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Anti-Diabetic Potentials Of Vernonioside E Saponin; A Biochemical Study

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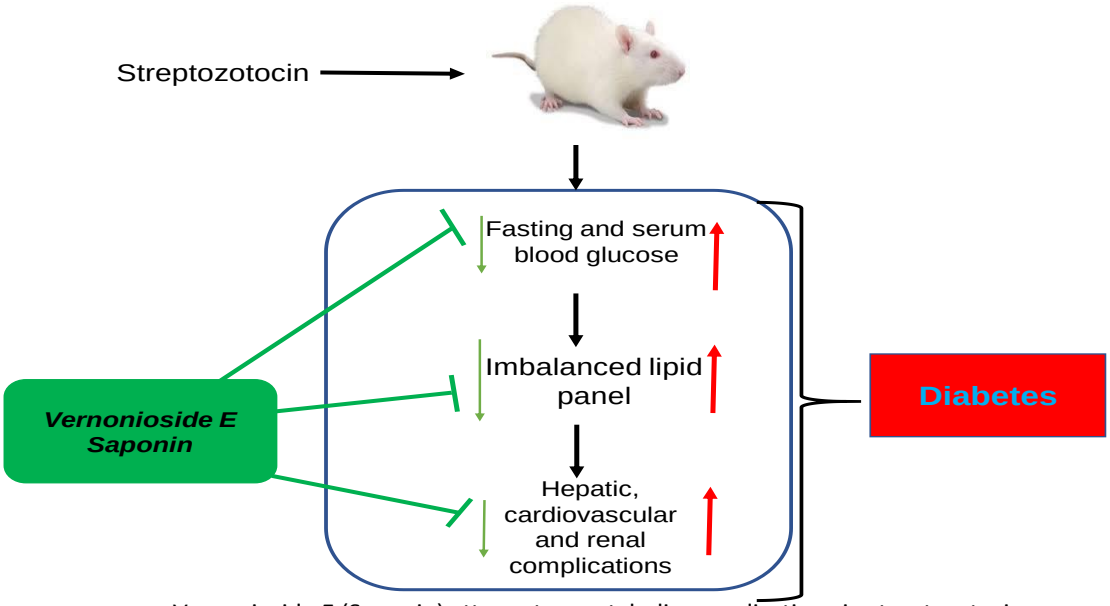
Abstract

Aim: Saponinshave been reported to demonstrate glucose lowering potentials in diabetic conditions. This study was aimed at investigating the effect of Vernonioside E (VE), a steroidal saponin extract from Vernoniaamygdalina(VA) in diabetic rats.

Main Methods: A total of 42 Wistarrats used in the study were distributed into seven (7) groups (n=6). Treatment with varying concentrations of Vernonioside E (VE1, VE2, and VE3), controls, normal (NC) and

diabetic (DC) were given placebo treatments, insulin (5 Iμ/kg b.w) (IP) given to the standard control (SC), and VA crude extract (400mg/kg b.w) per oral to the other group for 21 days.

Key findings: The results revealed diabetic rats that were presented with hyperglycemia and drastic weight loss had these features ameliorated (P<0.05). VE also attenuated dyslipidemia, decreased atherogenic coefficient, coronary risk indices, and increased cardio-protective index in diabetic rats. Furthermore, VE significantly (P<0.05) decreased serum urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). **Significance:**The findings in this study suggest that VE could be responsible for the reported antidiabetic potentials ofVernoniaamygdalina in diabetes.



Vernonioside E (Saponin) attenuates metabolic complications in streptozotocin-induced diabetic rats

1. Introduction:

Diabetes mellitus (DM) is a global health challenge. It is the most common endocrine disorder, characterized by chronic hyperglycemia. According to the World Health Organization projections, the diabetes population is likely to increase to 300 million or more by the year 2025 [1]. Diabetes mellitus is classified as Type 1 (Insulin-dependent diabetes mellitus-IDDM) and type 2 (non-insulin-dependent diabetes mellitus-NIDDM), gestational diabetes and Specific types of diabetes due to other causes with each type having a different mechanism of occurrence [2] and these may lead to various complications [3]. The insulin deficiency and glucose under-utilization in type-I diabetes could affect insulin-dependent glucose utilization tissues and glucose over-utilization in insulin-independent tissue with grievous consequences [3]. Treatment of DM is mainly by diet and exercise, insulin replacement therapy, and oral hypoglycemic agents (sulfonylureas and biguanides) as major

drugs[4]. Although there is progress in the treatment of diabetes by these methods, the search for alternatives continues due to the limitation of existing ones [5]

Reports have revealed that the hypoglycemic activity of most antidiabetic medicinal plants is due to their rich content of saponins [6]. According to these reports, saponins show diverse mechanisms of actions towards lowering hyperglycemia, including inhibition of hepatic glycogen phosphorylase and glucose 6 phosphatase activities and expression of their genes, improves insulin resistance, increases pyruvate kinase activity, inhibits carbohydrate digestion/absorption via inhibition of α-amylase and α-glucosidase activity, increase glycogen accumulation and GLUT4 translocation into membranes.

It has been reported that Platyconic acid (PA), a steroidal saponin from Platycodi radix (9), and Arjunolic acida triterpenoid saponin isolated from T. Arjuna[7], effectively increased insulin-stimulated glucose uptake in 3T3-L1 adipocytes, possibly by functioning as a peroxisome proliferator-activated receptors (PPARγ activator). PA is said to increase glycogen accumulation and decrease triacylglycerol storage in the liver, enhanced hepatic insulin signaling and potentiate the expression of adiponectin and PPARγ in adipose tissue, improved insulin signaling, and increased glucose transporter 4 (GLUT4) translocation into the membranes both in vivo and in vitro[8],.

The ability of saponins to reduce elevated plasma blood glucose has made saponins an excellent candidate in the research for the treatment of diabetes mellitus.Vernonioside E used in this study is a saponin that was isolated from Vernonia amygdalina. It is a stigmastane type of steroid glycosides[9]. Thus, the present study was aimed at investigating the anti-diabetic and potentials of VE (saponin) against in vivo as this could provide another window of opportunity in the search for ant-diabetic therapy

2. Materials and methods

2.1. Reagents and chemicals

Streptozotocin used for type 1 diabetes induction was purchased from Sigma Chemical Company, St. Louis, Missouri, USA. All other reagents and chemicals used were of analytical grade.

