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Hyptis verticillata attenuates dyslipidaemia, oxidative stress and hepato-renal damage in streptozotocin-induced diabetic rats

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ABSTRACT

Aims: Chronic hyperglycaemia in diabetes mellitus (DM) increases the production of free radicals which results in oxidative stress and related disorders such as cardiovascular diseases, compromised hepatic and renal functions. *Hyptis verticillata* reportedly demonstrated glucose lowering activity in previous studies. The present study therefore evaluated the effect of *H. verticillata* on hyperglycaemia-induced dyslipidaemia, hepatorenal distortions, oxidative stress, as well as calculated indices of cardiovascular function.

Methods: Wistar rats employed for this study consisted of normoglycaemic and diabetic rats in nine experimental groups. The normoglycaemic and diabetic rats were either treated with metformin (500 mg/kg b.w.), quercetin (10 mg/kg b.w.), or ethanol extract of *H. verticillata* leaf (250 mg/kg b.w. and 500 mg/kg b.w.) administered orally for 28 days.

Key findings: Results revealed that *H. verticillata* significantly lowered blood glucose level, attenuated dyslipidaemia, decreased atherogenic coefficient, atherogenic and coronary risk indices, and increased cardioprotective index in diabetic rats. Also, *H. verticillata* significantly decreased serum urea, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and unconjugated bilirubin levels, relative to untreated diabetic rats. Further, *H. verticillata* increased serum superoxide dismutase, catalase and glutathione peroxidase activities and glutathione level, and decreased malondialdehyde level in diabetic rats in a manner similar to metformin and quercetin. Histopathological investigation of the liver and kidney revealed restored hepatocytes and amelioration of congested interstitial blood vessel of the Bowman's space of the kidneys upon intervention with *H. verticillata*.

Significance: H. verticillata in addition to its anti-hyperglycaemic activity ameliorates oxidative stress, dyslipidaemia, atherogenicity and hepatorenal lesions in DM.

1. Introduction

Oxidative stress occurs as a result of an imbalance between the rate of free radical scavenging activity by the antioxidant system and excessive formation of free radicals. There is substantial evidence to support the relationship between hyperglycaemia-induced oxidative stress and several health complications including cardiovascular disorders [1]. Diabetes mellitus (DM) is a multifactorial disease that results from compromised insulin action or damage to the beta cells of the pancreatic islet and is associated with chronic hyperglycaemia and dyslipidaemia [2]. The chronic hyperglycaemia of DM eventually results in increased generation of free radicals such as reactive oxygen species (ROS) from autoxidation of glucose [3] and glycosylation of protein [4] which further complicates the diabetic state [5].

Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH) are known antioxidants capable of scavenging free radicals by breaking down ROS. Evidence exists that supports the link between DM and defective free radical scavenging activity [1,2]. Antioxidants have shown desirable pharmacological approach in complementing anti-hyperglycaemic agents in the management of oxidative stress and DM [6,7], hence the search for antioxidants has increased in the past decades thereby attracting interest to ethnobotanicals that have demonstrated antioxidant potentials.

Hyptis verticillata is an annual shrub belonging to the Lamiaceae,

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mint family and widely distributed in the United States from Texas to North Carolina, Florida, and Columbia as well as across the Caribbean. It is commonly called John Charles in Mexico and Costa Rica, or Wild Mint in Jamaica [8]. *H. verticillata* has been cultivated and traditionally used in the treatment of conditions that affect the respiratory system, digestive tract and gynaecological system [9]. It is commonly used as cold and colic remedy to relieve indigestion [10]. Additionally, it is used externally as topical medication for skin conditions [10]. Studies on *H. verticillata* have provided evidence of significant activity to support its use in the treatment and management of various disease conditions [10]. These studies demonstrated the presence of key phytochemical components particularly in different plant preparations of *H. verticillata* to include lignans, triterpenes, diterpenes, sesquiterpenes, monoterpenes, flavonoids, polyphenol and alkaloid [8–10]. Interestingly, the plant extract and isolated phytoconstituents in *H. verticillata* have shown varying therapeutic activities [11,12]. Reports from a preliminary study on *H. verticillata* in our laboratory indicated significant amounts of vitamins A, C and E as well as relatively high concentration of flavonoids and phenols, while saponins, alkaloids and tannins occurred in appreciable concentration in the plant extract [13,14], an observation that is consistent with an earlier report [10]. *H. verticillata* was reported to have anti-hyperglycaemic and anti-glycation activity on streptozotocin-induced diabetic rats [14]. The bioactivity of *H. verticillata* is strongly associated with the presence of it active principles including flavonoids, tannins and triterpenoids [14]. In a study by Hamada and colleagues [15], isolated compounds from *H. verticillata* inhibited proliferation of leukaemia cells and induced apoptosis. Also, *H. verticillata* has demonstrated potent inhibitory effects on inflammatory response as well as scavenging reactive oxygen species and suppressing cytochrome P450 activity [16].

Streptozotocin (STZ) induces DM by selectively inducing toxicity on the beta cells of the pancreas leading to generation of free radicals [17]. This is further compounded because the activity of antioxidant enzymes is low in the pancreatic islet cells. It is on this basis that we aimed to evaluate the role of *H. verticillata* in improving diabetes-related complications in STZ-induced diabetic rats. As the pharmacological activities of *H. verticillata* are not unrelated to its rich composition of bioactive principles, it is plausible to hypothesize that *H. verticillata* may ameliorate hyperglycaemia-induced oxidative stress, lipid complications, as well as hepato-renal disturbances consistent with diabetes. Therefore, this research work was carried out to evaluate the effect of ethanol leaf extract of *H. verticillata* on hyperglycaemia-induced oxidative stress, hyperlipidaemia, hepatic and renal function disturbances.

2. Materials and methods

2.1. Reagents and chemicals

Streptozotocin was purchased from Sigma Chemical Company, St. Louis, Missouri, USA. All other reagents and chemicals used for the present study were of analytical grade.

2.2. Plant material

H. verticillata was brought from Hungary and grown in Calabar, Cross River State, Nigeria. The leaves were harvested in May 2016, during the rainy season, from a home stead garden in Calabar and transported to Department of Botany, University of Calabar for botanical identification and verification by Mr. Frank Apojeye, a botanist. Voucher specimens were deposited in the herbarium of the same department (BOT/HV/2016/001) for future reference.

2.3. Preparation of plant extract

Fresh leaves of *H. verticillata* were rinsed thoroughly in clean tap water, before rinsing in distilled water, dried under shade for 7 days, blended into powder and stored in air-tight plastic containers. Thereafter, 1220 g of the powdered leaves was macerated in 98% ethanol at room temperature for 48 h as per our previous report [14]. The extract was double filtered, followed by rotary evaporation to yield 127.9 g of crude extract, which led to a percentage yield of 10.5%. The extract was then refrigerated at 4 °C pending usage. Phytochemical analysis of *H. verticillata* extract revealed the presence of flavonoids (19.67 ± 1.53%) and phenols (13.20 ± 0.40 mg/g dry weight), while steroids, triterpenoids, saponins, alkaloids as well as tannins were detected using GC–MS analysis [13].

2.4. Experimental animals

Seven to nine weeks old healthy male rats of the Wistar strain weighing 80–150 g were purchased from the animal house of the Department of Biochemistry, University of Calabar, Calabar and used for this study. The rats were housed in properly ventilated experimental cages. The experimental animals were maintained on tap water and standard pellets and were treated twice daily (10:00 am and 4:00 pm) for 28 days. Animal handling throughout the experimental duration was based on guidelines of the National Institute of Health publication (1985) for laboratory animal and ethical approval was also obtained from the Faculty Animal Ethics Committee, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.

