used to determine albumin concentration. Globulin concentration was determined by subtracting albumin concentration from the total protein. Serum total and direct bilirubin was determined by the methods stated in the diagnostic kit as described by Jendrassik and Grof [31].

2.6. Determination of serum lipid profile

Serum total cholesterol (TC) was determined by method of Stein [32]. *Procedure:* Exactly, 1000 µl of the cholesterol reagent (4-Aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and buffer) was added into three test tubes labelled as test, standard and reagent blank containing 10µl serum, 10µl standard reagent and 10µl distilled water respectively. The content of each tube was mixed and incubated at 37ºC for 5 mins. The absorbance was read against the reagent blank at 500nm and total cholesterol calculated.

Serum triacylglycerol (TAG): Concentration of TAG was determined by the method described by Stein [32]. *Procedure:* Exactly, 1000 µl of TAG reagent (buffer and enzyme reagent) was added into test tubes containing 10µl serum, and 10µl standard. Also, 1000µl of the tricylglycerols reagent was added to clean test tube (serving as reagent blank). The content in each of the test tubes was mixed and incubated at 37ºC for 5 mins. The absorbance was read against the reagent blank at 500 nm and TAG calculated.

Serum high density lipoprotein-cholesterol (HDL-c): The serum HDL-c concentration was determined by the method of Wacnic and Albers [33]. *Procedure:* Exactly, 0.5ml of reagent1 (R1) (phosphotungstic acid and magnesium chloride) was added into two test tubes; containing 0.2ml of serum and 0.2ml of standard, the content in each tube was mixed and allowed to stand at room temperature for 10 mins, centrifuged at 4000 rpm for 10 mins and the supernatant collected. Thereafter, 1.0ml of reagent 2 (phosphate, cholesterol esterase, cholesterol oxidase, peroxidase, 4-Aminoantipyrine, sodium cholate and dichlorophenolsulfonate) was added into test tubes containing 0.05ml of sample supernatant, 0.05ml standard supernatant and an empty test tube (reagent blank), mixed and incubated at room temperature for 30 mins. The absorbance was read against the reagent blank at 500nm and HDL-c concentration calculated.

Serum very low and low density lipoprotein-cholesterol (VLDL and LDL-c): VLDL cholesterol was calculated as triglycerides/5 and the concentration of LDL-c was calculated in mg/dl according to the protocol of Friedewald *et al*. [34] using the equation: LDL-c = (TC - TG/5) – HDL-c.

2.7. Statistical Analysis

The data generated were expressed as mean±standard deviation, and analysed using One–way analysis of variance (ANOVA) with the aid of SPSS 20 software. The PostHoc test was done with Least Significant Difference (LSD). Means with $p < 0.05$ was considered statistically significant and were indicated by different alphabets in superscript.

3. Results and Discussions

The result of liver assessment (Figures 1) showed elevated activities of liver function enzymes which is an indication of cellular leakage and loss of functional integrity of hepatocellular membrane [35,36,37.38]. Hepatocellular damage, is one of the characteristic changes in diabetes mellitus. The result shows that untreated Diabetic control rats (D.C) presented significantly elevated activity for AST (116.51± 2.34 U/L) compared to phenolic extract of *V. doniana* treated diabetic groups: D-200 (52.2±5.0 U/L), D-400 (47.7±5.5 U/L) and D-Std (55.75±8.22). Also, untreated Diabetic control rats showed elevated activity for ALT (78.34 ±13.04 U/L) compared to PEVD treated diabetic groups; D-200 (26.1±6.5 U/L), D-400 (23.8±3.3 U/L) and D-Std (24.82±3.07 U/L). Furthermore, untreated Diabetic control rats presented elevated ALP activity (673.90± 147.66 U/L) compared to PEVD treated diabetic groups; D-200 (40.02±3.22 U/L), D-400 (288.04±28.63 U/L) and D-Std (115.61±31.39U/L). The recorded values shows that phenolic extract of *V. doniana* and glibenclamide treated groups significantly lowered serum activities of AST, ALT and ALP, within the values of Normal Control group. These aminotransferases (AST and ALT) are markers of liver functions and any significant increase in their activities is indicative of cellular leakage, loss of functional integrity of hepatocellular membranes [36,39,40,41], and are also associated with necrosis of many tissues [40].