2.2. Collection and preparation of plant material and pure form of Vernonioside E

Fresh leaves of Vernonia amygdalina were obtained from the Endocrine Research Farm of Biochemistry Department, University of Calabar. A voucher specimen of this plant had been deposited in the herbarium of the University of Ibadan, Nigeria, in an earlier study [10]. The leaf sample was rinsed thoroughly in clean tap water and dried under shade for 7 days, blended into powder, and stored in air-tight plastic containers. Thereafter, 2000 g of the powder was soaked in absolute ethanol in a ratio of 1:3 (sample: solvent) at room temperature for 48 h [11], then filtered firstly with a cheese material and afterward with Whatman No. 2 filter paper. The filtrate was then

concentrated in a rotary evaporator (45–50 °C) to about 1/10th of the original volume, after which the concentrate was allowed to evaporate to complete dryness in a water bath (45 – 50 °C). The dried extract was weighed and it gave a percentage yield of 13.5% (Weight of extract/ Weight of starting material × 100 %). The extract was then refrigerated at 4 °C pending usage. The pure form of Vernonioside E (Acetylated E1 from Vernonia amygdalina) was obtained according to the method earlier described by Igile et al. [9] and used for the study. Briefly; exactly 600 g of V. amygdalina leaf was extracted with 4000 mL of methanol for 48 hours and evaporated to dryness with a percentage yield of 13.56 %. The residue obtained was macerated with hot 30 % methanol and centrifuge for 20 min at 3,000 rpm which gave a clear orange-brown supernatant that was condensed in vacuo and submitted to Merk short-glass column with dimensions 5 cm× 5 cm i.d Si gel. These were washed with aqueous solvents graded with increasing amounts of methanol from 30 % to 100 %. Vernonioside E was eluted from the column with 70 % methanol and then subjected to reversed-and-normal-phase liquid chromatographic purification with graded solvents from 65 % to 80 % methanol, trichloromethane, and water (65:14:1) as eluting solvents. The purified fraction was dried and precipitated from a minute volume of methanol and water (3:1) with about 3.8 % yield and used for the study. The purity level of the extract was confirmed using using nuclear magnetic resonance (NMR) analysis as earlier reported [9]

2.3. Determination of LD_{50} for Vernonioside E.

The LD_{50} for Vernonioside E was determined using experimental female mice that were obtained from the Department of Pharmacology, College of Medical Sciences, the University of Calabar using the method recommended by [12]. Vernonioside E was solubilized in 2% dimethylsulfoxide (DMSO) and administered (p.o) to experimental mice in three groups (n=3) at 1, 2, and 4mg/kg.b.w and monitored for 24 hrs for signs of toxicity. It was observed that the 2 mg/kg.b.w produced 2 deaths out of three animals This dose was repeated for another 2 mice for confirmation of toxicity and 50 % death was recorded. Hence, this was taken as the highest dose that caused mortality in animals while 1mg/kg.b.w was taken as the highest safe dose, hence, 1.4mg/kg.b.w was calculated as the LD_{50} for Vernonioside E

2.4 Laboratory animals

Forty-two (42) Wistar albino rats of both sexes weighing between 158 to 180 g were purchased from the Department of Physiology Animal House, University of Calabar, Cross River State, Nigeria, and used for this study. They were fed with rat pellets and tap water ad libitum and acclimatized for one week before the commencement of the experiment. The rats were divided into seven (7) groups

(N=6), based on their weights. The study protocol and animal handling complied with the guidelines of the National Institute of Health (NIH) publication [13] for laboratory animal care and use, the U.K. Animals (Scientific Procedures) Act, 1986 and Associated Guidelines, and the EU Directive 2010/63/EU for animal experiments. Moreover, the study was approved by the Faculty of Basic Medical Sciences Animal Research Ethics Committee (FAREC- FBMS), University of Calabar, Nigeria.

2.5 Induction of experimental diabetes.

Diabetes was induced in the rats after a twenty-four-hour fast [14] with 55mg/kg.b.w streptozotocin (STZ) using 0.5M sodium citrate buffer pH 4.5 administered intraperitoneally. Seventy-two (72) hours after administration, diabetes was confirmed with fasting blood glucose (FBG) concentration of > 200mg/dl using a one Touch ® Glucometer (Lifescan, Inc. 1996 Milpas, California, U.S.). Before diabetes induction, the fasting blood glucose of the animals was measured and repeated after 72 h, then three days interval for 21 days

2.6. Experimental design

The rats were divided into seven groups of six (6) animals each as shown below.

Group1: Normal glycaemic rats treated with 0.2 mL of vehicle

Group2: Diabetic control rats treated with 0.2 mL of vehicle

Group3: Diabetic rats treated with 5iμ/kg.b.w insulin

Group4: Diabetic rats treated with 400mg/kg.b.w Vernonia amygdalina leaf extract

Group5: Diabetic rats treated with Vernonioside E (0.70 mg/kg.b.w)

Group6: Diabetic rats treated with Vernonioside E (0.35mg/kg.b.w)

Group7: Diabetic rats treated with Vernonioside E (0.18mg/kg.b.w)

2.6 Treatment procedure.

The treatments were administered p.o in 0.2 mL of 2% DMSO (vehicle). The dose for V. Amydalina extract was selected based on a previous study [15]. The insulin dose of NPH(5iu/kg.b.w) administered intraperitoneally (IP)), was as previously used by Sonia and Scrinivasan [16] and also to simulate human regimen (therapeutic course) while the dose for Vernonioside E was obtained as explained in section 2.3. Treatment was given once daily at 9 am for 21 days. Fasting blood glucose was measured before induction and repeated at three (3) days interval in the course of the treatment. At the end of 21 days of treatment, the rats were fasted overnight and sacrificed under chloroform anesthesia. Whole blood was then collected via cardiac puncture and the serum was obtained and used for biochemical assays.