2.5. Induction of type 1 diabetes mellitus in experimental Wistar rats

Streptozotocin was dissolved in 0.1 M sodium citrate buffer, pH 4.5 and administered intraperitoneally to the experimental rats at a dose of 55 mg/kg b.w. in 1 mL of buffer as reported in our earlier investigation [14]. The control group received only 1 mL of sodium citrate buffer. Prior to treatment with STZ, the rats were fasted for 16–18 h but had ad libitum access to drinking water. Blood was collected via a single puncture of tail vein for assessment of fasting blood glucose (FBG) level using a glucometer (URight TD-4279 Blood Glucose Monitoring System, Germany). Animals with FBG level higher than 200 mg/dL 72 h post-STZ injection were selected for the present study.

2.6. Experimental design

Fifty-four male Wistar rats divided into nine groups ($n = 6$) of normoglycaemic and diabetic groups were employed for this study as follows:

Group 1: Normoglycaemic control (NC) rats treated with 0.2 mL of vehicle.

Group 2: Normoglycaemic rats treated with 10 mg/kg b.w of quercetin (N + QZ).

Group 3: Normoglycaemic rats treated with 250 mg/kg b.w of *H. verticillata* extract (N + HV250).

Group 4: Normoglycaemic rats treated with 500 mg/kg b.w of *H. verticillata* extract (N + HV500).

Group 5: Diabetic control rats treated with 0.2 mL of vehicle (DC).

Group 6: Diabetic rats treated with 500 mg/kg b.w of metformin (D + Met).

Group 7: Diabetic rats treated with 10 mg/kg b.w of quercetin (D + QZ).

Group 8: Diabetic rats treated with 250 mg/kg b.w of *H. verticillata* extract (D + HV250).

Group 9: Diabetic rats treated with 500 mg/kg b.w of *H. verticillata* extract (D + HV500).

The treatments were administered per os in 0.2 mL of 2% DMSO (vehicle). The dose for *H. verticillata* extract was selected based on our previous study where the LD_{50} was found to be > 5000 mg/kg body weight [14]. At the end of 28 days of treatment, the rats were fasted overnight and sacrificed under dimethyl ether anaesthesia. Whole

blood was then collected via cardiac puncture and the serum was obtained and used for biochemical analysis. The liver and kidneys of the experimental animals were excised and weighed with a multi-functional precision weighing balance (APOLLO/GF-A, Australia) and used for histological assessment.

2.7. Body weight change, weights and relative organ weights

Body weight was monitored weekly. The body weight change was calculated using the formula: [body weight change (g) = final body weight (g) − initial body weight (g)]. The liver and kidneys of the experimental animals were excised and weighed using a multi-functional precision weighing balance (APOLLO/GF-A, Australia) to obtain their absolute weights, while their relative weights were calculated using the formula:

$$\text{Relative liver weight (\%)} = \frac{\textit{Absolute liver weight (g)}}{\textit{Final body weight (g)}} \times 100$$

$$\text{Combined relative kidney weight (\%)} = \frac{\textit{Left + right absolute kidney weights (g)}}{\textit{Final body weight (g)}} \times 100$$

2.8. Estimation of serum lipid parameters

Estimation of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) in serum were achieved using colorimetric assay kits (Sigma-Aldrich, USA) following the manufacturer's protocols [18–20]. Cholesterol (0, 20, 40, 60, 80, 100 μg/mL) standard was used to quantify TC and HDL-C, while triglyceride (0, 40, 80, 120, 160, 200 nmol/mL) standard was used to quantify TG. For each assay, the reaction mixture was incubated at 37 °C for 60 min in the dark, before reading the absorbance at 570 nm using a microplate reader (Thermo Fisher Scientific, Finland). The concentrations of serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were estimated based on calculations previous described [21]. Furthermore, assessment of cardioprotective index (CPI) was based on HDL-C/LDL-C ratio, while atherogenic coefficient (AC), atherogenic (AI) and coronary risk (CRI) indices were determined using the formula: AC = [TC − HDL-C / HDL-C], AI = Log [TG/HDL-c] and CRI = [TC/HDL-C], respectively, as described earlier [22].

2.9. Estimation of serum antioxidant activity and malondialdehyde level

The effect of crude ethanol extract of *H. verticillata* extract on antioxidant enzymes in the serum samples was evaluated. The method used in the determination of the activity of superoxide dismutase (SOD) was as previously described by Crosti et al. [23] and is based on the autoxidation of 6-hydroxydopamine monitored at 490 nm using a spectrophotometer (Genesys 10S, Thermo Electron Scientific, Germany). Catalase (CAT) activity was assessed following the method of Aebi [24] which is based on decomposition of hydrogen peroxide, and absorbance was read at 240 nm using a spectrophotometer (Genesys 10S, Thermo Electron Scientific, Germany). Also, glutathione peroxidase (GPx) activity was evaluated in accordance with the method of Ellerby and Bredesen [25] which is based on tert-butyl hydroperoxidase-dependent NADPH oxidation monitored at 340 nm using a spectrophotometer (Genesys 10S, Thermo Electron Scientific, Germany). Glutathione (GSH) level was measured spectrophotometrically as previously described [26]. This method is based on the reduction of 5-thio-2-nitrobenzoic acid formed by the reaction of 5,5-dithiobis-2-nitrobenzoic acid with the sulfhydryl group of GSH. Absorbance was read at 412 nm using a spectrophotometer (Genesys 10S, Thermo Electron Scientific, Germany), with GSH (0, 5, 10, 15, 20, 25 μg/mL) as standard. Furthermore, the extent of lipid peroxidation was estimated using a spectrophotometric assay kit (Bioxytech MDA-586™, bioWorld, USA) to measure the formation of malondialdehyde (MDA) in the serum following the manufacturer's protocol. In this assay, tetramethoxypropane (0, 0.5, 1.0, 2.0, 3.0, 4.0 μM) was used as standard and absorbance was measured at 586 nm using a spectrophotometer (Genesys 10S, Thermo Electron Scientific, Germany).

2.10. Estimation of serum markers of hepatic and renal function

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed using spectrophotometric assay kits (Sigma- Aldrich, USA) based on previous reports [27]. Total bilirubin, conjugated bilirubin and unconjugated bilirubin were estimated spectrophotometrically [28]. Serum analysis for Na^+, K^+, CL^- and HCO_3^- was performed with Agappe assay kit (Agappe diagnostics, India) using an automatic analyser ROCHE module Cobas 6000 (C-501 and C-601) (Roche diagnostics, North America). The levels of creatinine, urea, albumin, globulin, and total protein were determined in the serum using Agappe assay kits (Agappe diagnostics, India).

2.11. Histopathological assessment of liver and kidney

The histological examination of the liver and kidney of the rats were carried out using hematoxylin and eosin staining procedure. The liver and kidneys were excised and fixed in neutral formaldehyde solution for 48 h, dehydrated by passing through graded series of alcohol, and embedded in paraffin blocks. Preparation of 4 μm thick sections was achieved using a semi-automated rotary microtome (Leica Biosystems, USA).

2.12. Statistical analysis

GraphPad prism software, version 7.0 (GraphPad Software, San Diego, CA, USA) was used to analyse data for statistical significance using One-way analysis of variance. This was followed by a Tukey post hoc test and results were considered significant at $p < 0.05$. All data were expressed as mean ± standard error of mean (SEM).

3. Results

3.1. Fasting blood glucose

The outcome of glycaemic control following treatment of experimental animals is summarized in Table 1. No significant difference was observed in the initial fasting blood glucose level prior to STZ treatment across all experimental groups compared to the normal control, NC. Intraperitoneal administration of STZ resulted in nearly fourfold alteration of fasting blood glucose level 72 h post-STZ treatment in the DC, D + Met, D + QZ, D + HV250 and D + HV500 groups to confirm induction of DM. Upon intervention with metformin, quercetin and plant extract, FBG declined significantly ($p < 0.05$) in all treated diabetic groups relative to DC with the 250 mg/kg b.w dose of *H. verticillata* extract showing the greatest impact by significantly ($p < 0.05$) causing a 4.74 and 1.16-fold decrease in FBG relative to DC and NC, respectively (Table 1).

