Original Research Article

JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY

Phenolic leaf extract of *Vitex doniana* ameliorated hepatorenal tissues dysfunctions in diabetic Wistar rats

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Abstract

This study evaluated hepatorenal tissues biomarkers of diabetic rats treated with phenolic leaf extract of *Vitex doniana*. Diabetes mellitus (DM) was induced in the rats by single dose intraperitoneal injection of alloxan monohydrate in phosphate buffer saline (PBS) (pH 7.4); dosage = 100 mg/kg; body weight (*bw*). A total of 36 male Wistar rats were divided into 6 groups of 6 rats each. Separate DM-rat groups were treated with 100 mg/kg; *bw*, 200 mg/kg; *bw* and 300 mg/kg; *bw* of phenolic leaf extract of *V. doniana* as well as standard anti-diabetic drug: 500 mg/kg; *bw* dimethylguanide for 28 days. Levels of serum hepatorenal tissues biomarkers, namely, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities as well as serum concentrations of total bilirubin, total protein, albumin, urea and creatinine were measured using spectrophotometric methods. The results showed dose dependent significant reductions ($p < 0.05$) in serum ALT, AST and ALP activities as well as total bilirubin, urea, creatinine concentrations in treated DM-rat groups. Conversely, treated DM-rat groups exhibited significant increases ($p < 0.05$) in serum total protein and albumin concentrations compared with untreated DM-rat group. The present study showed that phenolic leaf extract of *V. doniana* ameliorated hepatorenal tissues dysfunctions associated with alloxan-induced diabetic rats.

Keywords: Biomarkers; hepatorenal; phenolic; *Vitex doniana*

1 Introduction

Diabetes mellitus (DM) is linked with failure of insulin secretion mechanism and/or inaction of insulin in peripheral tissue and primarily characterized by hyperglycemia [1]. Epidemiological studies showed that DM is a public health concerns in developing countries where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable [2-3]. The increasing rate in DM incidence correlates with increasing trends in urbanization and lifestyle changes, including indulging in "Western-style" diet in developing countries [2]. Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to excessive intracellular formation of free radicals [4]. Free radical damage and oxidative stress are the major causative factors of hepatorenal tissue damage [5]. Increases in plasma activities of non-functional plasma enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST)), and alkaline phosphatase (ALP) are indicators of acute and chronic hepatic dysfunctions [6]. Hepatic enzymes are released into the bloodstream following cellular necrosis and increased cell membrane permeability and therefore, are used as diagnostic indicators of tissue damage [5]. Additionally, compromised renal function is characterized by elevated levels of plasma low threshold substances, namely, plasma urea and creatinine. According to previous studies, delay and impeding the progression of nephropathy could be achieved by good metabolic control against hyperglycemia in that prolonged hyperglycemia engenders diabetic nephropathy that is not easily reversed [7].

There is an urgent need for new, affordable and efficient compounds that could serve as primary agents for treatment and management of DM. Plant materials are pivotal in drug discovery and development required for the management and alleviation of several diseases. Studies showed that the isolation of new bioactive compounds from medicinal plants, based on traditional use or ethnomedical data, is indicating a rewarding approach and prospects [7]. The risks of chronic diseases in humans are ameliorated by the use secondary plant metabolites as therapeutics [8]. In recent years, there has been a considerable interest in natural antioxidants from plant materials because there are concerns that synthetic antioxidants could induce toxicity. Presently, the exploitation and use of drugs of plant origin as well as 'leads' in the production of more potent drugs are receiving renewed and growing interest [9]. Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [7-10]. Recently, phenolics have been considered powerful antioxidants *in vitro* and proven to be more potent antioxidants than Vitamin C and E and carotenoids [11].