2.7. Body weight, weight change, and relative organ weights

Bodyweight 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:

[Absolute liver weight (g)] / [Final body weight (g)] × 100

2.8. Estimation of serum lipid parameters

Estimation of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-cholesterol) was done using Randox assay kit (CHOD-PAP Method) based on NCEP [17], while triacylglycerol estimation was done using Randox assay Kit (GPO-PAP) method based on Tietz [17, 18]following manufacturers instruction. The tubes were mixed, incubated for 5 minutes at 37^0C, and absorbance measured at 546 nM against reagent blank within 60 minutes. Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein-cholesterol (VLDL-C) were estimated based on calculations [19, 20]. The assessment of cardioprotective index (CPI) was based on HDL-C/LDL-C ratio, while atherogenic coefficient (AC), atherogenic (AI), and coronary risk indices (CRI) 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 [21]

2.9. Estimation of serum indices of hepatic and renal function

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assayed using spectrophotometric assay kits (Randox) based on [22]. Serum analysis for Na^+ and K^+ was performed with TECO diagnostics kits based on Tietz [23] while Cl^- assay was performed with assay kits from DALAB based on [24] using an automatic analyzer ROCHE module Cobas 6000 (C-501 and C-601) (Roche diagnostics, NorthAmerica). The levels of creatinine, urea, albumin, globulin, and total protein were determined in the serum using Agappe assay kits (Agape diagnostics, India). Serum globulin was estimated by calculating the differences between serum total proteins and serum albumin given as Albumin (g/dl) = Total protein (g/dl)-Albumin (g/dl).

2.10. Estimation of serum glucose concentration

The serum glucose concentration was estimated using the enzymatic colorimetric test. GOD-PAP reagent kit (USA Randox Laboratories san Francisco). Briefly, the principle involves the enzymatic

oxidation of glucose in a sample by the enzyme, glucose oxidase, to generate hydrogen peroxide and gluconic acid. The H_2O_2 released whose concentration is proportional to the initial amount of glucose in the sample reacts, under the catalysis of peroxides, with phenol and 4-aminophenazone to form a red-violet quinone imine dye whose color intensity reflects the concentration of glucose in the sample.

2.11. Statistical analysis

The data obtained were analyzed by one way ANOVA using the GraphPad prism 7, followed by a post hoc Turkey test. All data were expressed as mean ± SEM (n=6), and the hypothesis was tested at a 95% level of significance.

3. Results

3.1. Effect of treatment on fasting blood glucose

Table 1 shows the changes in fasting blood glucose (FBG) of streptozotocin (STZ)-induced diabetic rats treated with VE at different doses of 0.7, 0.35, and 0.18mg/kg.b.w for 21 days. The diabetic control group (DC) group which had an initial FBG of 330.83±2.97 mg/dL remained high throughout with values of about (335.17±0.84mg/dL FBG). Values that were significantly higher than NC ($p<0.05$)(80.17±0.16 mg/dl) to the end of the experiment. However, this increase in FBG was reversed in VE-treated rats (1,2&3) to values of 93 ± 2 mg/dL, which is similar to that observed in insulin treatment(91.33 ± 2.03) mg/dL and VA whole extract treatments (90.33 ± 2.03) mg/dL ($p<0.05$).

3.2. Effect of treatment on body weight change and organ weights

Table 2 gives a clear picture of the effect of treatment with VE on body weight (absolute and relative) and organs weight (absolute and relative) in the various groups. While there was an appreciable increase in weight in all the treatment groups (VE 1,2&3, VA & INS) like the NC group, the reverse was observed in DC where there was a constant decrease in body weight from initial values of 169.90±0.86 to 145.50±0.69. Table 2 compares and contrasts between the different experimental groups. The weight of DC which was higher than NC at the start of the experiment was significantly lower than NC ($p<0.05$) at the end of the experiment. In the insulin, VA, and VE 1-3 treatments, there was a significant ($P<0.05$) increase in weight from initial values in all treatment groups. However, the increase was more pronounced in the VE treatments compared to insulin and VA whole extract treatments and similar to NC ($P<0.05$). Also, there was a significant ($P<0.05$) reduction in liver weight of DC compared to NC. This reduction was restored to normal in VE treated

rats at all doses (VE1-VE3), an effect which was significantly (P<0.05) higher than that observed in VA but similar to the insulin treatment group. The results showed that the weight of kidneys increased significantly (P<0.05) in DC compared to NC (p<0.05) but treatment with VE at all the doses, significantly decreased (P<0.05) absolute kidney weights and compared significantly (p<0.05)with that observed in insulin and VA treatments.

3.3 Effect of treatment on serum lipid profile

Figures 1A, B, C, D, and E show the effect of 21 days treatment of streptozotocin-induced diabetic rats with VE on serum lipid profile. Serum triacylglycerol (TG) increased by 50% in DC compared to NC (p<0.05) but decreased by 35.5 %, 54 %, and 47 %at all doses of VE (0.7, 0.35 & 0.18) mg/kg.b.w treatment, respectively. These percentages were better than those observed in INS and VA extract treatments. Serum total cholesterol (TC) increased by 47% in DC compared to NC (p<0.05) but decrease by 59 %, 36 % & 34 % in VE treatment at all doses of 0.7, 0.35 & 0.18mg/kg.b.w, respectively. This result was similar to that observed in insulin treatment where TC decreased by 34% while VA reduced it by approximately 25.08%. Similarly, high density lipoprotein (HDL) decreased by 66% in DC compared to NC (p<0.05) treatment which increased significantly (P<0.05) by 67, 45 & 44% at all doses of VE treatment (0.7, 0.35 & 0.18) mg/kg.b.w, respectively compared to DC (p<0.05). The effect of VE treatment on HDL was better than that observed in insulin and VA treatments which recorded only about 28 & 26 % increase, respectively, compared to DC. Low-density lipoprotein (LDL-c) increased by 26 % in DC compared to NC (p<0.05), decreased by 41 and 40% in VE treatments 2&3, respectively. INS treatment caused a significant (P<0.05) reduction of LDL-c by 78 % compared to NC while VA treatment reduced it only by 17 % compared to DC (p<0.05). Similarly, VLDL-c increased by 50% in DC compared to NC (p<0.05) but significantly (p<0.05) decreased by 32 %, 35 %, and 45 % in VE treatment doses of 1-3 compared to DC. These results are better than those observed in insulin and VA treatments significantly (p<0.05). reduced VLDL-c by 18 & 15%, respectively, compared to DC.