3.2. Body weight change, absolute and relative weight of liver and kidney

Body weight variations in experimental animals following treatment is shown in Table 2. From the result, the weight variation in the normal rats of both control and treated groups indicated a similar trend in weight appreciation over time. Conversely, in the STZ-induced diabetic animals, the untreated diabetic rats in the DC group visibly presented significant decline ($p < 0.05$) in body weight compared to NC group.

Table 1
Fasting blood glucose in all the experimental groups.

Groups	Fasting blood glucose (mg/dL)		
	Initial	72 h post-STZ	Final
Normoglycaemic groups			
NC	81.33 ± 3.25	79.00 ± 2.50	89.33 ± 3.19
N + QZ	84.00 ± 2.13	79.17 ± 1.82	79.67 ± 2.43
N + HV250	74.00 ± 4.26	79.50 ± 2.25	79.33 ± 1.17
N + HV500	81.50 ± 4.36	76.33 ± 2.91	80.70 ± 7.94
Diabetic groups			
DC	82.00 ± 2.18	422.80 ± 28.64[a]	490.00 ± 8.59[a] (5.49)
D + Met	84.17 ± 2.57	430.30 ± 27.39[a]	143.00 ± 8.85[a,d] (1.60) [3.43]
D + QZ	86.00 ± 5.29	388.00 ± 26.26[a]	117.00 ± 11.74[d] (1.31) [4.19]
D + HV250	82.17 ± 4.92	419.20 ± 27.12[a]	103.30 ± 2.70[b,d,x] (1.16) [4.74]
D + HV500	83.50 ± 3.94	422.50 ± 26.22[a]	236.50 ± 14.17[a,d,x,y,z] (2.65) [2.07]

NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, $n = 6$. [a]$p < 0.05$ vs NC, [d]$p < 0.05$ vs DC, [x]$p < 0.05$ vs D + Met, [y]$p < 0.05$ vs D + QZ, [z]$p < 0.05$ vs D + HV250 (one-way ANOVA followed by Tukey post-hoc test).

At the end of the 28-day experimental period, all diabetic treated groups presented increase in body weight which was significant ($p < 0.05$) compared to the DC group. The absolute liver weight increased significantly ($p < 0.05$) in the untreated diabetic group compared to NC but decreased significantly ($p < 0.05$) on treatment with metformin, quercetin and *H. verticillata* extract relative to DC as presented in Table 2. Also, the relative weight of liver and kidney did not differ significantly ($p > 0.05$) in the diabetic and normoglycaemic animals compared to NC. However, the diabetic treated groups showed significant ($p < 0.05$) increase in relative liver and kidney weight compared to DC group (Table 2).

3.3. Serum lipid profile

The effect of *H. verticillata* leaf extract on lipid parameters in normoglycaemic and diabetic rats is presented in Figs. 1–3. The normoglycaemic rats showed no significant change ($p > 0.05$) in the levels of TC, TAG and HDL-C following treatment with quercetin and *H. verticillata* extract compared to NC (Fig. 1 A1, B1 and C1), while LDL-C and VLDL-C were observed to decrease significantly ($p < 0.05$) in the extract treated groups compared to NC and N + QZ (Fig. 2 A1 and B1). Furthermore, for the diabetic rats, significant elevation ($p < 0.05$) in the levels of TC, TAG, LDL and VLDL ensued in the untreated diabetic group, DC with a concomitant decline in HDL which was significant ($p < 0.05$) compared to NC as presented in Figs. 1 A2, B2 and C2 and 2 A2 and B2. Interestingly, treatment with *H. verticillata* significantly declined ($p < 0.05$) TC and TAG while significantly increasing HDL-C in a manner consistent with metformin and quercetin. This observation was comparable to NC. Also, LDL-C and VLDL-C was significantly decreased ($p < 0.05$) to almost normal in all treated diabetic groups relative to DC (Fig. 2 A2 and B2).

The normoglycaemic rats showed no significant change ($p > 0.05$) in AC, CRI index and CPI compared to NC (Fig. 3 A1, B1 and C1), except for atherogenic index which declined following treatment and this decline was significant for the N + HV500 group (Fig. 2 C1). For the diabetic rats, it was observed that relative to DC, treatment with *H. verticillata* attenuated elevated levels of AI (Fig. 2 C2), AC and CRI in a manner that compared well with NC, metformin and quercetin, while CPI was significantly raised ($p < 0.05$) in all treated groups compared to DC (Fig. 3 A2, B2 and C2).

3.4. Serum antioxidant activity and malondialdehyde level

From the result in Table 3, considerable ($p < 0.05$) elevated activities of SOD, CAT and GPx, and GSH level occurred in all normoglycaemic treated groups compared to NC with quercetin indicating a remarkably higher activity compared to the N + HV250 and N + HV500 groups. For the diabetic groups, a similar trend with regards to variations in the activities of SOD, CAT, GPx and GSH was observed across the experimental groups. Results show a considerable ($p < 0.05$) decrease in the activities of SOD, CAT and GPx, and GSH level in all treated groups as well as DC except for D + QZ which indicated no significant ($p > 0.05$) change compared to NC. Again, when compared to DC, the activities of these antioxidants increased significantly ($p < 0.05$) following treatment. It is worthy to note that although both doses of *H. verticillta* increased the activities of these enzymes, the D + HV500 group presented greater impact which compares well with the standard antioxidant drug, quercetin. Furthermore, administration of quercetin and the two doses of extract significantly ($p < 0.05$) decreased the level of MDA in normoglycaemic rats. However, for the diabetic rats, a considerable elevation in MDA level in

Table 2
Body weight, weight and relative liver and kidney weights in all the experimental groups.

Groups	Body weight (g)		Body weight change (g)	Liver weight		Combined kidney weight	
	Initial	Final		AW (g)	RW (%)	AW (g)	RW (%)
Normoglycaemic groups							
NC	184.2 ± 1.8	230.3 ± 2.1	46.2 ± 1.0	4.90 ± 0.14	3.00 ± 0.06	0.57 ± 0.04	0.33 ± 0.01
N + QZ	185.0 ± 2.1	216.0 ± 2.1[a]	31.0 ± 3.4[a]	4.52 ± 0.27	3.02 ± 0.13	0.52 ± 0.06	0.34 ± 0.03
N + HV250	188.3 ± 1.7	200.5 ± 2.5[a,b]	12.2 ± 1.6[a,b]	4.07 ± 0.23[a]	3.03 ± 0.14	0.46 ± 0.04	0.33 ± 0.02
N + HV500	183.7 ± 1.4	208.7 ± 2.9[a]	25.0 ± 2.6[a,c]	4.35 ± 0.09	3.04 ± 0.04	0.52 ± 0.02	0.35 ± 0.01
Diabetic groups							
DC	184.2 ± 2.2	124.2 ± 1.5[a]	−60.0 ± 2.2[a]	6.09 ± 0.18[a]	4.23 ± 0.13[a]	0.77 ± 0.02[a]	0.42 ± 0.02[a]
D + Met	183.3 ± 2.5	179.2 ± 2.0[a,d]	−4.2 ± 3.5[a,d]	3.77 ± 0.31[a,d]	2.81 ± 0.16[d]	0.53 ± 0.03[a,d]	0.39 ± 0.01[d]
D + QZ	185.0 ± 2.8	180.8 ± 1.5[a,d]	−4.2 ± 3.3[a,d]	5.56 ± 0.30[a,d]	3.08 ± 0.17[d]	0.60 ± 0.01[a,d]	0.33 ± 0.01[d]
D + HV250	187.5 ± 3.9	186.0 ± 2.3[a,d]	−1.5 ± 4.6[a,d]	4.85 ± 0.22[a,d]	2.61 ± 0.12	0.55 ± 0.04[a,d]	0.37 ± 0.02[d]
D + HV500	185.3 ± 2.6	186.3 ± 2.2[a,d]	1.0 ± 4.3[a,d]	4.46 ± 0.11[a,d]	2.93 ± 0.04[d]	0.57 ± 0.03[a,d]	0.36 ± 0.01[d]

AW: absolute weight, RW: relative weight, NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, $n = 6$. [a]$p < 0.05$ vs NC, [b]$p < 0.05$ vs N + QZ, [c]$p < 0.05$ vs N + HV250, [d]$p < 0.05$ vs DC (one-way ANOVA followed by Tukey post-hoc test).