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Vitex doniana (Verbanaceae) is one of the medicinal plants used in the management of diseases associated with oxidative stress [12]. The rainforest belt of tropical West Africa and some East African countries including Uganda, Kenya and Tanzania are regions where *V. doniana* are widely distributed. In Nigeria, the plant is particularly found growing in the wide forests of Kogi and Benue States, as well as parts of the savannah regions of Kaduna, Sokoto and Kano States [13]. Some common names of the plant include: *vitex* (English), "*dinya"* (Hausa), "*dinchi"* (Gbagyi), "*ucha koro"* (Igbo), "*oriri"* (Yoruba) "*ejiji"* (Igala) and "*olih"* (Etsako) [13]. Hot aqueous leaf extracts of *V. doniana* are used for ameliorating disorders and diseases such as stomach and rheumatic pains, inflammatory response, diarrhea, dysentery and diabetes [14]. Additionally, extracts of the roots and leaves are remedies for epilepsy, nausea and colic pain [14]. Based on the enormous therapeutic usefulness of *V. doniana*, the present study evaluated the capacity of phenolic leaf extract of *V. doniana* to ameliorate hepatorenal tissue dysfunctions in alloxan-induced diabetic rats.

2 Materials and methods

2.1 Collection and preparation of plant materials

Fresh leaves of *V. doniana* were harvested from natural habitats in Mgbaleze, Onicha LGA, Ebonyi State of Nigeria (Latitude 6°15'N; Longitude 8°05'E). The plant specimens were identified and authenticated by Professor S.C. Onyekwelu of Department of Biology, Ebonyi State University, Abakaliki, Nigeria. The collected leaves were rinsed in clean water and dried at room temperature for 2 weeks. The dried leaves were pulverized using Thomas-Willey milling machine (ASTM D-3182; India), and the powdered sample was stored in a polyethylene bag at ambient laboratory temperature of 25 ± 5°C pending extraction.

2.2 Preparation of aqueous phenolic extract

About 400 g of the pulverized sample was soaked in 2 L of distilled water (1:5; *w/v*) and was allowed to stand for 24 h at 25 ± 5°C [15]. The extract was filtered and thereafter distilled and retrieved in rotary evaporator (Büch Rotavapor R-200) under reduced pressure for 12 h at 60°C. The crude extract was suspended in 50 mL of distilled water and partitioned with ethylacetate using column chromatography technique to yield phenolic extract [16]. The extract yield was 9.81% *w/w*. The phenolic extract was kept in sealed containers and stored at refrigerated temperature of 4°C until administered to the experimental rats. Finally, the phenolic extract was reconstituted in distilled water before appropriate doses were administered to the experimental rats.

2.3 Determination of total phenolic content

The total phenolic content was measured using spectrophotometric method of Nithiyanantham *et al*., [16]. In brief, 1.0 mL of sample (1.0 mg/mL) was mixed with 1.0 mL of Folin-Ciocalteu's phenol reagent and allowed to stand for 5 min. Next, 10 mL of 7% Na_2CO_3 solution was added to the mixture after which 13 mL of deionized distilled water was added and mixed thoroughly. The reaction mixture was allowed to stand in the dark for 90 min at 25°C. The absorbance of the sample was measured at maximum wavelength (λ_{max}) = 750 nm. The total phenolic content (TPC) was measured by comparing the absorbance of the sample with that of standard calibration curve of gallic acid solution. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample. The equation of the calibration curve is presented thus:

$$Y = 0.0231X + 0.0011 \quad \text{(Equation 1)}$$

Y: Absorbance of medium

X: Concentration of gallic acid (mg/mL)

$R^2 = 0.946$

Standard concentration range of gallic acid = 4.3 – 7.02 mg/mL

2.4 Experimental animal/ethics

Healthy male Wister albino rats were obtained from the animal house of Department of Veterinary Medicine, University of Nigeria, Nsukka. The rats were acclimatized at room temperature in Abia State University animal house, and were given growers mash (GM) and water *ad libitum* for two weeks.

The Ethical Committee on the use of animals for research, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria (Ethics Approval Number: FUTO/ECC/2018/56X) approved the present study. The standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978) were followed in handling of the rats as well as other experimental protocols.