3.4. Effect of treatment on indices of cardiovascular function

Indices of cardiovascular function studied included atherogenic coefficient (AC), atherogenic index (AI), coronary risk index (CRI), and cardioprotective index (CPI) (Figures 2 A, B, C, and D, respectively). The results showed that VE treatments protected the rats against cardiovascular risk indices and compared favorably with standard treatment insulin but better than VA whole extract. Increased cardiovascular risk indices (AC, AI, and CRI) observed in DC significantly (p<0.05) decreased

across all VE treatments while decreased CPI observed in DC treatment significantly ($p<0.05$) increased on treatment with VE doses.

3.5. Effect of treatment on serum enzyme concentrations

Serum indices of liver function are shown in Figure 3 A, B, C and D. Measured alanine aminotransferase activity (ALT) was higher in DC rats (6.5%) than in the NC ($p<0.05$). However, treatment with VE at 0.7 and 0.35mg/kg.b.w lowered the activity by 11.3 &11.1% respectively ($p<0.05$). Similarly, treatment with VA at 400mg/kg.b.wt lowered the activity by 6% like insulin treatment. Aspartate aminotransferase activity (AST) lowered in DC was restored towards normal by 8.18 % and 8 % in insulin and VA treatment groups with close values observed in VE2 and VE3 treatments. The ratio of AST/ALT activity increased in DC was restored in all VE and VA treatments like insulin. Measured alkaline phosphatase activity (ALP) which was increased by 26.8% in DC compared to NC ($p<0.05$), was significantly lowered by 23 ± 3 % in all the treatment groups when compared to DC ($p<0.05$).

3.6. Effect of treatment on serum blood glucose

Figure 3 E shows the effect of the treatment with Vernonioside E (VE) at different doses of (VE1-VE3) for 21 days on serum blood glucose (SBG). There was a significant rise in SBG of diabetic control (DC) group compared to normal control (NC) with values of. This increase was decreased in all the VE treatments (VE1-VE3) like insulin and VA treatments.

3.7 Effect of treatment on serum electrolytes, proteins, urea, and creatinine

Table 3 shows the result of the effect of 21 days treatment of streptozotocin (STZ) induced diabetic rats treated with Vernoniosede E on serum electrolyte, protein, urea, and creatinine levels. No significant reduction was observed in the serum K^+ concentration (5%) of DC compared to NC, VA, VE1 &VE3 groups. However, in insulin and VE2 treatments, there was a significant reduction in K^+ concentration ($p<0.05$), a noticeable 30 and 20% reduction in each case respectively. Serum Cl^- concentration was increased by 42.9% in DC compared to NC ($p<0.05$). This was significantly decreased in VE treatments compared to DC ($P<0.05$). There was a 10% reduction in serum Na^+ concentration ($p<0.05$) in DC compared to NC. This was restored to 10% in all VE and VA treatment groups and 20% in insulin treatment ($p<0.05$). More so, table 3 shows the result of the serum protein concentrations of the different experimental groups used in the study. No significant difference was observed in the DC group compared to NC in serum total proteins (,$p<0.05$). However, serum albumin was increased by 36% in the VE3 test group compared to DC, NC, and

other test groups (VA, VE 1 and 2 &INS). No significant reduction was observed in serum globulin in DC compared to NC ($p<0.05$). At VE 1, there was a significant decrease (51.6%)($p<0.05$) in serum globulin compared to DC and NC. Serum urea was increased by 43% in DC compared to NC($p<0.05$). This increased in urea was lowered by 41.73% and 41.67% in VE treatment groups 1&2 respectively, a result better than that observed in insulin and VA treatment groups ($p<0.05$). In the same vein, serum creatinine which was increased by 34.64% was significantly lowered in VE treatment groups. A result similar to that observed in insulin treatment where there was a 32.64% reduction in serum creatinine.

4.1. Discussion

Hepatic and renal dysfunctions alongside cardiovascular complications are frequently found in diabetes and hyperlipidaemic subjects [25]. Therefore, the present study investigated the impact of the potential diabetes therapy (Vernonioside E-VE) on hepatic, cardiovascular, and renal functions in rat's model of diabetes by evaluating hepatic enzymes activities, lipid panel, electrolytes and serum level of proteins, creatinine, urea, and glucose profiles in albino rats. Results showed elevated fasting blood glucose and serum blood glucose in diabetic control (DC) (Table 1 and Figure 3E) decreased in all treatments with Vernonioside E-VE and compared significantly with normal and standard controls (NC and SC). This observed glucose regulation with VE support earlier reports which states that saponins have the potential to stimulate insulin secretion, β-cells regeneration, and enhancement of the activity of enzymes involve in glucose metabolism [26]. Another reported mechanism of saponins possibly employed by Vernonioside E in glucose regulation is increased glucose utilization by the liver, lowered gluconeogenesis by inhibition of glucose-6-phosphate and fructose-1, 6 bisphosphatases, and activating glucose-6-phosphate dehydrogenase via the shunt pathway which improves glucose oxidation [26].

This study also assessed the effect of the treatment on body weight change and organ weights (Table 2). There was a constant weight reduction observed in DC rats as opposed to a constant increase in weight seen in NC and VE treatments. The results support earlier reports which stated that diabetes could cause decreased bodyweight due to decreased glucose utilization and increased tissue wasting [27]. However, a higher weight gain was observed in insulin treatments compared to VA and VE treatments, a major concern associated with dependence on insulin therapy [27]. Moreover, epidemiological studies have demonstrated a correlation between hyperinsulinemia and the risk of macrovascular disease [28]. This has raised the possible specter of insulin therapy accelerating macro-vascular diseases; however, this has not been reported in VA studies on diabetes, hence presenting Vernonioside E as a safe treatment for diabetes. The increased weight

observed in insulin treatment may be attributed to the high lipogenic activity of insulin as reported by Yanget al.[29] The observed inhibition of decrease in weight in this study is in agreement with earlier reports by several workers who demonstrated this in rats [30]. The results obtained in this study showed a similar trend in body weight and growth rate changes.