The increase in AST activities can also be viewed as an indication of myocardial cell [42,43]. Decreased enzyme activities recorded in PEVD treated groups (D200, D400 and D.std) and within the Normal control values indicate that the phenolic extract of *V. doniana* impacted positively to the liver and reduced hepatic damage. This finding is in line with other studies that reported reduction in blood glucose and liver marker enzymes on administration of aqueous, ethanol, n-hexane and methanol leaf extracts of *V. doniana* in diabetic rats [44,45]. Similarly, some studies used extracts of Cinnamon and alfalfa to reduce blood glucose and liver enzyme activities [46,47]. Plant polyphenol has membrane stabilizing activity [48], therefore, the reduced serum enzyme activities in phenol extract treated diabetic rats can also be attributed to the polyphenols. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [48,49,50]. However, treatment with 100 mg/kg bwt PEVD recorded no significant decrease in transaminase activity compared with the normal control rats, indicating concentration dependant action of PEVD. The increase in activity of alkaline phosphatase, a membrane bound and liver marker enzyme employed to assess the integrity of plasma membrane and endoplasmic reticulum in the untreated Diabetic control rats, confirms membrane damage and compromised membrane integrity [51,52]. Alkaline phosphatase activity can also rise as a result of large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver [41,53].

Total protein concentration (Figure 2) decreased significantly in Diabetic control (50.08±7.60 g/dl), non-significantly in diabetic treated groups: D-100 (61.72±4.23 g/dl), D-200 (56.39±0.25 g/dl), D-400 (64.19±1.38 g/dl) and D-Std (58.99±0.84 g/dl) when compared to 71.95±7.11 g/dl in Normal control group. The decreased protein concentration recorded for Diabetic control can be attributed to liver damage, which could result in decreased synthesis of various proteins such as serum total protein, albumin, and/ or globulin [41,54]. Total protein concentration in phenolic extract *Vitex doniana* -treated diabetic rats tending towards the values presented by rats in Normal control group, indicates positive effect of PEVD and showing possible enhancement of liver functions. Obasi, *et al.* [45] reported increased total protein in rats treated with methanolic extract of *V. doniana*. Serum albumin (Figure 2) decreased significantly in Diabetic control (26.96±4.54 g/dl) but varied non-significantly in PEVD treated diabetic groups; D-100 (32.50±2.07 g/dl), D-200 (33.32±2.73 g/dl) and D-400 (28.99±1.57 g/dl) when compared to the Normal control, and an indication of restored synthetic function of the liver stimulated by phenolic leaf extract *Vitex doniana*.Serum total protein measures the total amount of albumin and globulin and they serve as transport proteins and for immunologic responses and biomarkers of disease state [55,56]. Albumin is manufactured in the liver, while some globulins are synthesize in liver, but most are made in reticuloendothelial system [56].

However, 400 mg/kg PEVD treated diabetic rats presented significantly higher globulin concentration compared to other treated diabetic groups. This is a dose dependent effect by PEVD and is in line with the work of Elizabeth, *et al.* [57] that reported increased globulin concentration in methanol extract of *Moringa oleifera* treated diabetic rats. The non-significant variation in serum albumin concentration in the treated diabetic rats when compared to the Normal control is an indication of restored synthetic function of the liver stimulated by phenolic leaf extract *Vitex doniana*. Globulin decreased significantly in diabetic rats when compared to normal control. The serum concentration of total bilirubin (Figure 3) of Diabetic control (1.97±0.15 g/dl) increased significantly compared to Normal control (1.35±0.25g/dl), PEVD treated diabetic rats; D-100 (1.17±0.03 g/dl), D-200 (1.05±0.22 g/dl), D-400 mg/kg (1.04±0.20 g/dl) and D-Std (1.15±028 g/dl). Bilirubin is a product of breakdown and destruction of red blood cells with biological and diagnostic values [58,59]. The significantly increased concentration of bilirubin in Diabetic control group indicate possible hepatobiliary damage [60] or decreased uptake of bilirubin by the hepatic cells due to decreased concentration of protein-albumin (Figure 2) needed for its transport [60]. With values of the increased ALP activities recorded, the increased bilirubin concentration can be attributed to the pathological alteration of biliary flow [41.53]. However, the markedly reduced bilirubin in the PEVD-treated groups indicates protection from hepatobiliary damage [60], and inhibition of haemoglobin destruction and restoration of normal bilirubin uptake by the liver. This is in line with diabetic studies that reported decrease in elevated concentration of bilirubin

when treated with aqueous and methanol extract of *V. doniana* [44,45,61,62].

