IAA Journal of Scientific Research 4(1):75-90, 2018. ISSN: 2736-7319

The Effects of Crude Ethanol Root Extract and Fractions of *Sphenocentrum jollyanum* on Liver and Kidney Function Parameters of Streptozotocin-Induced Diabetic Wistar Albino Rats.

[1]Ugwu Okechukwu P.C.,[2]Onyeneke E.C., [1]Igwenyi, I. O., [1]Aja P. M., [3]Ugwuoke, Kenneth C., [1]Okon Michael B. and [1]Onyeke S.C.

[1]Department of Biochemistry, Ebonyi State University Abakaliki, Nigeria.

[2]Department of Biochemistry University of Benin, Edo State, Nigeria

[3]Biochemistry Department, Federal University Wukari, Nigeria.

ABSTRACT

The effects of ethanol root-extract and fractions of *Sphenocentrum jollyanum* (SJ) on liver and kidney function parameters of streptozotocin (STZ) induced diabetic Wistar albino rats were carried out with a total of 88 albino rats and 32 mice. Forty eight male albino rats were randomly assigned into eight groups, each containing six animals. Diabetes was induced by intraperitoneal injection of a single dose of 70mg/kg body weight of STZ. The treatment started after confirmation of diabetes and lasted for 21 days. Groups 1, 2 and 3 served as positive control (diabetic rats treated with 0.5 ml of normal saline), standard control (diabetic rats treated with 0.5mg/kg body weight of glibenclamide) and negative control (non diabetic rats treated with 0.5ml of normal saline) respectively. Groups 4, 5 and 6 rats were induced with diabetes and were treated with 250, 500 and 1000 mg/kg body weights of the crude ethanol extract of SJ respectively while rats in groups 7 and 8 were induced with diabetes and treated respectively with 250 mg/kg body weight of methanol and ethylacetate fractions of SJ. The liver and kidney function parameters were determined using standard laboratory procedures. The treatment of STZ induced diabetic albino with crude ethanol root-extract of *Sphenocentrumjollyanum* at doses of 250, 500 and 1000 mg/kg body weight and 250 mg/kg body weight of ethylacetate and methanol root fractions of *Sphenocentrumjollyanum* significantly ($p<0.05$) increased the levels of total protein and albumin. STZ-induced diabetic albino rats treated with the extract and fractions significantly ($P<0.05$) decreased the activities of gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), the level of bilirubin and significantly ($P<0.05$) increased the levels of total protein and albumin when compared with the positive control. The treatment of STZ-induced diabetic albino rats with the extract and fractions significantly ($P<0.05$) decreased the level of urea, creatinine, sodium (Na^+), bicarbonate (HCO_3^-) and increased the level of chloride (Cl^-), calcium (Ca^{2+}) and potassium (K^+)relative to positive control. The result also showed no significant ($p>0.05$) changes in the values of urea, creatinine, Na^+ and HCO_3^- in the crude extract relative to fractions except at the dose of 250 and 500 mg/kg body weights. Also it showed a significant ($p<0.05$) decrease in the levels of Ca^{2+} and Cl^- in crude extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) changes relative to the fractions. The effect on the crude extract was dose dependent and the value for standard control showed no significant ($p<0.05$) changes relative to the negative control.

Keywords: *Sphenocentrum jollyanum,* liver, kidney, albino rats

INTRODUCTION

A medicinal plant is a plant that any of its parts can be used for therapeutic purposes and the constituents can be used as a precursor for the synthesis of useful drugs [1]. Medicinal plants have continued to play many roles in the

management, treatment and cure of ailments and diseases [2].

Sphenocentrum jollyanum is an erect shrub that belongs to the family Menispermacea [3]. It is called "Ezeogwu" in Igbo, "Aduro Koroo" or "Okramankote" in the Akan Language in Ghana [4]. *Sphenocentrum jollyanum* has been shown to have antihypertensive, antioxidant, antinociceptive, antiviral and anti-angiogenic effects in animals [5]; [6]. The plant is also documented for its use against chronic coughs, worms and other inflammatory conditions as well as tumors [7]; [8]. The plant is traditionally used as remedy for feverish conditions as well and as an aphrodisiac [9]; [10]. Studies have shown that the leaves posses significant antipyretic and analgesic activities [11]. The roots and leaves have been reported to be active against polio [12].

It is also believed to be emetic and purgative agents especially when poisoning is suspected; the sap is believed to relieve stomach ache and constipation and also boost appetite and sexual desire [13]. In Ivory Coast, pounded roots are taken against high blood pressure, while the boiled roots are given against epileptic fits [14]. In Ghana, the pulped root is used to treat breast tumors [15]. Different parts of the plant have been used extensively for the treatment of various ailments in Western African sub-regions [16]. Extracts from the root have been used for the relief of constipation, as stomachic, for sickle cell disease, rheumatism and other inflammatory conditions [17]. The fruits are used as an anti-fatigue snack [18]. It has been also reported by [19] that the methanolic extract of the root of *Sphenocentrum jollyanum* increased the testosterone levels in a dose-dependent manner and also reduced the count, motility and viability of spermatozoa in albino rats.