2.5 Induction of diabetes mellitus

The rats were deprived of food and water for 16 h after which the initial fasting plasma glucose concentrations (FPGC) were measured using a glucometer (Roche, Mexico City) prior to induction of DM. Experimental DM was induced by single dose intra-peritoneal (*i.p.*) injection of 100 mg/kg alloxan monohydrate (Sigma, St Loius, USA.) in normal saline; pH 7.4. The rats with FPGC greater than 110 mg/dL were selected for the experiment [17].

2.6 Experimental design

A total of 36 adult male Wister rats (*Rattus norvegicus*) weighing between 180 and 230 g were divided into 6 groups of 6 rats each. The separate DM-rat groups were treated at regular time intervals of 12 h for a period of 28 days by oral administration of different doses of phenolic leaf extract of *V. doniana* and standard anti-DM drug – dimethylguanide.

The experimental rats were divided into 6 groups based on the treatment.

- Group I: DM-rats were provided GM + water *ad libitum* + phenolic extract of *V. doniana* (100 mg/kg in normal saline; *i.p.*).
- Group II: DM-rats were provided GM + water *ad libitum* + phenolic extract of *V. doniana* (200 mg/kg in normal saline; *i.p.*).
- Group III: DM-rats were provided GM + water *ad libitum* + phenolic extract of *V. doniana* (300 mg/kg in normal saline; *i.p.*).
- Group IV: DM-rats were provided GM + water *ad libitum* + metformin (500 mg/kg in normal saline; *i.p.*).
- Group V: Normal rats were provided GM + water *ad libitum*.
- Group VI: DM-rats were provided GM + water *ad libitum*.

2.7 Blood sample collection and preparation

After treatment, the rats were deprived of food and water for 24 h, sacrificed and blood samples were collected by cardiac puncture and allowed to clot in sterile vials. The clotted blood was centrifuged at 2000 *rpm* for 10 min and stored in the refrigerator at 4°C until further analysis.

2.8 Hepatorenal tissues biomarkers

The method of Reitman and Frankel, [18] according to the instructions of Randox kit manufacturers (Randox R Laboratories Ltd. Ardmore, United Kingdom), was used for measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Serum alkaline phosphatase (ALP) activity was measured using the methods of Shaheen *et al.*, [19]. Serum total and direct bilirubin concentrations were measured using the methods previously described [20]. The Biuret method was used to measure serum total protein concentrations as described by Gornall *et al.,* [21] using Randox kits (Randox R Laboratories Ltd. Ardmore, United Kingdom) and previously reported [17]. The Doumas *et al.,* [22] method was used to measure serum albumin concentration using Randox kits (Randox R Laboratories Ltd. Ardmore, United Kingdom). Serum urea and creatinine concentrations were measured using the Randox colorimetric standard methods according the manufacturer's instructions.

2.9 Statistical analyses

All data obtained were stated as mean ± standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA) and the posthoc test was done using Tukey's multiple comparison tests. The probability level was set at $p < 0.05$. The Graph Pad Prism version 7.0 software was used for all the statistical analysis.

3 Results

3.1 Total phenolic content of aqueous leaf extract of V. doniana

The total phenolic content of aqueous leaf extract of *V. doniana* was 354 ± 0.58 mg GAE/g dry samples.

3.2 Serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphate (ALP) activities

There was significant reduction ($p < 0.05$) in serum ALT, AST and ALP activities of Group IV, Group III, Group II and Group I compared with that of Group VI (Figures 1-3). Figure 1 showed that serum ALT activity of Group III was not significantly different ($p > 0.05$) from that of Group IV. Similarly, serum AST activity of Group II was not significantly different ($p > 0.05$) from that of Group IV (Figure 2). Serum ALP activities of the experimental groups was in the increasing order: Group V > Group IV > Group III > Group II > Group I.