Cholesterol concentration (Figure 4) reduced significantly ($p<0.05$) in diabetic groups administered phenol extract *V. doniana;*D-100 (154.49±6.90 mg/dl), D-200 (140.35±15.71 mg/dl), D-400 (127.93±13.77 mg/dl) in a dose related manner when compared to Diabetic control (175.79±5.70 mg/dl). The hypocholesterolemic activity of phenol of *V. doniana* leaf extract may be attributed to the inhibition of HMG-CoA reductases, stimulation of cholesterol-7-alpha-hydroxylase which converts cholesterol into bile acids or inhibition of cholesterol absorption from the intestine due to formation of complexes [63]. Numerous studies have shown hypocholesterolemic effect of plant phytochemicals [45,52,64]. Diabetes progresses with dyslipidemia, a significant risk factor of atherosclerosis and cardiovascular disease [7,65,66]. Furthermore, results showed that triacylglceride (TAG) concentration (Figure 4) reduced significantly in D-200 (19.23±1.03 mg/dl), D-400 (25.64±3.07 mg/dl) and glibenclamide (15.13±0.73 mg/dl) treated groups compared to Normal (38.92±3.57 mg/dl) and diabetic control (55.33±3.59 mg/dl) groups. The hypertriglyceridemia may be attributed to increased mobilization of free fatty acid (FFA) from peripheral depot [67]. The rise in serum triacylglceride of diabetic untreated rats indicates derangement of lipid metabolism and possible amplified incidence of cardiac dysfunction [68].The serum HDL-cholesterol (Figure 5) were significantly higher in D-200 (79.22±6.12mg/dl), and D-400 (123.06±11.16 mg/dl) phenolic leaf extract *V. doniana* treated rats compared to Diabetic control (65.96 ±1.40 mg/dl). The HDL-c in D-200 and D-400 PEVD treated rats were restored within the values for Normal Control (103.05±22.65 mg/dl) and glibenclamide (93.15±14.91mg/dl). The low HDL-cholesterol observed in untreated diabetic rats indicated significant dyslipidaemia. This is so because, HDL-cholesterol transports cholesterol from the arterial walls back to the liver for biosynthesis. Therefore, the low serum HDL-cholesterol reduces or inhibits its transport capacity in diabetic untreated group and increases the chance of atherosclerosis and vascular diseases [56]. Arvind et al. [69] reported that, decrease in HDL-cholesterol contributes to coronary artery disease. However, the elevated concentration of HDL-cholesterol in phenol extract of *V. doniana,* and glibenclamide treated groups, indicated significant amelioration of dyslipidaemia. This result corroborates other studies that showed decreased concentration of HDL-cholesterol in diabetic rats and increased HDL-cholesterol after administration of plant extracts [44,64]. Furthermore, phenolic extract of *Vitex doniana* treated rats presented significantly reduced LDL-cholesterol (Figure 5) and VLDL (Figure 6) concentrations compared to untreated Diabetic control rats. The phenolic leaf extract *V. doniana* may have induced increased catabolism of LDL- cholesterol via hepatic receptors for final elimination in the form of bile acids [70]. This could imply that phenolic extract of *V. doniana* could improve lipid profile, particularly for cholesterol and LDL-cholesterol in diabetic conditions. Lowering of serum lipid concentrations, especially LDL-cholesterol is an important strategy that has shown effectiveness in delaying the on-set of chronic disorders associated with hyperlipidemia [71].

Figure 6: shows that values of atherogenic index (LDL/HDLc ratio) reduced significantly in D-100 (0.76±0.15 mg/dl), D-200 (0.75±0.02 mg/dl), D-400 (0.21±0.05 mg/dl) PEVD treated diabetic rats and glibenclamide (0.54±0.14 mg/dl) compared to untreated Diabetic control (1.62±0.03 mg/dl). This suggest a dose dependent decrease of atherogenic index in diabetic rats administered phenolic extract of *Vitex doniana*. The atherogenic index is an excellent predictor of cardiovascular disease risk and can be deployed to efficiently monitor the effectiveness of lipid-lowering therapies [72,73], especially, deposition of cholesterol in animal tissues. Deposited LDL and cholesterol can be oxidized by free radicals resulting in irreversible damage to cell membranes. However, flavonoids and phenols have shown capacity to scavenge free radicals by inhibiting LDL oxidation [73,75], and this can protect vertebrate organisms from atherosclerosis [75]. Therefore, the phenolic extract of *Vitex doniana* can be attributed as an effective lipid-lowering remedy comparing favourably with the standard drug. The lowered values of atherogenic index observed in diabetic rats treated with phenolic extract of *Vitex doniana* agrees with studies which reported that natural phenolic compounds have hypolipidemic effects [56,72,76]. The results of the study have shown that induction of diabetes mellitus caused hepatocellular damage as observed in increased activities of liver enzymes, fluctuations in serum proteins, bilirubin and dyslipidaemia. However, the restoration and normalization of liver enzymes activities, concentrations of proteins and bilirubin indicate hepatoprotective potentials of phenolic leaf extract of *V. doniana.* Furthermore, the significant reduction in serum total cholesterol, triacylglycerides, LDL-cholesterol, VLDL, with concomitant significant increase in the HDL-cholesterol which generally lowered atherogenic index indicate the anti-dyslipidaemic potentials of phenolic leaf extract of *V. doniana*. The effects of phenolic leaf extract of *V. doniana* were observed in the 200 mg/kg and 400 mg/kg administration and were comparable with the standard drug- glibenclamide. Finally, with the growing interest for alternative remedies for the treatment of diseases such as diabetes, it is evident from the results recorded in this study that the phenolic leaf extract of *V. doniana* could be a potent chemotherapeutic agent for diabetes and its related diseases.