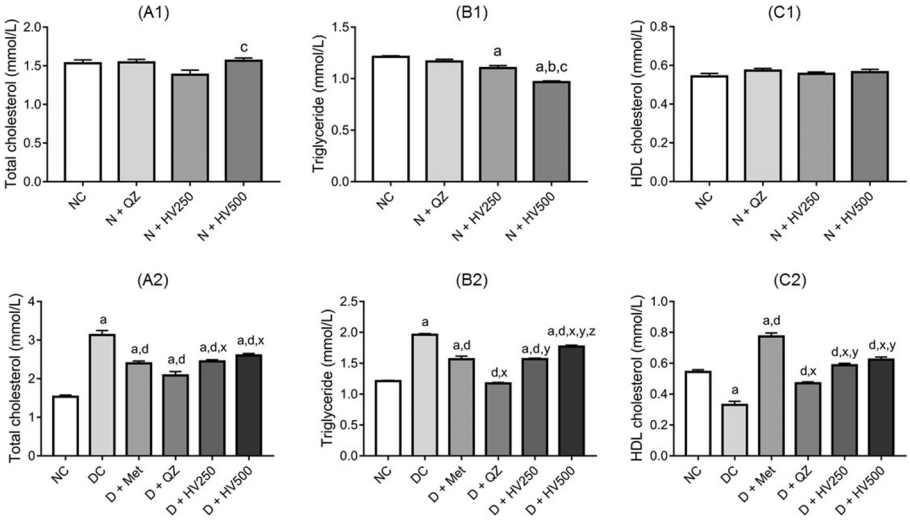

Fig. 1. Effect of *H. verticillata* on serum total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C) levels in normoglycaemic (A1, B1, C1) and diabetic (A2, B2, C2) rats. NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, n = 6. $^{a}p < 0.05$ vs NC, $^{b}p < 0.05$ vs N + QZ, $^{c}p < 0.05$ vs N + HV250, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met, $^{y}p < 0.05$ vs D + QZ, $^{z}p < 0.05$ vs D + HV250 (One-way ANOVA followed by Tukey post-hoc test).

DC as well as the D + Met and D + HV250 groups compared to NC. When compared to DC, all treated groups showed significant decrease in MDA level and again, quercetin showed a greater impact (Table 3).

3.5. Serum markers of hepatic function

The results of the effect of 28-day administration of ethanol leaf extract of *H. verticillata* on the serum activity of AST, ALT and ALP are presented in Table 4. From the result, no significant ($p > 0.05$) change was observed for AST, ALT and ALP in N + QZ, N + HV250 and N + HV500 relative to NC. The untreated diabetic group (DC) showed a significantly ($p < 0.05$) raised level of AST, ALT and ALP compared to NC which upon treatment was significantly ($p < 0.05$) reduced as observed in the D + Met, D + QZ, D + HV250 as well as D + HV500 experimental groups (Table 4).

There was no significant ($p > 0.05$) difference in the levels of total bilirubin, conjugated and unconjugated bilirubin in the normoglycaemic treated groups relative to NC and although the percentage conjugation of bilirubin increased in the treated normoglycaemic groups compared to NC, this increase was insignificant ($p > 0.05$). Conversely, the levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin were significantly increased ($p < 0.05$) in DC group compared to NC group and decreased significantly ($p < 0.05$) in the treated diabetic groups relative to DC group except for the level of conjugated bilirubin which was significantly elevated after 28-day treatment with metformin, quercetin and *H. verticillata* extract. Also, the percentage conjugation of bilirubin decreased significantly ($p < 0.05$) in the DC group compared to NC, whereas, it significantly ($p < 0.05$) increased in the treated diabetic groups compared to DC group (Table 4).

3.6. Serum markers of renal function

Compared to NC, the levels of urea and creatinine were observed to decrease significantly ($p < 0.05$) in N + QZ, N + HV250 and N + HV500 groups with a corresponding significant decrease ($p < 0.05$) in urea/creatinine ratio (Table 5). We observed a significant increase ($p < 0.05$) in urea and creatinine in the DC group relative to NC and although treatment with metformin, quercetin and *H. verticillata* extract significantly decreased ($p < 0.05$) serum levels of urea and creatinine in the treated diabetic groups, the negative effect of diabetes on the level of urea and creatinine was best improved by treatment with 250 mg/kg b.w of *H. verticallata* extract (Table 5). Also, urea/creatinine ratio significantly increased ($p < 0.05$) in DC group compared to NC group and decreased significantly ($p < 0.05$) following treatment with metformin, quercetin and *H. verticillata* extract (Table 5).

No significant change was observed for serum levels of total protein, albumin and globulin in the treated normoglycaemic rats compared to NC. For the diabetic groups, serum total protein, albumin and globulin levels decreased significantly ($p < 0.05$) in DC, D + Met, D + QZ,

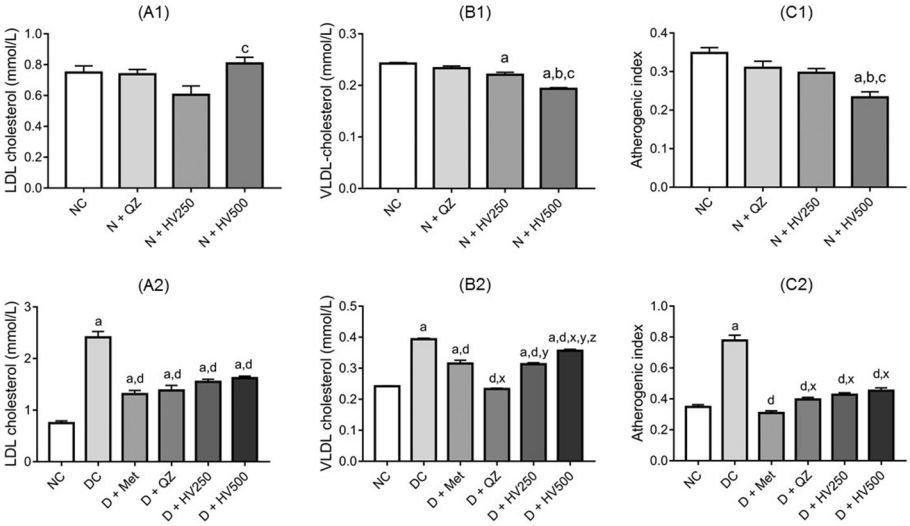

Fig. 2. Effect of *H. verticillata* on serum low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and atherogenic index (AI) in normoglycaemic (A1, B1, C1) and diabetic (A2, B2, C2) rats. NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, n = 6. $^{a}p < 0.05$ vs NC, $^{b}p < 0.05$ vs N + QZ, $^{c}p < 0.05$ vs N + HV250, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met, $^{y}p < 0.05$ vs D + QZ, $^{z}p < 0.05$ vs D + HV250 (One-way ANOVA followed by Tukey post-hoc test).