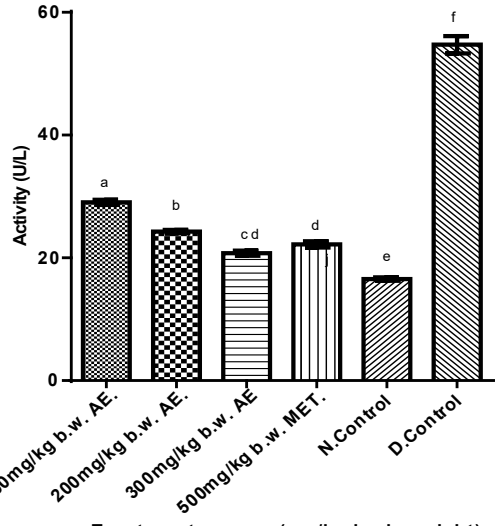

Figure 1: Serum alanine amino transferase (ALT) activity (U/L) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean ± standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic.

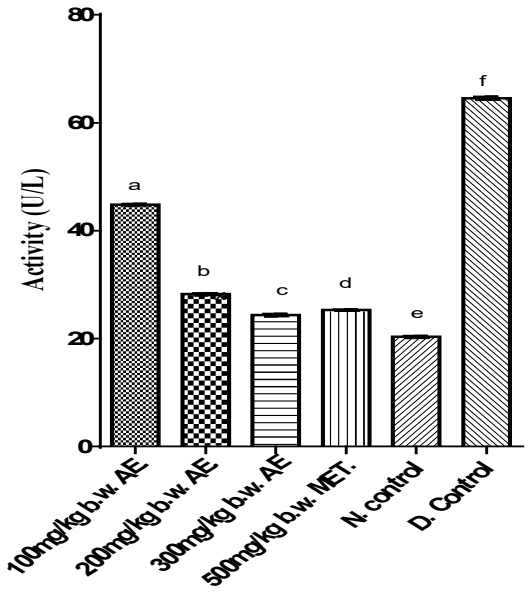

Figure 2: Serum aspartate amino transferase (AST) activity (U/L) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic.

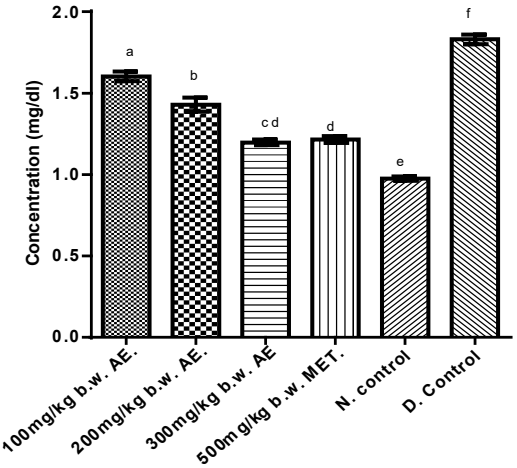

Figure 4: Serum total bilirubin (TBL) concentration (mg/dL) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic

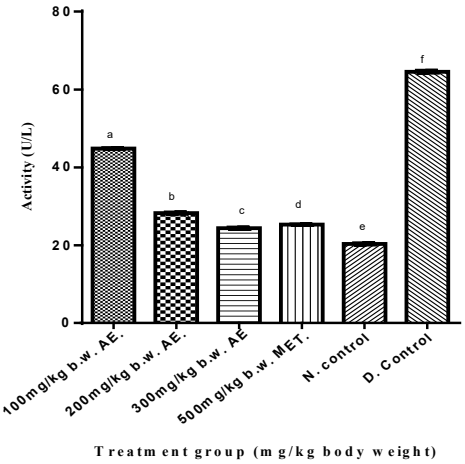

Figure 3: Serum alkaline phosphatase (ALP) activity (U/L) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic.

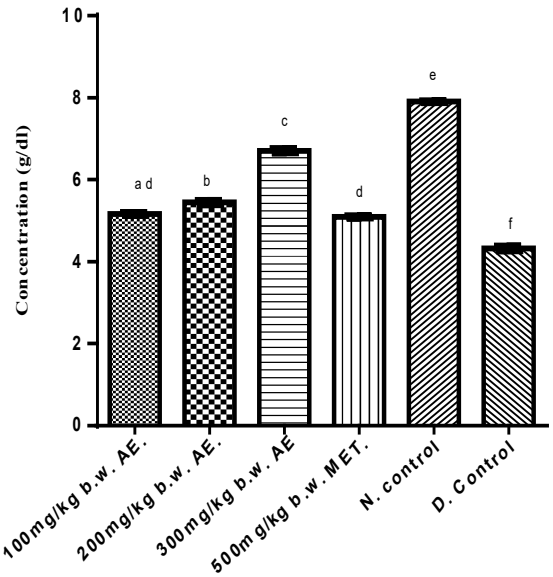

Figure 5: Serum total protein (TP) concentration (g/dL) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic

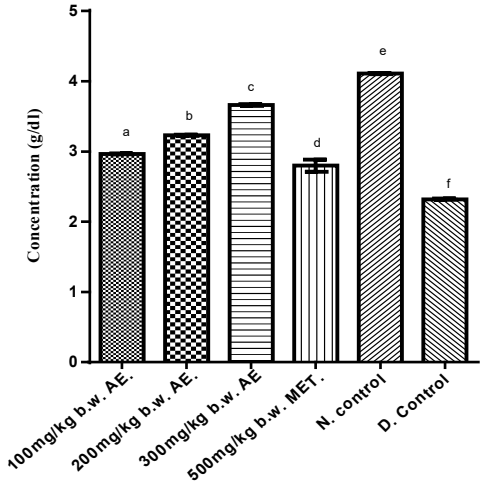

Figure 6: Serum albumin (ALB) concentration (g/dL) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend**:** b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic.

3.3 Serum total bilirubin, total protein, albumin, urea and creatinine concentrations

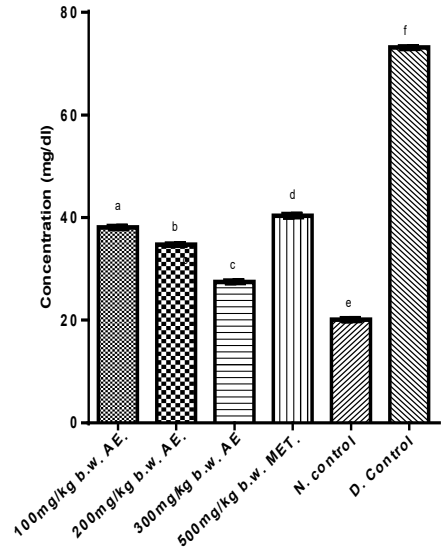

Figure 7: Serum urea (UR) concentration (mg/dL) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic.

Likewise, serum total bilirubin, total protein, albumin, urea and creatinine concentrations of Group IV, Group III,

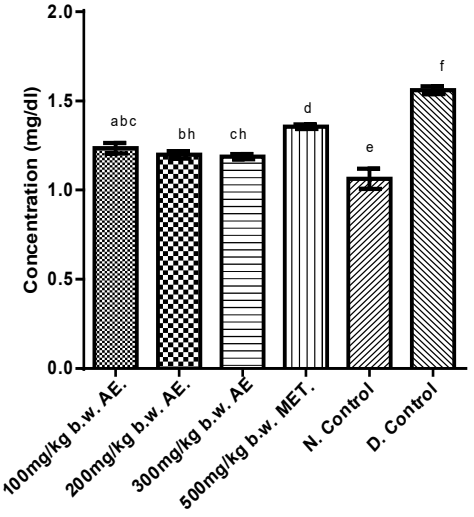

Figure 8: Serum creatinine (CREA) concentration (mg/dL) of alloxan-induced diabetic rats administered *V. doniana* aqueous phenolic leaf extracts and Metformin™.
Treatment with superscripts a, b, c, d, e showed significant difference ($p > 0.05$) compared with diabetic control rat group. Values are expressed as mean standard deviation (n =5). Values with the same superscript are not significantly different ($p < 0.05$).
Legend: b.w.: Body Weight; AE: Aqueous Extract; MET: Metformin™; N: Normal; D: Diabetic.

Group II and Group I were significantly lower ($p < 0.05$) than that of Group I (Figures 4-8). Figure 4 showed that serum total bilirubin concentration of Group III was not significantly different ($p > 0.05$) from that of Group IV. Serum total protein concentration of Group I was not significantly different ($p > 0.05$) from that of Group IV (Figure 5).