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin action, insulin secretion or both [20]. Insulin deficiency leads to chronic hyperglycemia with disturbances in fat, carbohydrate and protein metabolism.

Streptozotocin (STZ) is widely used to induce diabetes in various laboratory animals as it is particularly toxic to the pancreatic insulin-producing beta cells in mammals [21]; [22]. Aloxan and streptozotocin are the most prominent diabetogenic chemicals in diabetic research. Both are cytotoxic glucose analogues and their mechanism of beta cell selective action is identical [23]; [24]; [25]; [26].

The liver is an organ involved in many metabolic functions and is prone to xenobiotic induced injury because of its central role in xenobiotic metabolism [27]. Liver is also an important insulin-dependent tissue which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes [28]. The liver is an important site for insulin clearance and play an important role in maintaining normal glucose concentration in fasting and post prandial states. The liver is one of the tissues that bear the brunch of chronic hyperglycemia, since glucose is freely permeable to its cells (Mayes, 2000). This unrestricted entry, in the presence of excess and sustained glucose in blood, is bound to cause metabolic derangements which would express themselves on the gross architecture of the tissue.The kidney is an important organ in glucose homeostasis, which relies on the adequate production of insulin from pancreatic beta cells and action of insulin in peripheral tissue [29]. Both production of insulin and tissue sensitivity to insulin are impaired in the setting of chronic kidney diseases caused by diabetes [30].

MATERIALS AND METHODS

Collection of Biological Materials

The present study was carried out using the roots of *Sphenocentrum jollyanum* and albino rats and mice. Fresh roots of *Sphenocentrum jollyanum* were collected from Ovoko in Igbo-Eze South Local Government Area of Enugu State, Nigeria and was authenticated in the *Herbarium* Unit of Department of Botany, University of Nigeria, Nsukka by Mr O. Onyeukwu. Part of the authenticated plant was

deposited in the *herbarium* for reference purposes.

Eighty eight albino *wistar* rats and thirty two male albino mice were purchased from the Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were acclimatized for a period of two weeks at the animal house of the Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria prior to commencement of experiment. They were maintained at room temperature, 12hr day/night period and fed *ad libitum* on water and growers mash; weighed prior to commencement of experiment and daily till the end of the experiment.

Preparation of the Plant Extract

The roots of *Sphenocentrum jollyanum* were harvested and washed under tap water to remove contaminants and air dried under shade. They were pulverized using laboratory milling machine and sifted using 0.25 mm sieve. One thousand five hundred gram (1,500g) of the powdered root sample of *Sphenocentrum jollyanum* was soaked in 7500 ml of ethanol for 48 hours with agitation. The resulting ethanol root extract was filtered using muslin cloth and evaporated to dryness using rotary evaporator at a temperature of 45^{0}C. The concentrated ethanol root extract of *Sphenocentrumjollyanum*was used for subsequent analyses.

Fractionation of the Crude Extract of *Sphenocentrum jollyanum* Roots

The ethanol root extract of *Sphenocentrum jollyanum* (20 g) was fractionated in a glass column (150 cm x 1.5 cm) packed with 200 g of a slurry of silica gel G. (70-230 mesh). The column was eluted in succession with 500 ml ethyl acetate and 500 ml methanol to obtain ethyl acetate (EAF) and methanol (MF) fractions respectively. The resulting fractions were evaporated to dryness using rotary evaporator at a temperature of 45^{0}C. The concentrated ethyl acetate (EAF) and methanol root fractions of *Sphenocentrum jollyanum*were used for subsequent analyses.

Determination of Liver Function Status

Gamma-glutamyl transferase (γ-GGT)was determined as described by [31]. The aspartate transaminase, alanine transaminase, and ALP activity was determined using the method of [32]. Bilirubin was determined by the method of [33]. Serum total protein and albumin was determined by the method of [34].

Determination of Kidney FunctionStatus

Plasma Urea was determined using the method of [35]. Plasma creatinine was determined using the method of [35]. The plasma sodium and potassium levels were analyzed using flame emission spectrophotometric method of [56]. The determination of calcium ion was done by the method of [37]. Plasma Bicarbonate (HCO_3^-) was determined using [38] method. Plasma chloride was determined using [39].

Statistical Analysis

Results were expressed as mean± standard deviations where applicable. The data were subjected to one-way analysis of variance (ANOVA), followed by Post hoc Duncan multiple comparison test using SPSS software version 21 and $p < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Effect of Crude Ethanol Root-Extract and Fractions of *Sphenocentrum jollyanum* on Liver Function Parameters in STZ-induced Diabetic Albino Rats