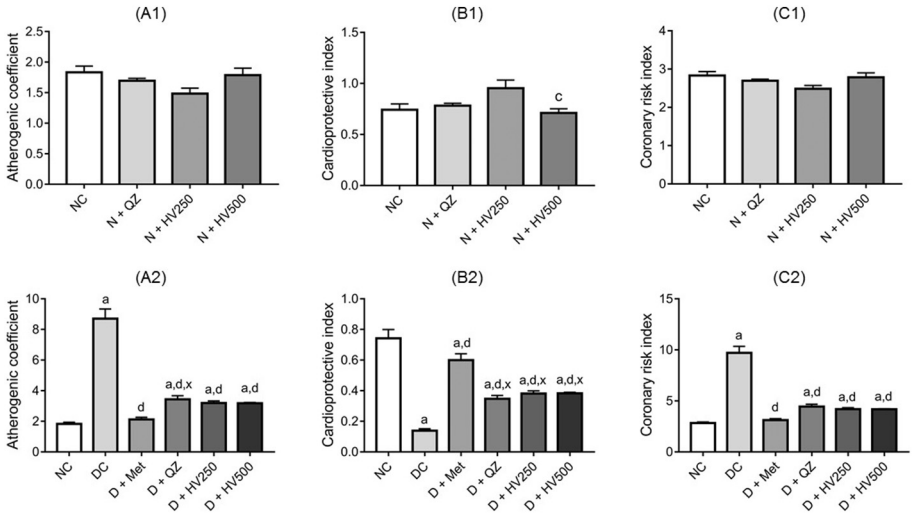

Fig. 3. Effect of *H. verticillata* on atherogenic coefficient (AC), cardioprotective index (CPI) and coronary risk index (CRI) in normoglycaemic (A1, B1, C1) and diabetic (A2, B2, C2) rats. NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, n = 6. $^{a}p < 0.05$ vs NC, $^{c}p < 0.05$ vs N + HV250, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met (One-way ANOVA followed by Tukey post-hoc test).

D + HV250 and D + HV500 compared to NC. However, relative to DC group, total protein increased in D + Met and D + QZ and this increase was significant ($p < 0.05$) for D + Met and insignificant ($p > 0.05$) for D + QZ whereas, the extract treated groups presented significantly lower ($p < 0.05$) level of total protein compared to the DC. Additionally, albumin and globulin levels significantly increased in all treated diabetic groups relative to DC group (Table 5).

Although treatment of normoglycaemic rats with quercetin and *H. verticillata* extract caused insignificant increase ($p > 0.05$) in serum levels of potassium and bicarbonate as presented in Table 5, sodium and chloride levels were significantly elevated ($p < 0.05$) in all normoglycaemic treated groups except for N + QZ that showed a significant decrease ($p < 0.05$) in sodium level compared to NC. Serum sodium, potassium and chloride levels decreased significantly ($p < 0.05$) in DC compared to NC group and was insignificantly raised in the diabetic treated groups relative to DC group, except for D + Met and D + QZ which presented a significant increase ($p < 0.05$) relative to NC and DC. Furthermore, serum bicarbonate level significantly increased in DC, D + Met, D + QZ and D + HV250 groups relative to NC and decreased significantly ($p < 0.05$) in all treated diabetic groups compared to DC group (Table 6).

3.7. Histopathology

The photomicrograph as presented in Fig. 4a–f shows the effect of the 28-day treatment on the cellular architecture and integrity of the hepatocytes. From the photomicrograph, the untreated diabetic rats presented with enlarged and congested central veins and numerous degenerated hepatocytes with disintegrated nuclei. However, upon treatment with both doses of *H. verticillata* extract (D + HV250 and D + HV500), numerous normal hepatocytes with intact nuclei were observed. This observation compared well with the groups treated with metformin and quercetin.

The photomicrograph of the kidney of the untreated diabetic group (DC) revealed the presence of shrunken glomeruli with sparsely populated mesangial cells as well as pyknotic nuclei. The Bowman's space was enlarged, and interstitial blood vessels were congested compared to the NC, while the treated diabetic groups which were observed to have largely normal glomeruli (Fig. 5a–f).

4. Discussion

Medicinal plants serve as important resources across many countries to treat and manage DM-associated complications. Their use results from the rich composition of bioactive components which account for their safe and broad effectiveness . *H. verticillata* contains significant amount of bioactive components with potent therapeutic properties [10]. In this work, the study plant produced a remarkable decline in FBG comparable to metformin and quercetin. This observation could have possibly resulted from a pancreatic and extra-pancreatic

Table 3
Serum antioxidant enzymes activity and malondialdehyde level in all the experimental groups.

Groups	SOD activity (units/g protein)	CAT activity (units/g protein)	GPx activity (units/g protein)	GSH level (nmol/g protein)	MDA level (nmol/g protein)
Normoglycaemic groups					
NC	33.00 ± 0.89	4.58 ± 0.11	154.20 ± 1.45	26.17 ± 1.45	35.23 ± 1.59
N + QZ	75.17 ± 0.91^{a}	9.65 ± 0.13^{a}	383.30 ± 2.81^{a}	72.50 ± 1.52^{a}	9.23 ± 0.29^{a}
N + HV250	38.83 ± 1.14^{a,b}	5.48 ± 0.11^{a,b}	216.30 ± 3.65^{a,b}	43.67 ± 1.61^{a,b}	26.13 ± 0.87^{a,b}
N + HV500	45.67 ± 1.20^{a,b,c}	7.33 ± 0.14^{a,b,c}	285.30 ± 2.01^{a,b,c}	53.33 ± 2.29^{a,b,c}	18.63 ± 0.54^{a,b,c}
Diabetic groups					
DC	10.17 ± 0.60^{a}	1.20 ± 0.11^{a}	35.00 ± 1.75^{a}	8.33 ± 0.88^{a}	89.93 ± 2.83^{a}
D + Met	19.67 ± 1.36^{a,d}	2.40 ± 0.16^{a,d}	114.30 ± 2.91^{a,d}	16.50 ± 0.76^{a,d}	64.50 ± 2.25^{a,d}
D + QZ	31.67 ± 1.33^{d,x}	6.88 ± 0.26^{a,d,x}	202.80 ± 6.26^{a,d,x}	32.17 ± 1.11^{a,d,x}	22.89 ± 1.11^{a,d,x}
D + HV250	21.50 ± 0.99^{a,d,y}	3.23 ± 0.14^{a,d,x,y}	115.00 ± 3.64^{a,d,y}	19.00 ± 1.16^{a,d,y}	54.92 ± 1.89^{a,d,x,y}
D + HV500	25.83 ± 1.96^{a,d,x,y}	5.05 ± 0.23^{d,x,y,z}	145.00 ± 2.53^{d,x,y,z}	26.50 ± 1.31^{d,x,y,z}	42.15 ± 0.67^{d,x,y,z}

SOD: superoxide dismutase, CAT: catalase, GPx: glutathione peroxidase, GSH: total glutathione, MDA: malondialdehyde, NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, $n = 6$. $^{a}p < 0.05$ vs NC, $^{b}p < 0.05$ vs N + QZ, $^{c}p < 0.05$ vs N + HV250, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met, $^{y}p < 0.05$ vs D + QZ, $^{z}p < 0.05$ vs D + HV250 (one-way ANOVA followed by Tukey post-hoc test).