In this study, untreated diabetic (DC) (Figure 1) showed elevations in TC, TG, LDL-c, and VLDL-c and a concomitant decrease in HDL-c when compared to NC rats; there was also a concomitant rise in serum HDL-c in the NC compared to DC, the protective molecule. This rise in negative lipid panel was reversed (decreased) in VE treated rats like insulin and VA whole extract. A review by Suleiman et al. [31] reported the lipid-lowering effect of Vernonia amygdalina (VA) in rats fed high cholesterol diets. The result of this investigation, demonstrate a hypolipidaemic activity of both leaf extract of VA and VE like insulin treatments. Marrelli et al,[32]reported that saponins bind cholesterol in the intestinal lumen making them less readily reabsorbed and or bind with bile acids causing their fecal excretion. This excretion is offset by an enhanced conversion of cholesterol to bile acids, consequently lowering cholesterol in plasma. Another possible mechanism of the hypocholesterolemic effect of Vernonioside E is inhibition of 3-HMG-CoA reductase. Our earlier study showed biologically active molecules like saponins and polyunsaturated fatty acids can down-regulate the activity of HMG-CoA reductase in obesity complications and this is achieved via several suggested mechanisms [33]. Besides, increase expression of LDL-c receptors in the liver has been implicated to increase the clearance of LDL-c excretion from circulation. This mechanism might have been exhibited by the VA and VE used in this study. These findings support the work of Adaramoyeet al[34] who reported the hypolipidaemic effect of VA in high cholesterol diet-fed rats. The increased HDL-c and the reduced atherogenic index (AI), coronary risk index (CRI), atherogenic coefficient (AC) and increased cardioprotective index (CPI) (Figure 2) in the diabetic treated rats further demonstrate the anti-atherogenic activity of the VA and VE. High-density lipoprotein (HDL) possesses antioxidant properties that can prevent the oxidation of LDL-c. Elevated levels of HDL-c as seen in this study protect the arterial wall from the development of atherosclerotic plaques by preventing the macrophage-mediated accumulation of cholesterol deposit in response to endothelial injury.

Alteration in hepatic enzymes and hence liver dysfunction are frequently found in diabetes [35]. In this study, serum liver enzyme activities were evaluated to find out the potential diabetes therapy on liver function. Results show elevated serum aminotransferases and alkaline phosphatase activities with increased AST: ALT ratio in diabetic control compared to non-diabetic control, insulin treatment, and Vernonioside E treatment (Figure 3 A, B, C, and D), implying an associated liver dysfunction risk in diabetic condition. This result is in line with that of Atangwho et al. [15] who reported elevated enzyme activities in diabetic subjects and amelioration of disordered fat

mobilization on the administration of VA leaf extract. In this study, treatment with Vernonioside E ameliorated the effect on the activities of these enzymes like standard insulin treatment which is in agreement with earlier reports of Atangwho et al., [15], who reported the same ameliorative effect by whole crude extract of Vernonia amygdalina in alloxan-induced diabetic rats, indicating a reversal of potential hepatotoxic effect. This reversal capabilities of VA can be attributed to its rich content of antioxidants such as saponins [36]. The presence of H atom-donating group present in Vernonioside E molecule in both the aromatic ring and side chain of the aglycone structure [9] could be responsible for the antioxidative and hepatoprotective capabilities of saponin, as seen in this study. The liver cells may be protected by the acetoxy group of Vernonioside E saponin (C_{30} position) via a potential binding of the carboxyl group with the OH groups of amino acids and protein on hepatocytes, ensuring their protection from the action of ROS and RNS produced in glucose oxidation and streptozotocin metabolism.

Serum indices of kidney function (Table 3), creatinine, urea, and selected electrolytes (k^+, Cl^-, Na^+) were measured in this study. Creatinine level, a sensitive marker of kidney function was found to increase in diabetic rats, suggesting some form of kidney malfunction when compared to NC and all other treatment groups. However, VE treatment reduced the serum creatinine concentration better than VA, suggesting that pure form of VE may provide a better therapeutic property than whole crude leave extract. Serum urea also increased significantly ($P<0.05$) in DC rats while sodium significantly ($P<0.05$) decreased in DC compared to NC, and VE treatments. These observed effects (increase in serum urea) following induction of diabetes in rats were reversed (decreased) following VE treatment to values similar to that in NC, and VA treatment indicating an improvement in kidney function. Similarly, a decrease in sodium concentration in DC rats, indicating potential hyponatremia was normalized following treatment with VE like insulin treatment. The rise in serum chloride ion concentration in DC compared to NC indicates the risk of attaining hypertensive status in DC. However, the rise in serum chloride was attenuated in VE treated rats like insulin treatment. This result agrees with earlier findings by Akhtar et al.[37] who reported antihypertensive herbs and their mechanism of action to involve saponins indicating that the antihypertensive properties of VA are associated with saponins, a group of compounds in which Vernonioside E belongs.

5.1 Summary

This study showed that diabetic rats that presented with hyperglycemia, significant weight loss, hyperlipidemia, potential liver malfunction, cardiac and kidney damage had most of these features ameliorated on treatment with Vernonioside E. More so, in the course of this study, it was discovered that Vernonioside E is endowed with potentials that can be exploited in the development

of novel anti-diabetic drugs and the management of hyperlipidemia as well as diseases associated with the liver and kidney better than some of the current anti-diabetic drugs in circulation.

5.2 Conclusion

Conclusively, this study has demonstrated that Vernonioside E is an active principle and a saponin found in Vernonia amygdalinais responsible for some of the anti-diabetic, nephroprotective, and hepatoprotective activities reported to be associated with Vernoniaamygdalina leaf extract.

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Conflict of interest

The authors declare no conflict of interest

Author's contribution

DEU and GOI conceived and designed the study. DEU, RIO, and GUU performed literature searches, UNO, WAO, GUU, RIO, and ONO carried out the animal experiments and the laboratory analyses, GOI extracted the VE, DEU, GUU and ONO analyzed the data and prepared the manuscript.UAI proofread and edited the manuscript for intellectual content. All authors read and approved the final manuscript