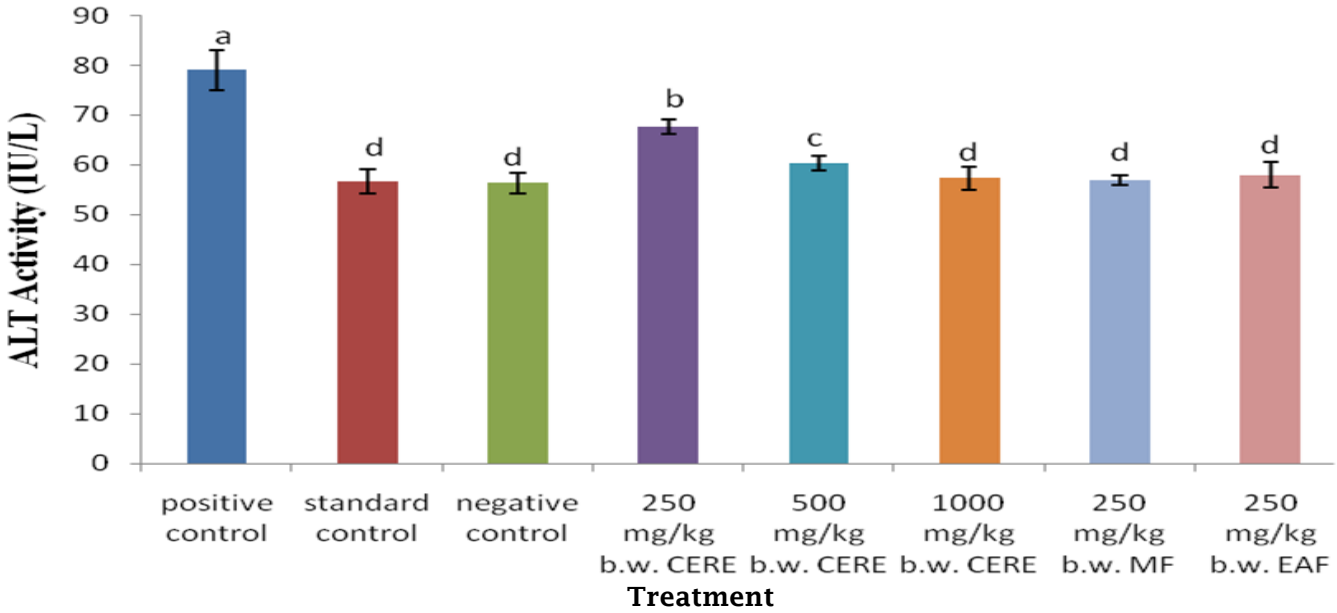

Figure 1: ALT activity in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are eshown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