Table 4
Serum markers of hepatic function in all the experimental groups.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total bilirubin (μmol/L)	Conjugated bilirubin (μmol/L)	Unconjugated bilirubin (μmol/L)	Percentage of conjugated bilirubin (%)
Normoglycaemic groups							
NC	38.83 ± 2.37	27.00 ± 1.16	174.00 ± 2.19	9.80 ± 0.19	5.47 ± 0.06	4.33 ± 0.25	55.94 ± 1.63
N + QZ	39.83 ± 3.05	28.33 ± 0.92	169.00 ± 6.06	10.40 ± 0.34	6.30 ± 0.13^{a}	4.10 ± 0.30	60.82 ± 1.87
N + HV250	40.17 ± 1.38	28.83 ± 1.08	164.00 ± 3.36	10.77 ± 0.38	6.07 ± 0.20	4.70 ± 0.50	56.85 ± 3.24
N + HV500	39.67 ± 2.16	26.83 ± 1.49	165.30 ± 4.25	10.15 ± 0.31	6.03 ± 0.18	4.12 ± 0.40	59.82 ± 3.04
Diabetic groups							
DC	159.30 ± 1.12^{a}	151.70 ± 1.52^{a}	436.70 ± 1.12^{a}	18.07 ± 0.21^{a}	8.77 ± 0.08^{a}	9.30 ± 0.22^{a}	48.56 ± 0.67
D + Met	88.67 ± 0.56^{a,d}	49.67 ± 0.92^{a,d}	257.30 ± 1.12^{a,d}	15.33 ± 0.29^{a,d}	10.92 ± 0.38^{a,d}	4.42 ± 0.55^{d}	71.39 ± 3.01^{a,d}
D + QZ	88.33 ± 0.42^{a,d}	62.33 ± 1.28^{a,d,x}	189.30 ± 1.48^{a,d,x}	14.38 ± 0.51^{a,d,x}	10.78 ± 0.17^{a,d}	3.60 ± 0.53^{d}	75.42 ± 2.85^{a,d}
D + HV250	52.00 ± 0.73^{a,d,x,y}	35.00 ± 0.37^{a,d,x,y}	219.30 ± 1.12^{a,d,x,y}	15.50 ± 0.035^{a,d,y}	10.73 ± 0.40^{a,d}	4.77 ± 0.51^{d}	69.40 ± 2.83^{a,d}
D + HV500	84.33 ± 0.92^{a,d,x,y,z}	46.67 ± 1.12^{a,d,y,z}	289.00 ± 1.67^{a,d,x,y,z}	13.40 ± 0.46^{a,d,x,z}	10.62 ± 0.33^{a,d}	2.78 ± 0.6.4^{d}	79.78 ± 3.90^{a,d}

AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase. NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, $n = 6$. $^{a}p < 0.05$ vs NC, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met, $^{y}p < 0.05$ vs D + QZ, $^{z}p < 0.05$ vs D + HV250 (One-way ANOVA followed by Tukey post-hoc test).

mechanism. Furthermore, steroids, triterpenoids, flavonoids, alkaloids, tannins and polyphenols were identified as the major constituents of the leaf extract [13,14]. These phytochemicals in plants play important roles in the mechanism of action of ethnobotanicals. Particularly, plants containing flavonoids, tannins and triterpenoids have demonstrated blood glucose lowering ability in diabetic state by stimulating pancreatic β-cell regeneration, insulin secretion and lowering gluconeogenesis [6,7,14]. Hence, it is plausible to suggest that the observed anti-hyperglycaemic effect of *H. verticillata* is partly as a result of the presence of these phytochemicals.

Weight loss is consistent with poorly managed DM basically as a result of sustained hyperglycaemia, hyperinsulinaemia, increased mobilization of proteins and fatty acids that eventually cause tissue wasting [28]. From this work, body weight of the untreated diabetic animals decreased significantly, but upon intervention with *H. verticillata* extract, there was a significant improvement in body weight comparable to the standard anti-diabetic drug, metformin, as well as quercetin, suggesting the ability of the study plant to protect against weight loss via improving glucose and energy utilization by tissues for protein synthesis and to build growth materials for tissue.

Significant increase in liver and kidney weights (hypertrophy) were observed in the diabetic rats relative to NC. Increased liver weight, fatty liver in DM is attributed to hypoinsulinaemia-induced accumulation of triacylglycerol and increased influx of fatty acids to the liver [29], resulting in enlarged liver. Also, renal hypertrophy in DM results possibly from over expression of transforming growth factor-beta 1 in the cells of the proximal convoluted tubule [30]. Interestingly, treatment with *H. verticillata* elicited a decrease in the weight of liver and kidney, suggestive of a possible amelioration of diabetes-induced fatty liver and renal hypertrophy. In addition to fatty liver and renal hypertrophy, poorly managed DM causes dyslipidaemia which eventually results in diabetic nephropathy. The elevated lipid levels were attenuated upon treatment with *H. verticillata*, in a manner consistent with metformin and quercetin, thus demonstrating the ability of the study plant to reverse dyslipidaemia and improve lipid metabolism. The hyperlipidaemia observed in the untreated diabetic rats in the present study is indicative of an increase in the mobilization of free fatty acids from the peripheral fat depots. This may probably have resulted from the uninhibited actions of the lipolytic enzyme, lipase caused by insulin deficiency characteristic of the diabetic state [31]. This observation was consistent with calculated indices viz., AI, AC, CPI and CRI, which were all attenuated following intervention with the study plant. These indices are valuable in assessing the risk of developing cardiovascular related conditions secondary to diabetes mellitus. Particularly, patients with elevated AC, AI and CRI are more predisposed to cardiovascular diseases [22]. The ability of *H. verticillata* to significantly decrease AI, AC and CRI, while improving CPI provides further proof regarding the potency of *H. verticillata* against atherogenicity in diabetic state. Also,

Table 5
Serum markers of renal function in all the experimental groups.

Groups	Urea (mmol/L)	Creatinine (μmol/L)	Urea/Creatinine (fold change)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)
Normoglycaemic groups						
NC	6.63 ± 0.15	68.03 ± 0.30	1.00 ± 0.02	64.67 ± 0.42	37.00 ± 0.67	27.67 ± 0.21
N + QZ	4.37 ± 0.06^{a}	76.43 ± 0.43^{a}	0.59 ± 0.01^{a}	63.67 ± 1.38	38.83 ± 0.70	32.67 ± 0.42
N + HV250	3.53 ± 0.04^{a,b}	57.10 ± 0.44^{a,b}	0.64 ± 0.01^{a}	60.00 ± 0.93	38.83 ± 0.31	30.67 ± 0.56
N + HV500	3.42 ± 0.11^{a,b}	67.20 ± 0.40^{b,c}	0.52 ± 0.02^{a,b,c}	61.83 ± 0.70	37.33 ± 1.05	29.00 ± 0.86
Diabetic groups						
DC	9.57 ± 0.06^{a}	108.30 ± 0.85^{a}	1.18 ± 0.01^{a}	44.67 ± 0.42^{a}	26.33 ± 2.43^{a}	15.33 ± 0.84^{a}
D + Met	8.67 ± 0.13^{a,d}	96.57 ± 0.74^{a,d}	0.92 ± 0.02^{a,d}	50.33 ± 0.56^{a,d}	35.00 ± 0.37^{d}	22.67 ± 0.21^{a,d}
D + QZ	8.62 ± 0.08^{a,d,x}	83.07 ± 0.77^{a,d,x}	0.52 ± 0.01^{a,d,x}	44.00 ± 0.37^{a,x}	35.50 ± 0.37^{d}	23.83 ± 0.79^{a,d}
D + HV250	5.53 ± 0.26^{a,d,y}	76.30 ± 1.12^{a,d,x,y}	1.14 ± 0.03^{a,x,y}	35.00 ± 0.37^{a,d,x}	31.83 ± 2.15	20.83 ± 0.91^{a,d,y}
D + HV500	8.07 ± 0.11^{a,d,y}	81.67 ± 0.43^{a,x,y,z}	1.01 ± 0.01^{d,x,y,z}	37.33 ± 0.42^{a,d,x,y,z}	25.00 ± 0.37^{a,x,y,z}	22.33 ± 0.76^{a,d}

NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Urea/creatinine ratio is expressed as fold change relative to NC group. Values are mean ± SEM, $n = 6$. $^{a}p < 0.05$ vs NC, $^{b}p < 0.05$ vs N + QZ, $^{c}p < 0.05$ vs N + HV250, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met, $^{y}p < 0.05$ vs D + QZ, $^{z}p < 0.05$ vs D + HV250 (One-way ANOVA followed by Tukey post-hoc test).

Table 6
Serum electrolyte levels in all the experimental groups.