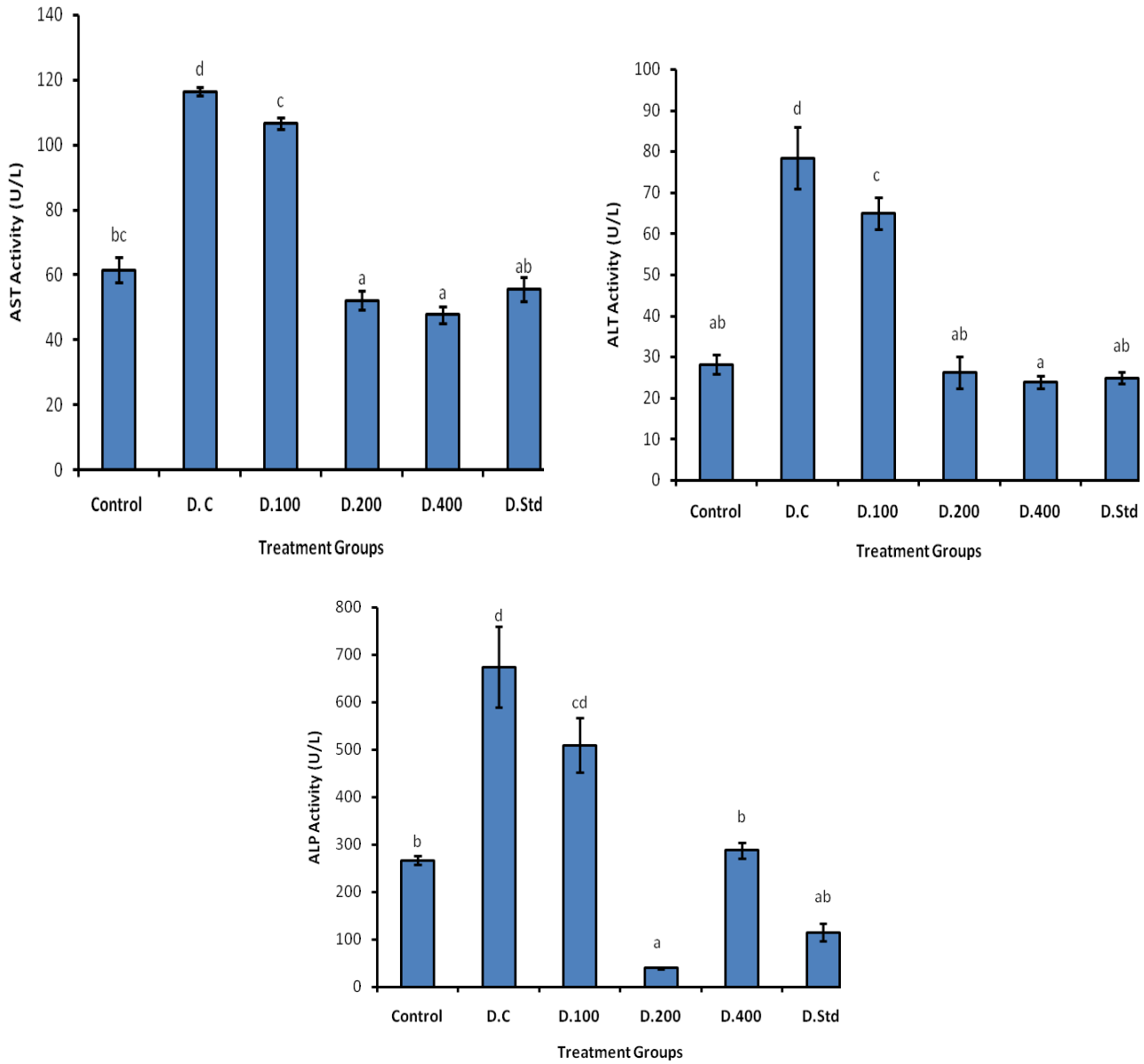

Figure 1: Effect of phenolic extract of *Vitex doniana* (PEVD) on AST, ALT and ALP activities of alloxan-induced diabetic rats. Bars represent mean ±S.D of five determinations. Bars with different letters are significantly different at $p<0.05$. Groups: Control, DC: Diabetic control, D-100: Diabetic rats +100 $mgkg^{-1}$ PEVD, D-200: Diabetic rats + 200 $mgkg^{-1}$PEVD, D-400: Diabetic rats +400 $mgkg^{-1}$PEVD, D-Std: Diabetic rats + Glibenclamide.