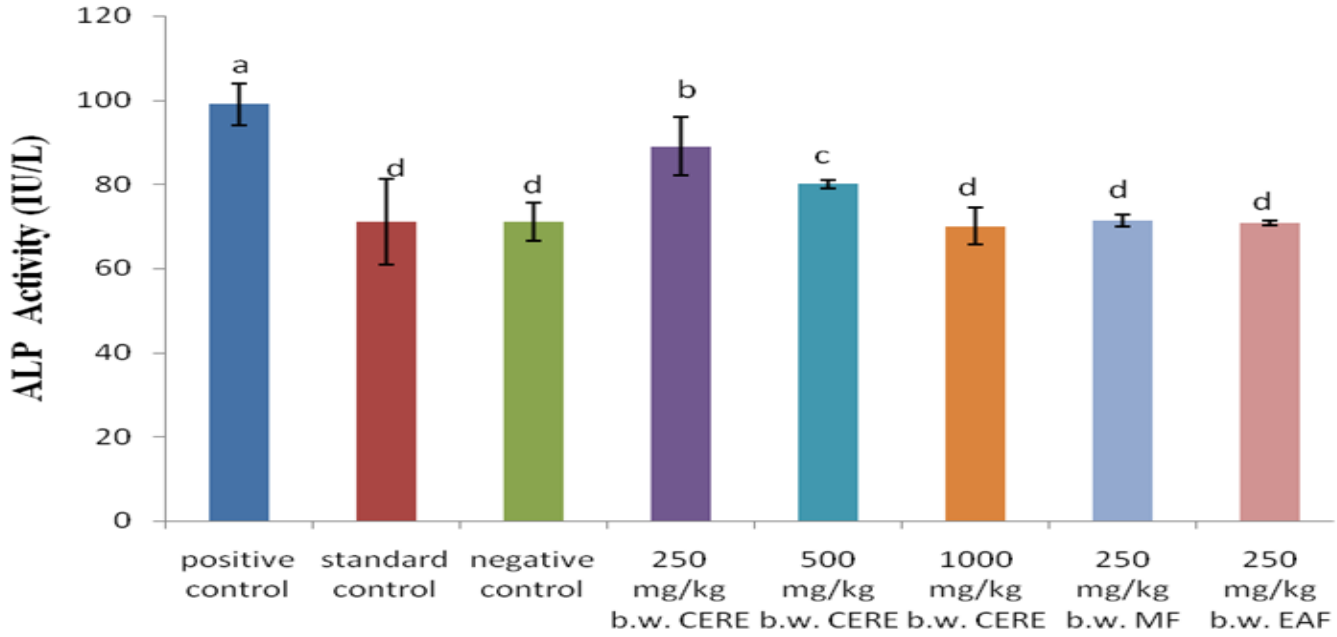

Figure 2: ALP Activity in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

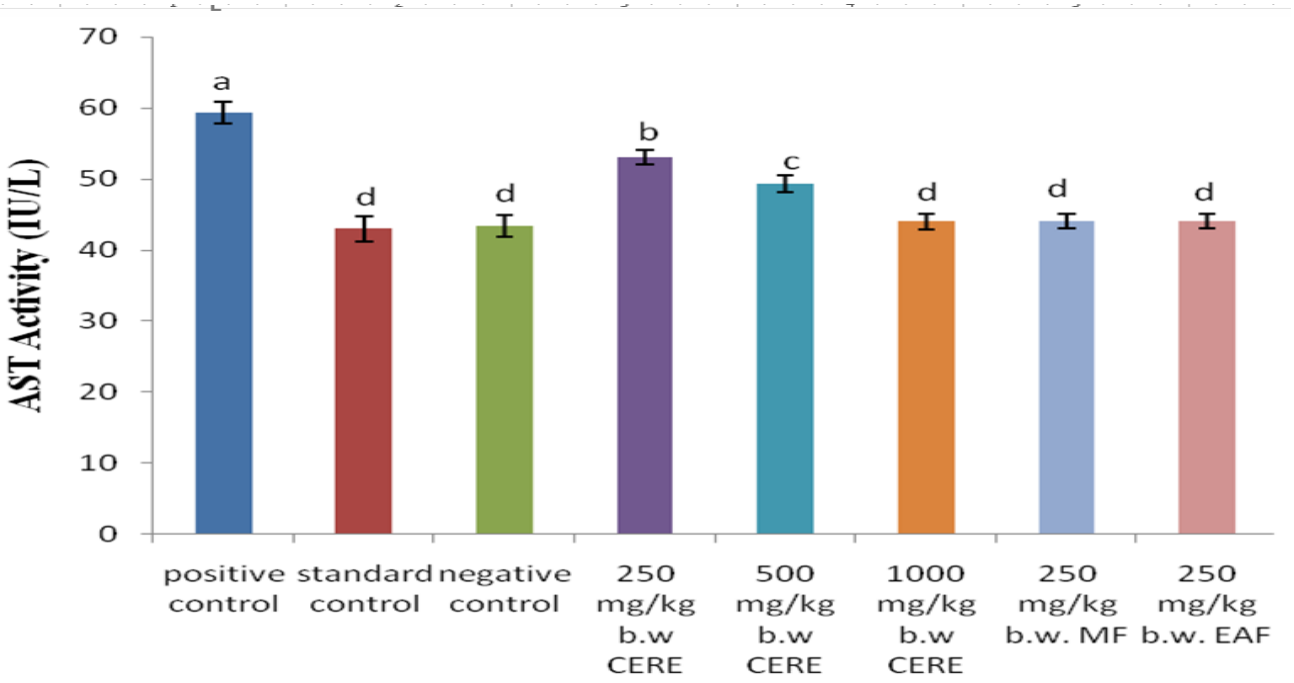

Figure 3: AST activity in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

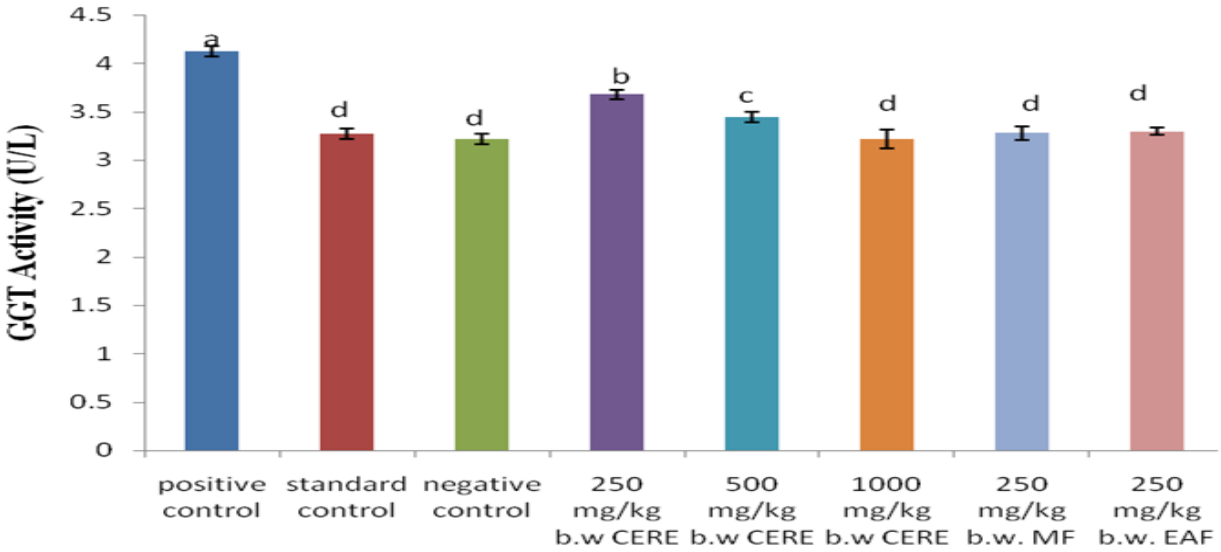

Figure 4: GGT activity in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

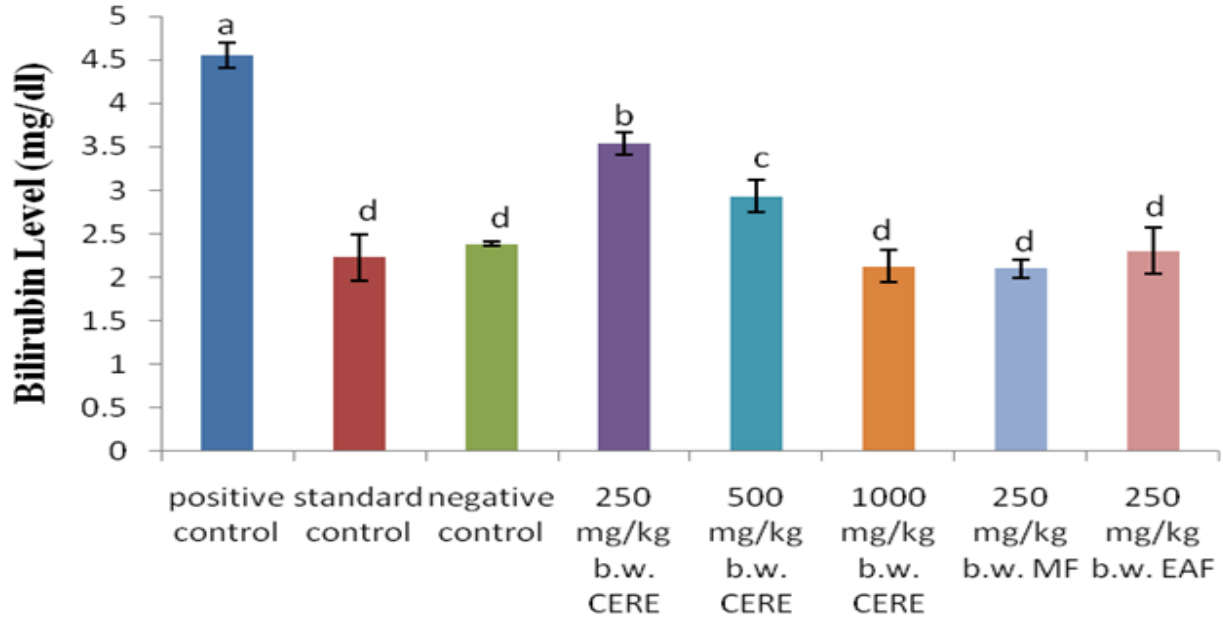