Figure 6 showed that Group VI gave the lowest serum albumin concentration, whereas that of Group V presented the highest serum albumin concentration. Serum urea concentration among the experimental rat groups was in the increasing order: Group VI > Group IV > Group I > Group II > Group III > Group V (Figure 7).

Finally, serum creatinine concentrations of Group I, Group II and Group III showed no significant difference ($p > 0.05$), whereas serum creatinine concentrations of the remaining rat groups showed significant difference in the increasing order: Group V > Group IV > Group VI (Figure 8).

4 Discussion

Phenolics are potent antioxidants *in vitro* and have been proven to be more potent antioxidants than vitamin C, vitamin E and carotenoids [11]. Previous studies have shown that evaluations of hepatic enzymes in serum of animal models are diagnostic indices for ascertaining the capacities of herbal remedies to ameliorate chemically-induced liver damage [23-24].

The significant reduction ($p < 0.05$) in serum ALT, AST and ALP activities of the treated DM-rat groups when compared with that of the untreated DM-rat group was an obvious indication of regeneration of damaged hepatocytes

as previously reported [25-26]. Accordingly, Chikezie *et al*., [7] reported that plant extracts reversed hepatic injuries by accelerating regeneration of parenchymal cells. Ojiako *et al.,* [17] reported that the capacity of plant extracts to lower serum levels of activities of hepatic enzymes in a dose-dependent manner could be attributed to bioactive principles such as phenols, flavonoids, alkaloids, saponins and tannins.

Hyperbilirubinemia occurs following massive intravascular haemolysis and haem catabolism as well as blockage of biliary tract. In addition, blockage of the biliary tract impedes conjugated bilirubin in the hepatocytes engendering hyperbilirubinemia [17]. The results of the present study showed a significant reduction ($p < 0.05$) in serum total bilirubin concentration of diabetic rats administered with various doses of phenolic leaf extract of *V. doniana* when compared with untreated diabetic rats (Group VI). This finding was in concord with previous reports [17]. The significant reduction ($p < 0.05$) in serum total bilirubin concentration of diabetic rats administered with various doses of phenolic leaf extract of *V. doniana* appeared to suggest that the extract offered protection to the hepatocytes against DM-induced necrosis. According to Ojiako *et al.,* [17] diabetic rats exhibited significantly raised ($p < 0.05$) serum urea and creatinine concentrations compared to healthy non-diabetic rats, which was diagnostic of compromised renal function.

Related previous studies had reported the capacity of diverse plant extracts to reverse hepatorenal tissue injuries associated with DM, which conformed to the outcome of the present investigations. For instance, aqueous leaf extract of *Carica papaya* [27] and flower extract *Chamomile recutita* [28] at administered dose of 500 mg/kg *bw*, within 21 days experimental time, ameliorated hyperglycemia, dyslipidemia and oxidative stress in DM rats. Additionally, Chinese and Brazilian propolis prevented hepatorenal injury by inhibiting lipid peroxidation and enhancing the activities of antioxidant enzymes in DM rats that exhibited nephropathy and liver disease [29].

5 Conclusion

The present study showed that phenolic leaf extract of *V. doniana* reduced serum urea and creatinine concentration in the DM-rat. Likewise, elevated levels of serum albumin and total protein of DM rats were significantly lowered ($p < 0.05$) following the administration of various doses of phenolic leaf extract of *V. doniana*. The finding was an obvious indication that phenolic leaf extract of *V. doniana* reversed renal tissue dysfunction.

The present study showed that phenolic leaf extract of *V. doniana* ameliorated hepatorenal tissues dysfunctions associated with alloxan-induced diabetic rats. Notwithstanding, the scope of the present investigation was, to a large extent, limited to Type-1 DM model, which may not be applicable to the Type-2 DM pathophysiology. Furthermore, the DM rats did not exhibit full therapeutic benefits following treatment of phenolic leaf extract of *V. doniana* within the 28 days experimental time.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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