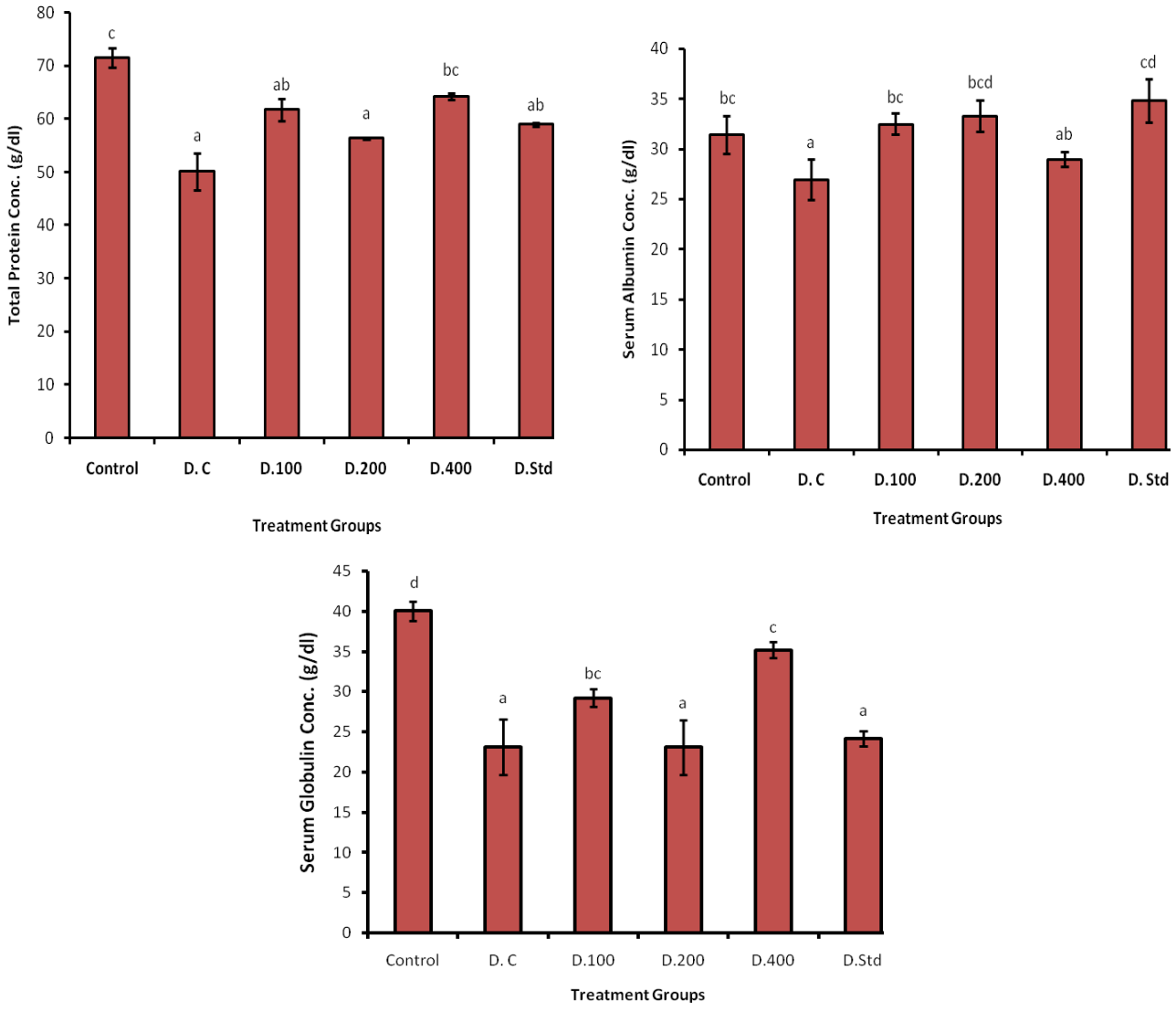

Figure 2: Effect of phenolic extract of *Vitex doniana* (PEVD) on total protein, albumin and globulin concentration of alloxan-induced diabetic rats. Bars represent mean ±S.D of five determinations. Bars with different letters are significantly different at $p<0.05$. Groups: Control, DC: Diabetic control, D-100: Diabetic rats +100 $mgkg^{-1}$ PEVD, D-200: Diabetic rats + 200 $mgkg^{-1}$PEVD, D-400: Diabetic rats +400 $mgkg^{-1}$PEVD, D-Std: Diabetic rats + Glibenclamide.