Figure 5: Bilirubin levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at $p<0.05$.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

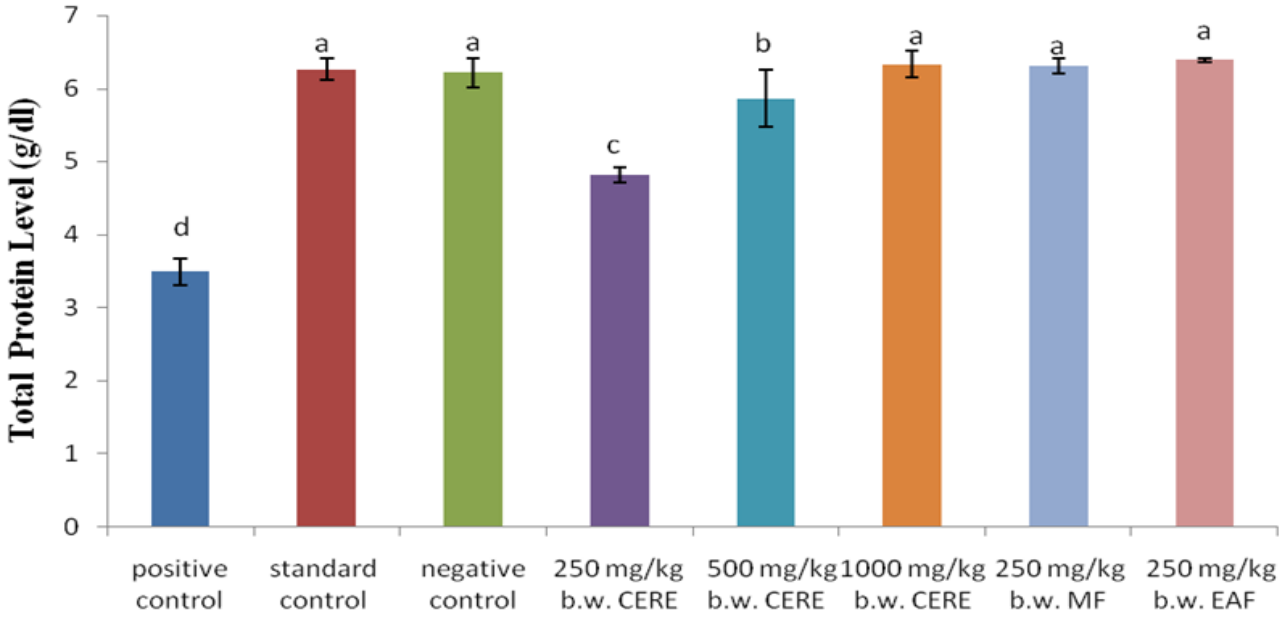

Figure 6: Total protein levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different Alphabet showed significant difference at $p<0.05$.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