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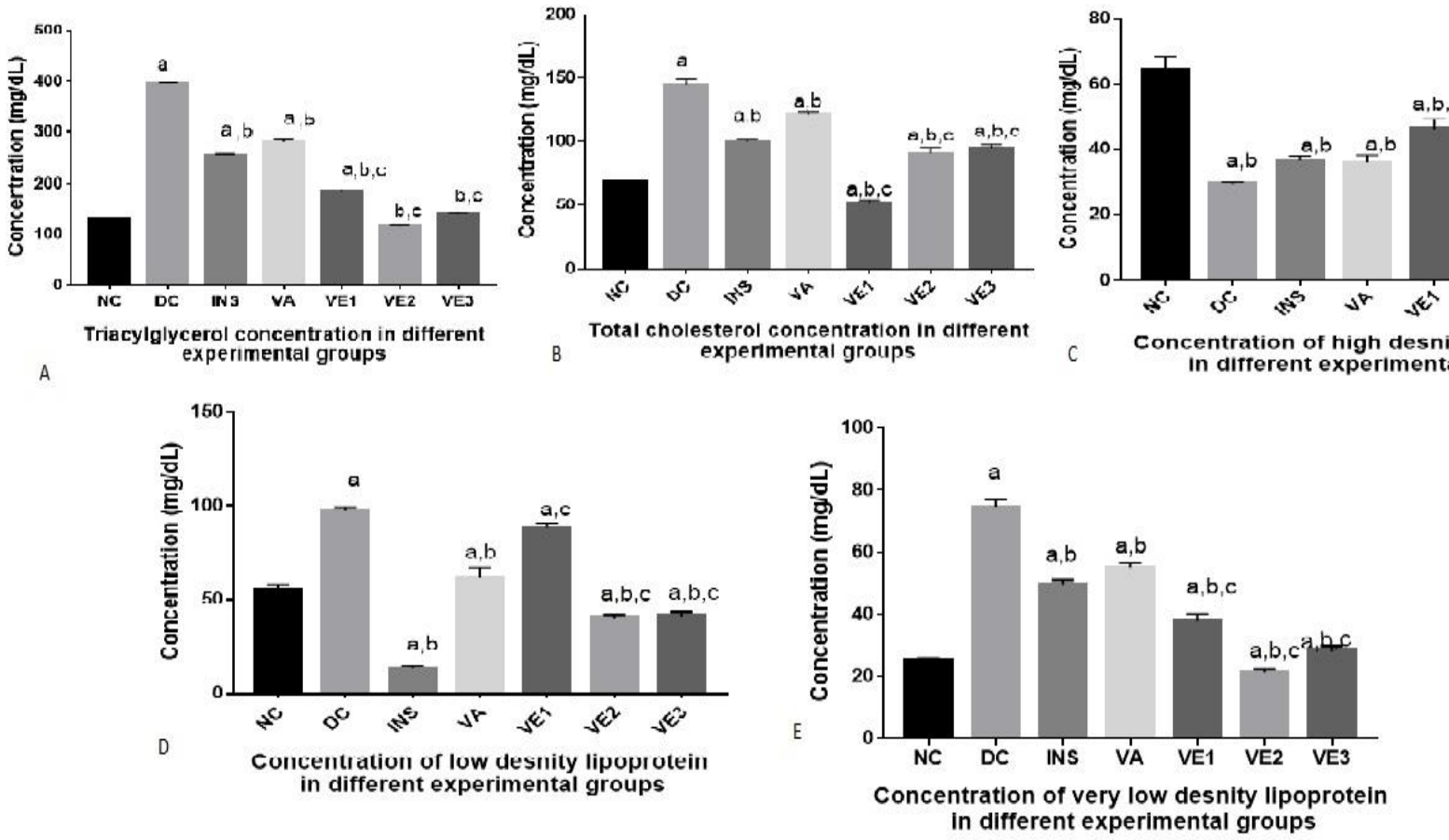

Figure 1: Serum lipid parameters of different experimental groups

Values are expressed as mean ±SEM, n = 6; [a]significantly different from NC at p<0.05; [b]significantly different from DC at p<0.05; [c]significantly different from VA, NC=Normal control, DC=Diabetic control, INS=Insulin treatment, VA= Venoniaamygdalina extract (400mg/kg.b.w), VE1-VE3=Varying concentrations of Venonioside E (0.70. 0.35 and 0.18 mg/kg.b.w) respectively