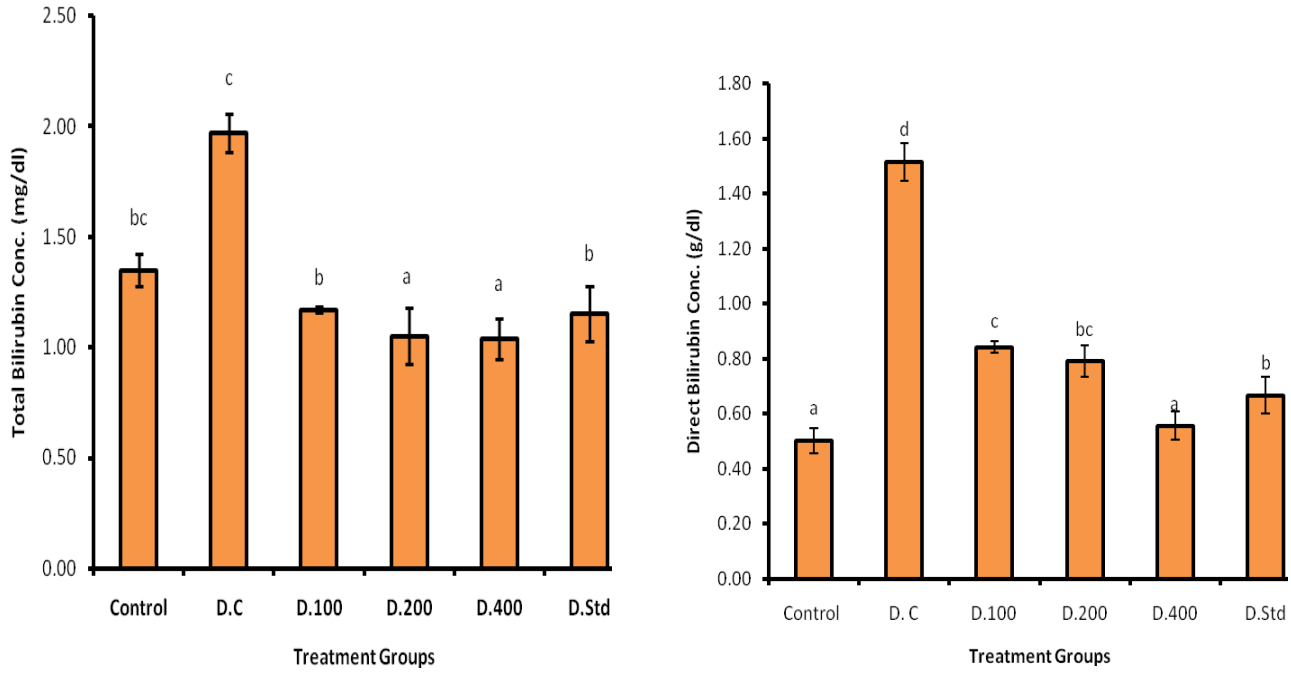

Figure 3: Effect of phenolic extract of *Vitex doniana* (PEVD) on Total and Direct bilirubin concentrations of alloxan-induced diabetic rats. Bars represent mean ±S.D of five determinations. Bars with different letters are significantly different at $p<0.05$. Groups: Control, DC: Diabetic control, D-100: Diabetic rats +100 $mgkg^{-1}$ PEVD, D-200: Diabetic rats + 200 $mgkg^{-1}$ PEVD, D-400: Diabetic rats +400 $mgkg^{-1}$PEVD, D-Std: Diabetic rats + Glibenclamide.