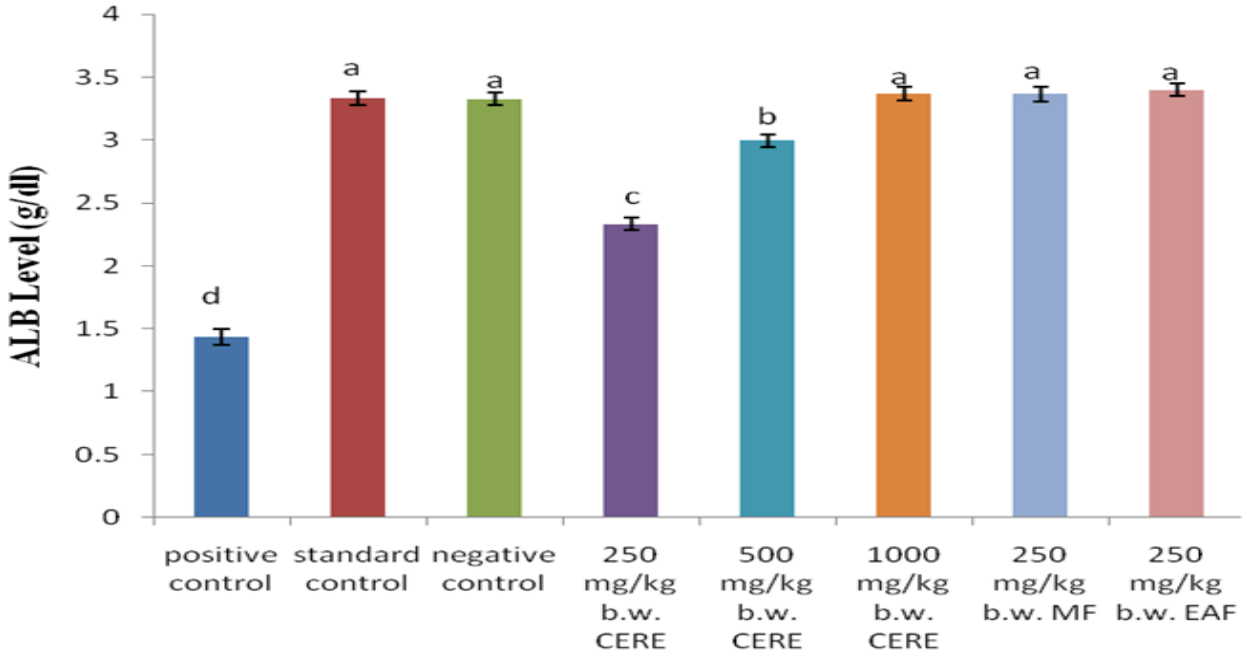

Figure 7: Albumin levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at $p<0.05$.

Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

Effect of Ethanol Crude Root-Extract and Fractions of *Sphenocentrum jollyanum* on Kidney Function Parameters in STZ-induced Diabetic Albino Rats

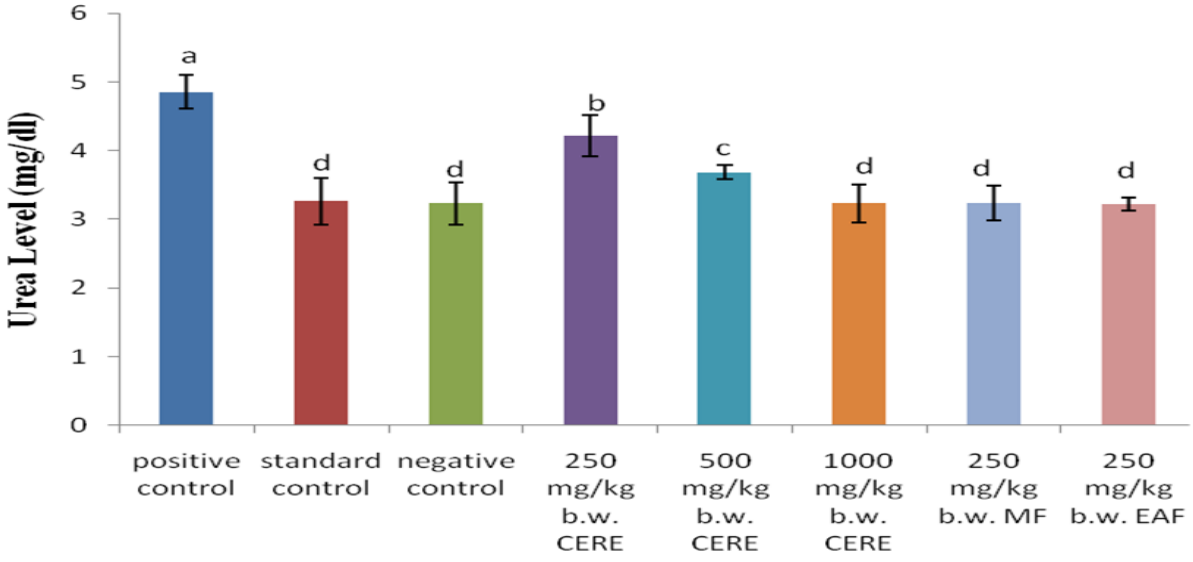

Figure 8: Urea levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=6). Mean values with different alphabet showed significant difference at $p<0.05$.

Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

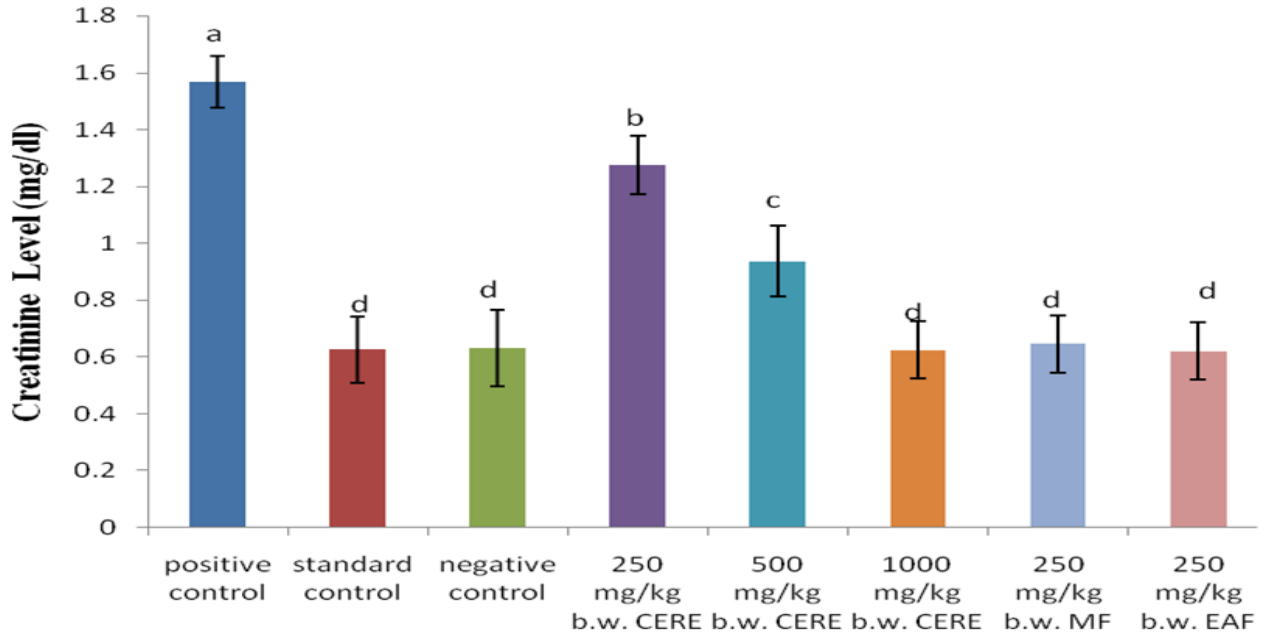

Figure 9: Creatinine levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and Fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction.

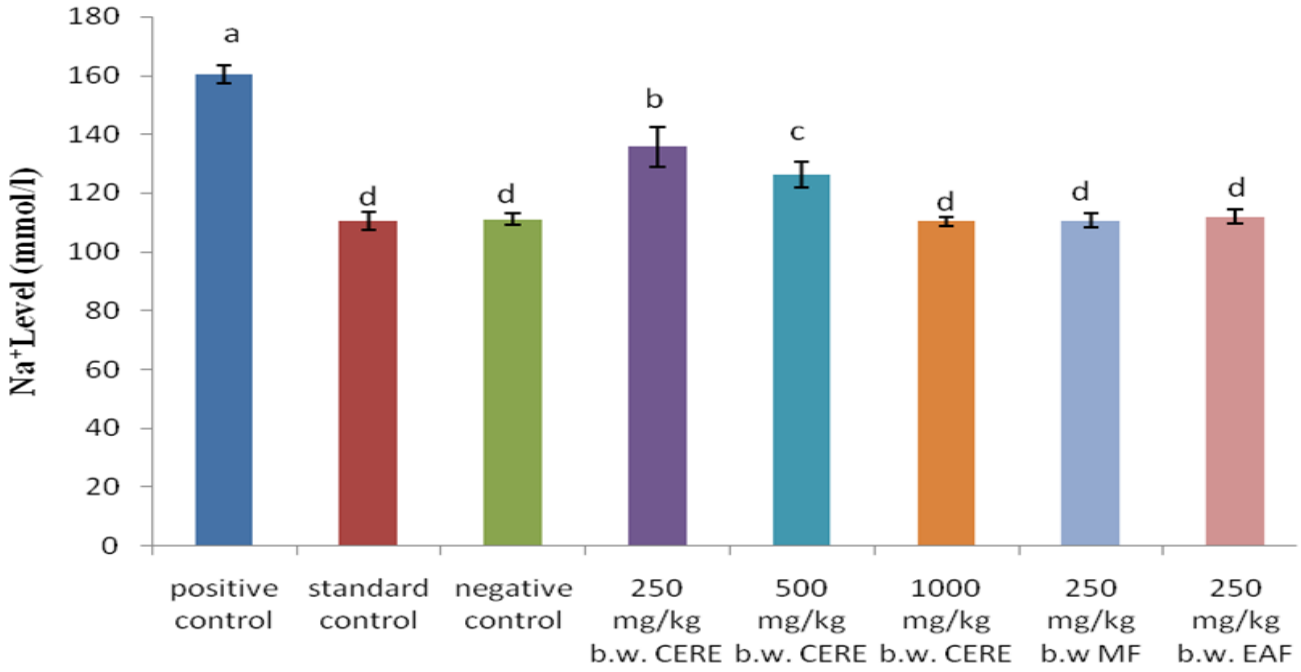

Figure 10: Sodium levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at p<0.05.