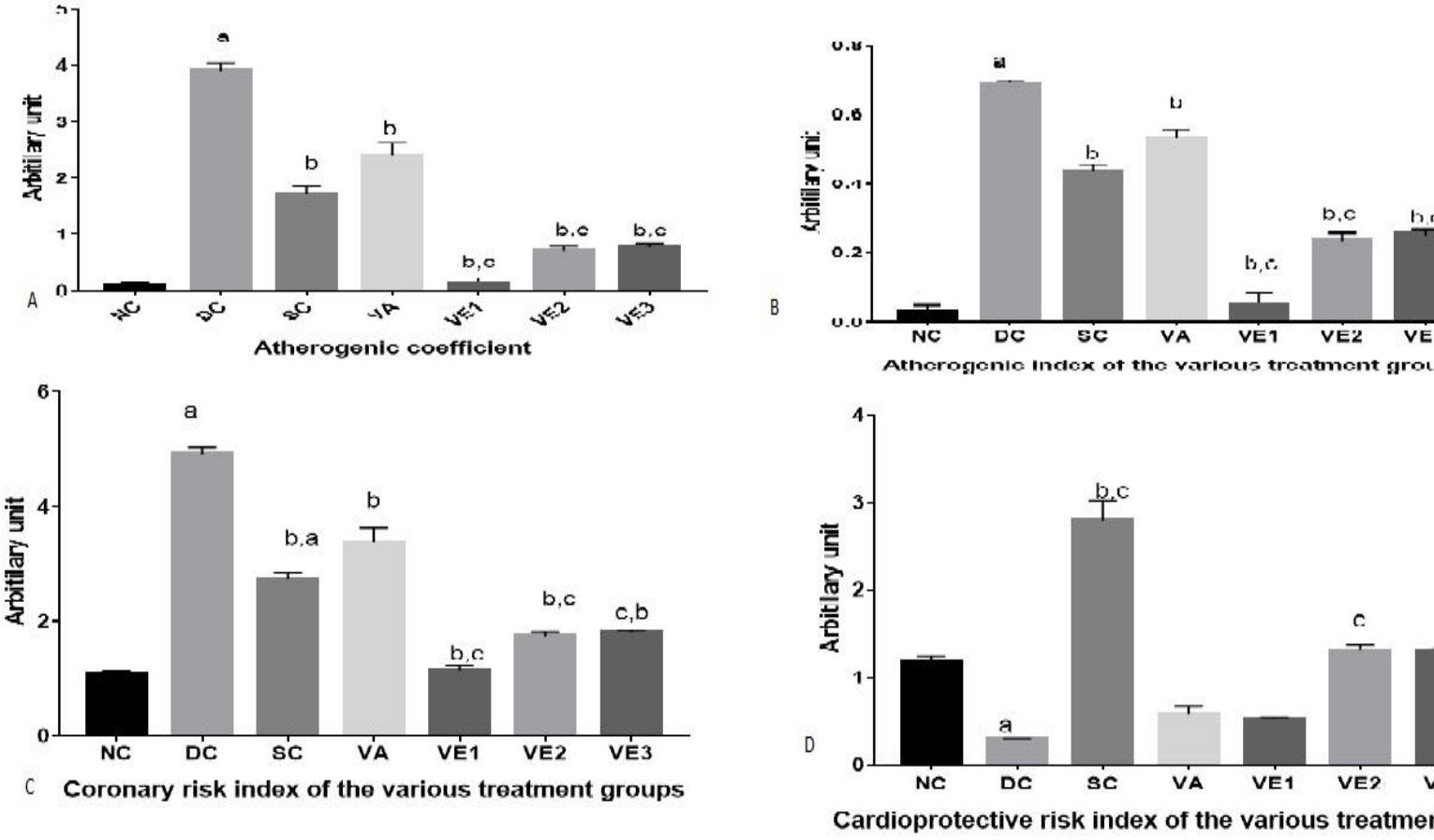

Figure 2: **Indices of cardiovascular function of different experimental groups**

Values are expressed as mean ±SEM, n = 6; [a]significantly different from NC at p<0.05; [b]significantly different from DC, [c]significantly different from VA, at p<0.05. AC=Atherogenic coefficient, AI= Atherogenic index, CRI= Coronary risk index, CPI=Cardioprotective index, NC=Normal control, DC=Diabetic control, INS=Insulin treatment, VA= Venoniaamygdalina extract (400mg/kg.b.w), VE1-VE3=Varying concentrations of Venonioside E (0.70. 0.35 and 0.18 mg/kg.b.w) respectively

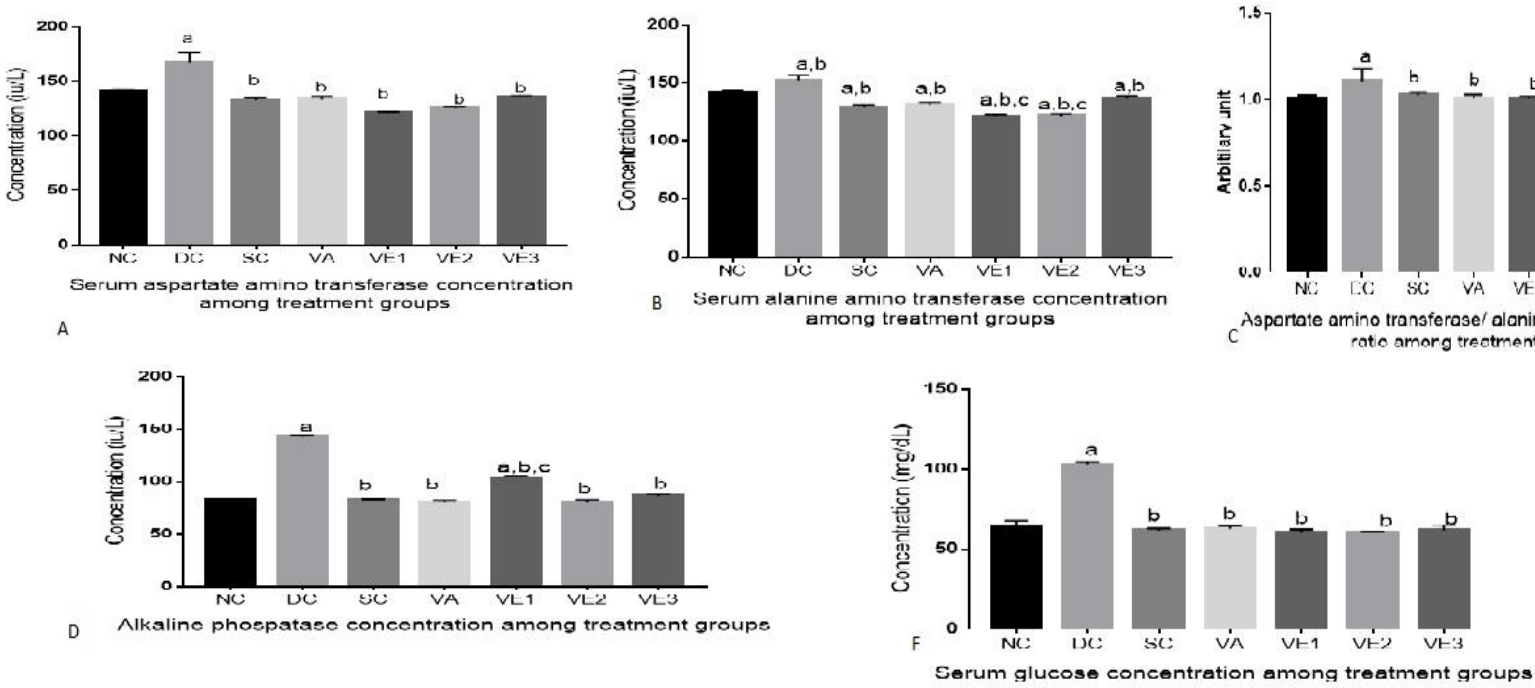

Figure 3: Hepatic enzymes and serum glucose concentration of different experimental groups

Values are expressed as mean ±SEM, n = 6.[a] significantly different from NC at p<0.05; [b] significantly different from DC, [c]significantly different from VA, at p<0.05. NC=Normal control, DC=Diabetic control, INS=Insulin treatment, VA= Venoniaamygdalina extract (400mg/kg.b.w), VE1-VE3=Varying concentrations of Venonioside E (0.70. 0.35 and 0.18 mg/kg.b.w) respectively

TABLE 1: Fasting blood glucose of different experimental groups (pre-induction, induction and post induction/treatment) (mg/dL)