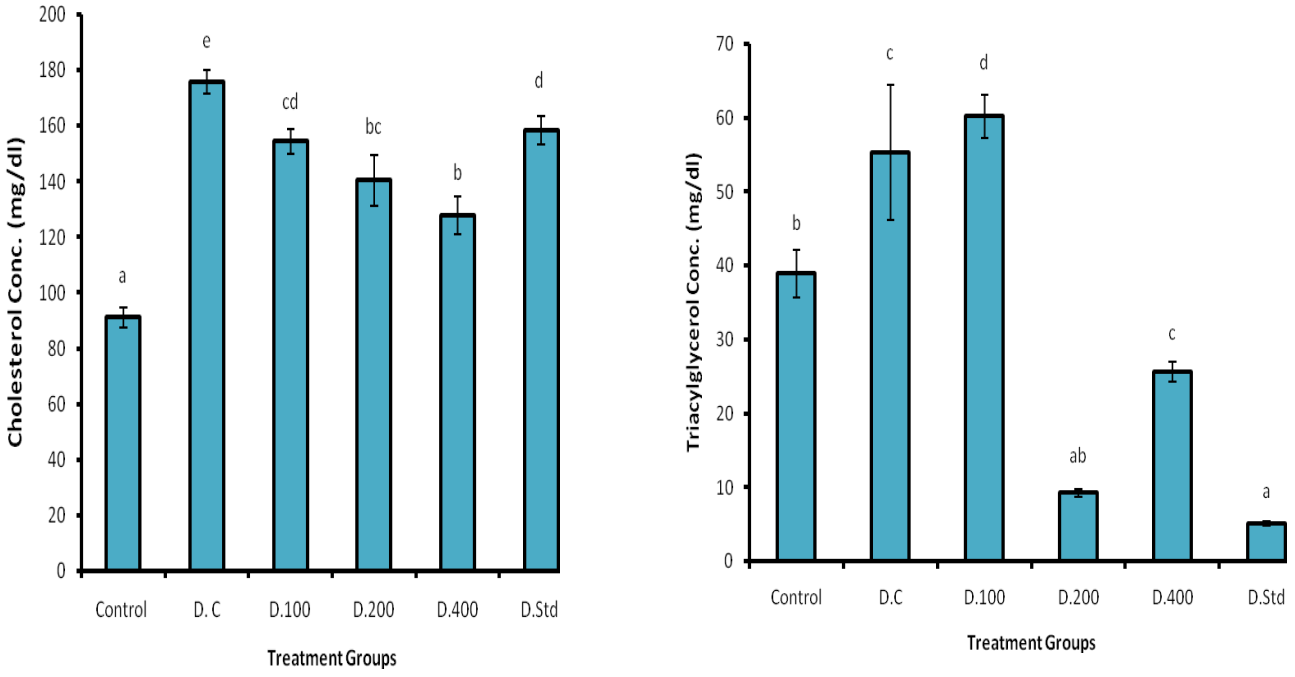

Figure 4: Effect of phenolic extract of *Vitex doniana* (PEVD) on total cholesterol and triacylglyceride of alloxan-induced diabetic rats. Bars represent mean ±S.D of five determinations. Bars with different letters are significantly different at $p<0.05$. Groups: Control, DC: Diabetic control, D-100: Diabetic rats +100 $mgkg^{-1}$ PEVD, D-200: Diabetic rats + 200 $mgkg^{-1}$ PEVD, D-400: Diabetic rats +400 $mgkg^{-1}$PEVD, D-Std: Diabetic rats + Glibenclamide.