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Normoglycaemic groups				
NC	135.30 ± 0.42	4.40 ± 0.04	108.70 ± 0.42	15.00 ± 0.37
N + QZ	134.70 ± 1.20	4.43 ± 0.18	105.20 ± 1.56	15.83 ± 0.48
N + HV250	136.00 ± 0.37	4.47 ± 0.04	106.20 ± 1.17	16.83 ± 0.65
N + HV500	134.70 ± 0.42	4.90 ± 0.04	105.30 ± 1.33	16.17 ± 0.48
Diabetic groups				
DC	129.00 ± 0.37^{a}	3.47 ± 0.06^{a}	104.70 ± 0.42^{a}	23.33 ± 0.21^{a}
D + Met	144.70 ± 0.42^{d}	4.00 ± 0.07^{a,d}	114.00 ± 0.73^{a,d}	15.33 ± 0.21^{d}
D + QZ	127.00 ± 0.37^{a,d,x}	4.54 ± 0.07^{d,x}	118.00 ± 0.73^{d}	18.00 ± 0.37^{a,d,x}
D + HV250	139.00 ± 0.37$^{\times,y}$	4.38 ± 0.04^{d,x}	105.00 ± 0.37	17.67 ± 0.62^{a,d,x}
D + HV500	137.30 ± 0.42^{a,x,y}	5.43 ± 0.02^{a,x,y,z}	109.33 ± 0.42^{d}	14.50 ± 0.43^{d,y,z}

NC: normoglycaemic control, N + QZ: normoglycaemic +10 mg/kg b.w quercetin, N + HV250: normoglycaemic +250 mg/kg b.w. *H. verticillata*, N + HV500: normoglycaemic +500 mg/kg b.w. *H. verticillata*, DC: diabetic control, D + Met: diabetic +500 mg/kg b.w. metformin, D + QZ: diabetic +10 mg/kg b.w quercetin, D + HV250: diabetic +250 mg/kg b.w. *H. verticillata*, D + HV500: diabetic +500 mg/kg b.w. *H. verticillata*. Values are mean ± SEM, $n = 6$. $^{a}p < 0.05$ vs NC, $^{d}p < 0.05$ vs DC, $^{x}p < 0.05$ vs D + Met, $^{y}p < 0.05$ vs D + QZ, $^{z}p < 0.05$ vs D + HV250 (one-way ANOVA followed by Tukey post-hoc test).

considering the duration of the present study, the observed decrease in the level of HDL-C in the untreated diabetic animals may have resulted from accelerated formation of advanced glycosylated end-products (AGEs), an occurrence that is consistent with uncontrolled hyperglycaemia. The anti-glycation potential of *H. verticillata* was demonstrated in our previous investigation and we observed that animals treated with the plant extract showed significant reduction in glycated haemoglobin relative to the untreated diabetic animals [14]. The study plant has also been reported to possess a rich profile of bioactive compounds including flavonoids, polyphenols, squalene, 9,12,15-octadecatrien-1-ol, 1-octadecyne as well as eicosane in considerable amounts [10,13,14]. These compounds have demonstrated hypocholesterolemic effects [32]. Consumption of squalene in diet was linked with hypolipidaemic effects specifically associated with decreased levels of LDL-C, TG and increased levels of HDL-C [33]. These bioactive volatile components, together with phytochemicals including tannins and alkaloids present in *H. verticillata* may have contributed to alleviating the dyslipidaemia and associated disturbances as observed in this study.

Oxidative stress occurs as a result of an imbalance between oxidants and antioxidants such that the concentration of oxidants far exceeds antioxidants. It is measured by reduction in antioxidant defence enzymes, increases in lipid peroxidation as well as damage to proteins and DNA. Oxidative stress has been singled out as a major cause of diabetic complication in humans and experimental animals [34]. Considering the deleterious effect of oxidative stress, it is not surprising that cells maintain a variety of defences in response to this condition, this they achieve via the activities of enzymatic (SOD, CAT, GPx, GST and GR) and non-enzymatic (GSH, vitamins C and E) antioxidants. Hence, it has been proposed that antioxidants which are potential scavengers of oxidants may play a positive role in alleviating diabetes as well as reducing secondary complications associated with this disorder. In the present study, the activities of GPx, CAT and SOD, and GSH level were significantly decreased in the untreated diabetic animals but on treatment with *H. verticillata* extract, metformin or quercetin, the activities of these enzymes were significantly raised and was almost normalized in the quercetin treated diabetic group, an observation that was not surprising for quercetin which is an already established antioxidant. A significant decline in GPx and GSH in the untreated diabetic rats is reflective of an impaired antioxidant defence and a concomitant increased susceptibility to oxidative stress, while the increased levels of these antioxidant enzymes observed in the treated groups suggests protective effect of *H. verticillata* against over production of ROS. Also, the marked decline in CAT level in the untreated diabetic animals reflects the inability of these animals to effectively handle and eliminate hydrogen peroxide. *H. verticillata* significantly increased the activities of CAT and SOD both in the diabetic and normal animals, further demonstrating the anti-oxidative potential of this plant. This observation is in line with earlier reports on the antioxidant potential of *H.*

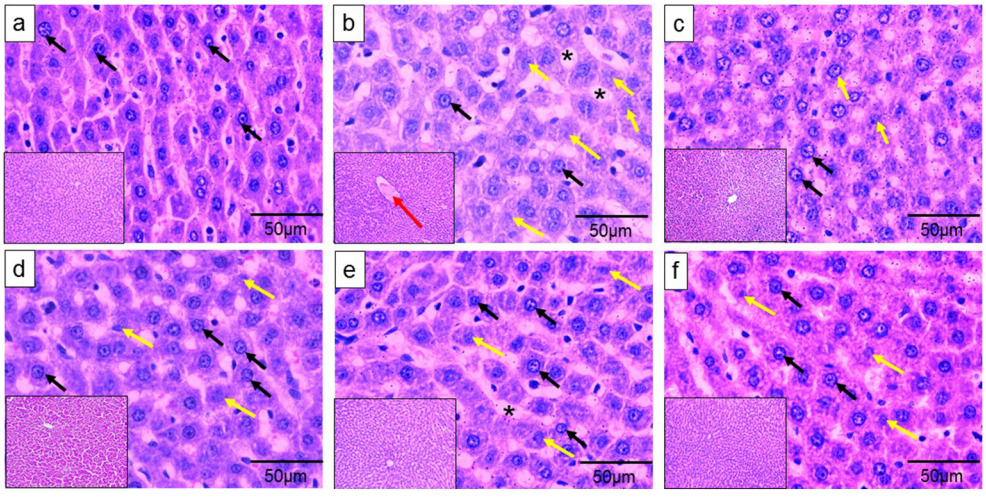

Fig. 4. Representative photomicrographs of hematoxylin & eosin-stained section of the liver of (a) normoglycaemic control, (b) diabetic control, (c) diabetic +500 mg/kg b.w. metformin, (d) diabetic +10 mg/kg b.w quercetin, (e) diabetic +250 mg/kg b.w. *H. verticillata* and (f) diabetic +500 mg/kg b.w. *H. verticillata* group. Photographs were taken using 10× (scale bar = 200 μm) and 40× (scale bar = 50 μm) objectives. Enlarged central vein (red arrow) was seen in diabetic control group (b) relative to normoglycaemic control and the treated diabetic groups. Large vacuolations (*) and numerous degenerated hepatocytes with disintegrated nuclei (yellow arrow) were seen in diabetic control group relative to normoglycaemic control and the treated diabetic groups, which had numerous normal hepatocytes with intact nuclei (black arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