	Pre-induction Induction		Post – induction/treatment						
	DAY0	72HRS	DAY3	DAY6	DAY9	DAY12	DAY15	DAY18	DAY21
N	82.5±0	81±0.18	81.5±0.2	81.17±0.	80.33±	81.17±0.	81.17±0.	80.5±0.	80.17±0
C	.31		3	19	0.25	07	13	17	.16
D	82.17±	330.83±	420.67±2	409.5±0.	418.83±	428.17±	412.83±	390.5±0	335.17±
C	0.25	2.97*	.80*	87*	0.76*	1.26*	1.30*	.57*	0.84*
IN	81.17±	337.33±	298.5±1.	209.67±	184.83±	138.83±	111.5±0.	101.5±0	91.33±0
S	0.36	2.10*	31*,a	0.84*,a	2.00*,a	0.53*,a	39*,a	.31[a]	.25[a]
V	81.5±0	337±2.0	265.83±0	203.33±	175.67±	136.5±0.	111.17±	101.5±0	90.33±0
A	.30	0*	.68*,a,c	1.90*,a	0.98*,a	83*,a	0.32*,a	.25[a]	.34[a]
V	82.17±	345.67±	258.17±0	208.83±	176.33±	134±0.7	109.17±	98.5±0.	90.83±0

E 1	0.22	1.74*	.86*,a,c	1.13*,a	0.88*,a	2*,a	0.34*,a	17a	.29a
V E 2	82.17± 0.25	337.17± 1.14*	258.17±1 .72*,a,c	209.7±1. 17*,a	178.33± 1.22*,a	135±0.6 2*,a	109±0.1 5*,a	99.5±0. 27a	90±0.24 a
V E 3	81.83± 0,19	338.33± 0.80*	258.17±1 .72*,a,c	215±1.3 7*.a	176.67± 0.83*,a	135.17± 0.76*,a	111.17± 0.49*,a	103.17± 0.46a	93.67±0 .14a

Values are expressed as mean ±SEM, n = 6.*significantly different from NC at p<0.05;a = significantly different from DC at p<0.05; c = significantly different from INS at p<0.05. NC=Normal control, DC=Diabetic control, INS=Insulin treatment, VA= Venonia amygdalina extract (400mg/kg.b.w), VE1-VE3=Varying concentrations of Venonioside E (0.70. 0.35 and 0.18 mg/kg.b.w) respectively

TABLE 2: Body weight, weight change, absolute and relative organ weights in all experimental groups

	Body weight(g)			Liver weight		kidney weight	Combine
	Initial	Final	Weight change(g)	ALW(g)	RLW(%)	AKW(g)	RKW(%)
NC	162.78±0,45	171.88±0.39	9.10±0.22	7.13±0.07	4.15±0.04	1.20±0.03	0.70±0.02
DC	169.90±0.86	145.50±0.69*	-24.40±1.05*	4.8±0.12*	3.30±0.08*	1.48±0.02*	1.02±0.01*
INS	165.15±0.10	171.43±1.17a	6.28±0.28a	6.73±0.05a,c	3.93±0.04a	1.37±0.02	0.80±0.01a
VA	168.70±1.15	175.83±1.45a	7.13±0.38a	5.88±0.06*,a	3.35±0.04	1.18±0.03a	0.67±0.02a
VE 1	165.22±1.09	171.10±0.94a	5.88±±0.18a,c	6.82±0.06a.c	3.99±0.04a	1.35±0.02	0.79±0.01a
VE 2	170.95±0.27	172.93±1.00a	1.97±0.92a,c,d,e,f	7.40±0.07a.c	4.29±0.05a	1.22±0.02a	0.70±0.01a
VE	164.85±0.6	169.63±0.64	4.77±0.21a,c	7.05±0.07a,	4.16±0.05	1.35±0.01	0.80±0.01

3	4	[a]	[c]	[a]	[a]

Values are expressed as mean ±SEM, n = 6.*significantly different from NC at p<0.05;a = significantly different from DC at p<0.05; c = significantly different from VA at p<0.05; d=significantly different from NIS at p<0.05;e = significantly different from VE1# at p<0.05; f = significantly different from VE3 at p<0.05 ALW=Absolute liver weight, RLW=Relative liver weight, AKW= Absolute kidney weight, RKW= Relative kidney weight. NC=Normal control, DC=Diabetic control, INS=Insulin treatment, VA= Venonia amygdalina extract (400mg/kg.b.w), VE1-VE3=Varying concentrations of Venonioside E (0.70. 0.35 and 0.18 mg/kg.b.w) respectively

Table 3: **Serum indicators of hepatic and renal function in the different experimental groups (electrolytes, proteins, urea and creatinine)**

	K^+ (mmol/L)	Cl^- (mg/dL)	Na^+ (mEq/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
NC	7.92 ±0.10	68.91±1.16	111.86±5.58	7.50± 1.46	2.69±0.41	6.21±1.68	82.07±1.54	0.95±0.13
DC	7.05±0.16*	75.09±2.17*	83.96±2.77*	7.03± 2.49	2.45±0.96	5.64±1.41	208.23±1.66*	1.93±0.22*
INS	3.70±0.17*,[a]	74.09±2.04*	126.39±1.90*,[a]	7.64± 1.60	3.42±0.90	5.24±1.46	158.03±1.09*,[a]	0.98±0.06*
VA	5.45±0.49*,[a,b]	42.48±1.02*,[a,b]	113.14±2.32[a,b]	8.09± 1.01	2.69±0.51	3.70±0.15	93.87±1.66*,[a,b]	1.29±0.30*
VE.1	6.05±0.25*,[a,c,d]	32.44±0.76*,[a,b]	109.97±4.82[a,b]	4.32± 0.79	2.25±0.75	1.80±0.32*,[a]	85.59±1.22[a,b,c]	0.92±0.06*
V	3.93±0.28*,[a,b,e]	40.15±0.94*,[a,b,d]	115.17±1.69[a,b]	7.26± 0.29	3.20±1.09	4.72±1.37	85.73±2.38[a,b,c]	0.98±0.05*

E.2	6.07±0.17	71.91±1.07^{c,d,e}	103.84±1.24^{a,b,e}	5.63±1.27	5.32±1.06*^{,a,c,d}	3.29±0.77	124.60±1.21*^{,a,b,c,d,e}	1.60±0.26*^{,b,d,e}
VE.3								

Values are expressed as mean ±SEM, n = 6.*significantly different from NC at p<0.05;a = significantly different from DC at p<0.05; b = significantly different from INS at p<0.05;c = significantly different from VA at p<0.05;d = significantly different from VE. 1 at p<0.05;e = significantly different from VE. 2 at p<0.05.