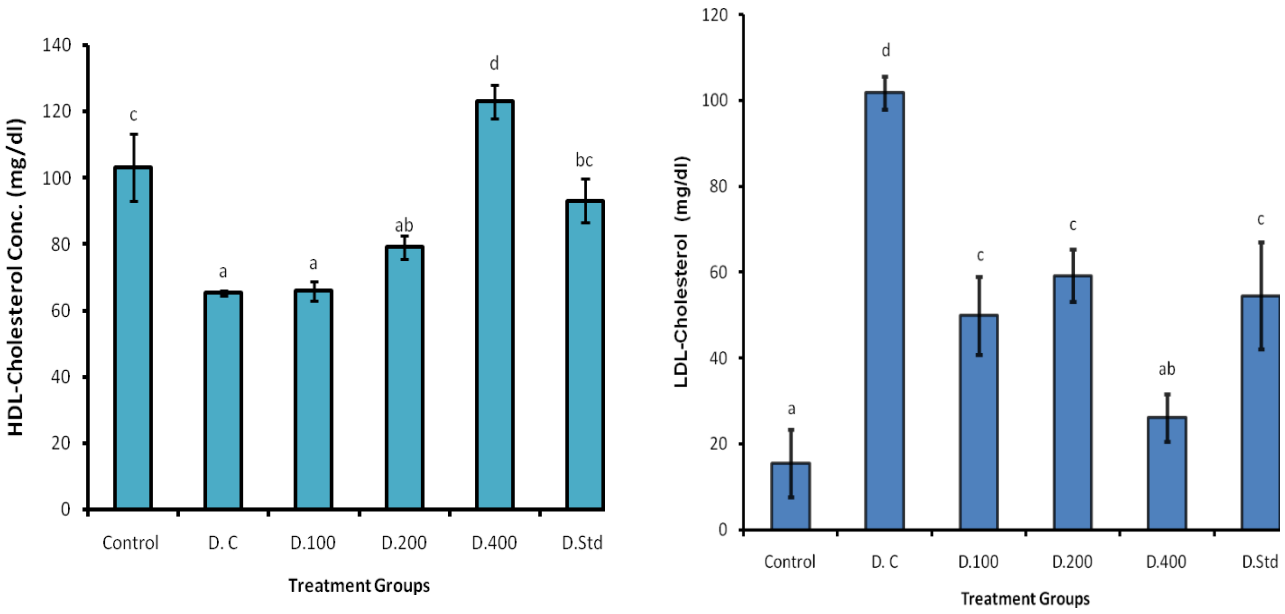

Figure 5: Effect of phenolic extract of *Vitex doniana* (PEVD) on HDL-cholesterol and LDL-cholesterol concentration of alloxan-induced diabetic rats. Bars represent mean ±S.D of five determinations. Bars with different letters are significantly different at $p<0.05$. Groups: Control, DC: Diabetic control, D-100: Diabetic rats +100 $mgkg^{-1}$ PEVD, D-200: Diabetic rats + 200 $mgkg^{-1}$ PEVD, D-400: Diabetic rats +400 $mgkg^{-1}$PEVD, D-Std: Diabetic rats + Glibenclamide.

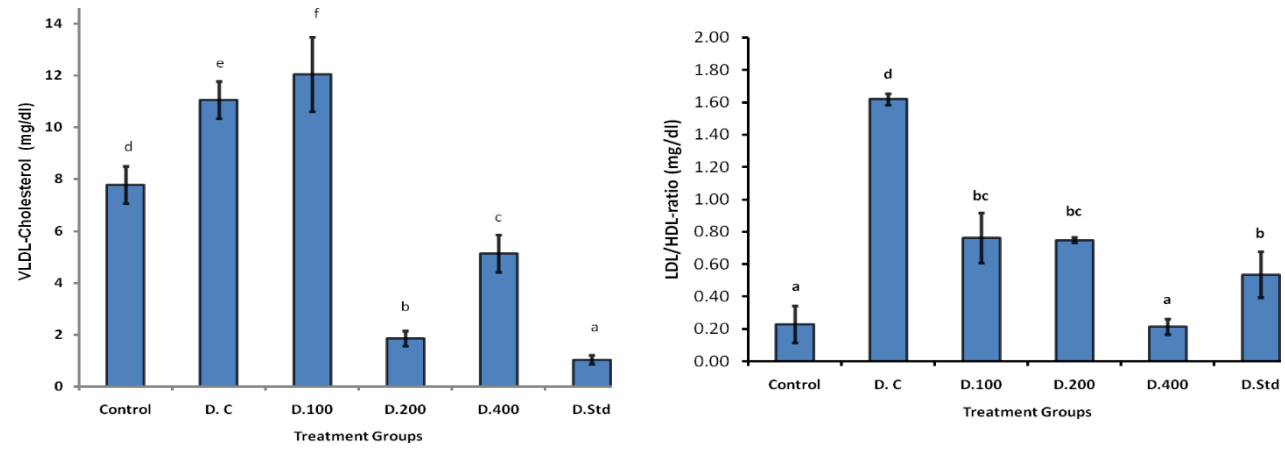

Figure 6: Effect of phenolic extract of *Vitex doniana* (PEVD) on VLDL-cholesterol and HDL/LDL-cholesterol concentration ratio of alloxan-induced diabetic rats. Bars represent mean ±S.D of five determinations. Bars with different letters are significantly different at $p<0.05$. Groups: Control, DC: Diabetic control, D-100: Diabetic rats +100 $mgkg^{-1}$ PEVD, D-200: Diabetic rats + 200 $mgkg^{-1}$PEVD, D-400: Diabetic rats +400 $mgkg^{-1}$PEVD, D-Std: Diabetic rats + Glibenclamide.

Conflict of interest

The authors declare no conflict of interest.

Author's contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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