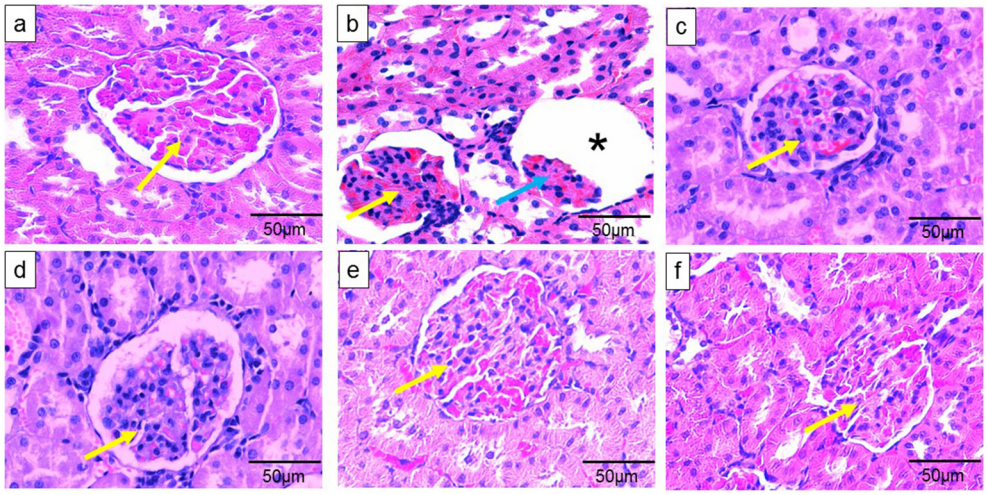

Fig. 5. Representative photomicrographs of hematoxylin & eosin-stained section of the kidney of (a) normoglycaemic control, (b) diabetic control, (c) diabetic +500 mg/kg b.w. metformin, (d) diabetic +10 mg/kg b.w quercetin, (e) diabetic +250 mg/kg b.w. *H. verticillata* and (f) diabetic +500 mg/kg b.w. *H. verticillata* group. Photographs were taken using a 40× objective (scale bar = 50 μm). The diabetic control group showed shrunken glomerulus (blue arrow) and enlarged Bowman's space (*), relative to normoglycaemic control and the treated diabetic groups which showed largely normal glomeruli (yellow arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

verticillata [10]. Again, ROS elicit degradation of membrane polyunsaturated fatty acids through step wise peroxidation process which ultimately yields MDA, one of the major peroxidation products [35]. MDA is an aldehyde which is highly unstable and induces oxidative stress by the formation of advanced lipid peroxidation end products [36]. MDA therefore serves as an important marker to measure the level of lipid peroxidation in tissues and elevated levels has been reported in diabetic rats [25,37,38] and is corroborated in the present study. Impairment of membrane fluidity as well as inactivation of some membrane proteins is a deleterious consequence of membrane lipid peroxidation. From our investigation in the present study, *H. verticillata* may prevent these negative effects by inhibiting the process of lipid peroxidation thus reducing the formation of MDA as observed in the significantly decreased level of MDA in the treated diabetic group relative to the untreated diabetic rats.

Alterations in enzymatic activity as well as concentration of amino transferases, viz., ALT, AST and ALP in the blood are important in diagnosis to confirm the occurrence of hepatocellular damage [39]. The deleterious effect of hyperglycaemia in diabetic state is estimated to account for a significant incidence of liver disease which may eventually lead to death [40]. Increase in serum AST, ALT and ALP in the untreated diabetic animals provides further evidence to support the adverse effect of hyperglycaemia on the liver. It is probable to attribute the progression of hepatic damage in the 28-day experimental period to increased oxidative stress and lipid peroxidation resulting from uncontrolled hyperglycaemia. Interestingly, the present study reports the hepatoprotective effect of *H. verticillata* as demonstrated by the significant reduction of serum levels of ALT, AST and ALP in the treated diabetic rats which were similar to the pattern of reduction observed for the groups treated with quercetin and metformin. Interestingly, the 250 mg/kg b.w. was the choice dose in ameliorating diabetes-induced liver damage by significantly decreasing serum concentration of amino transaminases compared to the other treated diabetic groups. Also, the increased concentration of total and unconjugated bilirubin provided further prove of compromised hepatic function and the liver's inability to conjugate or excrete bilirubin resulting in abnormal elevation and accumulation in the liver. Upon treatment, serum level of bilirubin was significantly decreased with a concomitant increase in percentage conjugation of bilirubin, indicative of a possible restoration of the liver's ability to handle bilirubin. Again, evidence to support hepatocellular damage as reflected in the decreased liver weight and increased serum concentration of transaminases was provided by the distorted histological integrity of the hepatic tissue of the untreated diabetic animals. Histological assessment of the hepatic tissue gives vital information regarding the functional integrity of the liver [41]. In the present study, the untreated diabetic rats were observed to have a compromised hepatic architecture with prominent congested central vein, numerous degenerated hepatocytes with disintegrated nuclei and cells that showed abundant cytoplasm with microvescicular steatosis. This observation is consistent with previous reports on altered integrity of the liver in untreated diabetic rats [6,40], which demonstrated a gradual recovery on treatment with quercetin, metformin and *H. verticillata* extract.

Serum levels of total protein, albumin as well as globulin were significantly decreased in the untreated diabetic animals compared to the normal control rats. This observation was however consistent with the decline in body weight in these animals, further evidence of tissue wasting. While these observations suggest possible renal damage, it is also probable that the decline in total protein, albumin and globulin levels may have resulted from liver toxicity as is consistent with uncontrolled diabetes [30]. However, treatment with *H. verticillata* did not seem to cause any significant increase in the level of total protein, globulin and albumin compared to the normoglycaemic control. Although the hepatoprotective role and the potential of *H. verticillata* to reverse hepatotoxicity has been documented [10], it is possible that the study plant required a longer duration to restore the synthetic ability of the liver as is the case with some medicinal plants [42].

Urea and creatinine are both non-protein nitrogenous components that arise from the catabolism of proteins and nucleic acids. Elevation of urea and creatinine levels in serum may be indicative of possible renal anomaly [43]. In the present study, serum urea and creatinine levels were significantly raised in the untreated diabetic group, however, treatment with *H. verticillata* resulted in a significant decrease in the urea and creatinine levels.

Diabetes is characterised by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to distortions in electrolyte homeostasis. In this study sodium, potassium and chloride concentrations in serum decreased significantly, while bicarbonate was significantly increased in the untreated diabetic rats. Diabetes-induced polyuria may have caused excessive loss of water in urine which resulted in the observed hyponatraemia. However, treatment reversed hyponatraemia and hypokalaemia, restoring the concentration of sodium and potassium comparable to normoglycaemic control rats. This is a desirable feature for *H. verticillta* because electrolyte imbalance is a predisposing factor to hypovolumic shock as it depresses the central nervous system leading to death in uncontrolled DM [44]. Furthermore, the concentration of bicarbonate ion in the blood serves as a useful index to monitor the acidity of body fluids. Disturbances in bicarbonate concentration are usually indicative of interferences with respiratory function as well as metabolic conditions. The increased bicarbonate level accompanied by significant decrease in the concentration of chloride ion in the

untreated diabetic animals as observed in the present study is an obvious indication of metabolic alkalosis that may have resulted from renal insufficiency. Although fewer cases of alkalosis have been reported relative to acidosis which occurs more frequently in DM [45], the observation in the present study is consistent with previous report [46] that dehydration combined with hyperglycaemia can result in metabolic alkalosis. However, treatment with the study plant elicited a significant decrease in the level of bicarbonate. The improvement in electrolyte levels by *H. verticillata* relative to the untreated diabetic group following the 28 day treatment is suggestive of a possible recovery from the derangement in electrolytes induced by hyperglycaemia.

5. Conclusion

The present investigation validates the potential of *H. verticillata* to ameliorate diabetes-related complications. *H. verticillata* is reported in the present study to possess anti-hyperglycaemic, anti-hyperlipidaemic and antioxidant activity and exhibit protective action against diabetes-induced hepatic and renal dysfunction. However, further investigations to clarify its exact mechanism of action at cellular and molecular levels are required, some of which are on-going in our laboratory.

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Competing interest

Authors declare no competing interest.

Authors' contribution

IO and GEE conceived and designed the research work; IO carried out the experiments and wrote the manuscript draft; GEE, IJA and EI performed literature search; IO and VUN analysed the data. All authors read and approved the final manuscript.

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