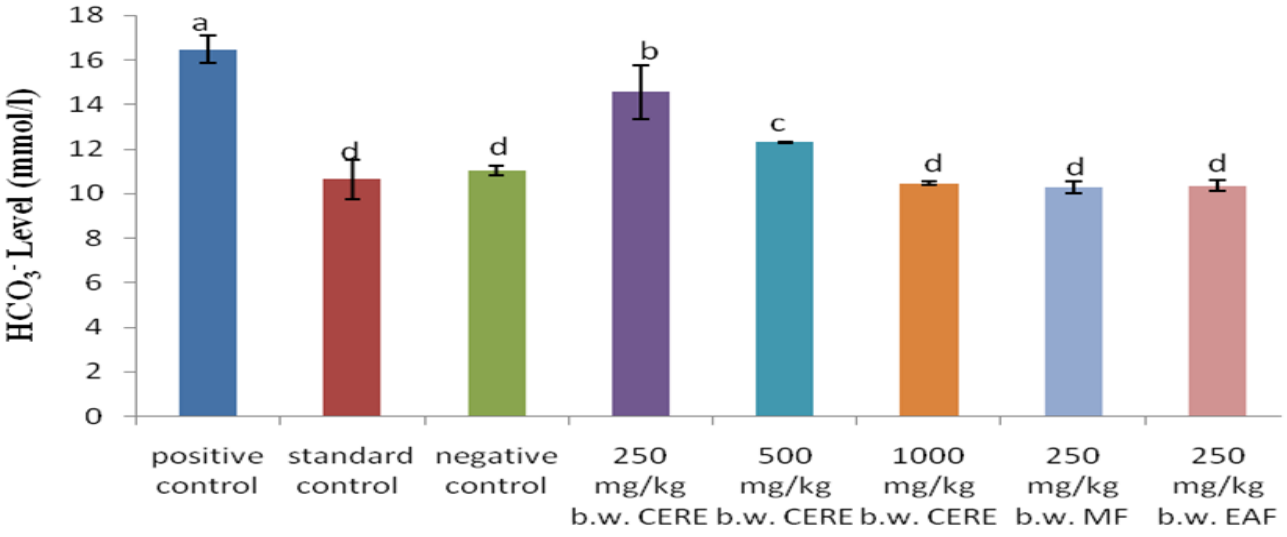

Figure 11: Bicarbonate levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different Alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

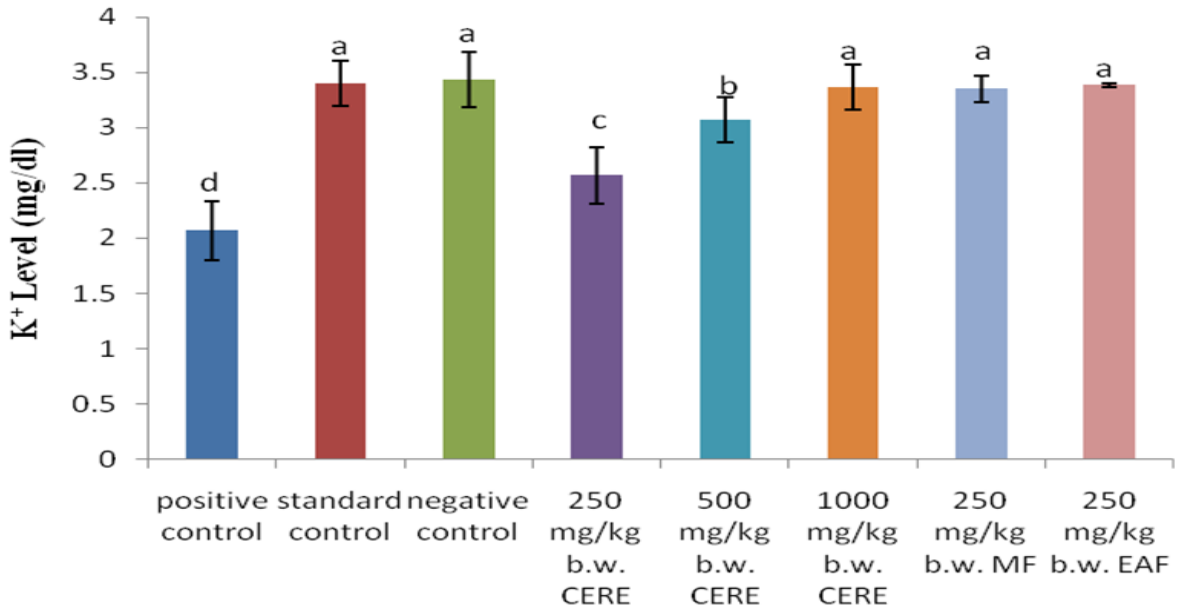

Figure 12: Potassium levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

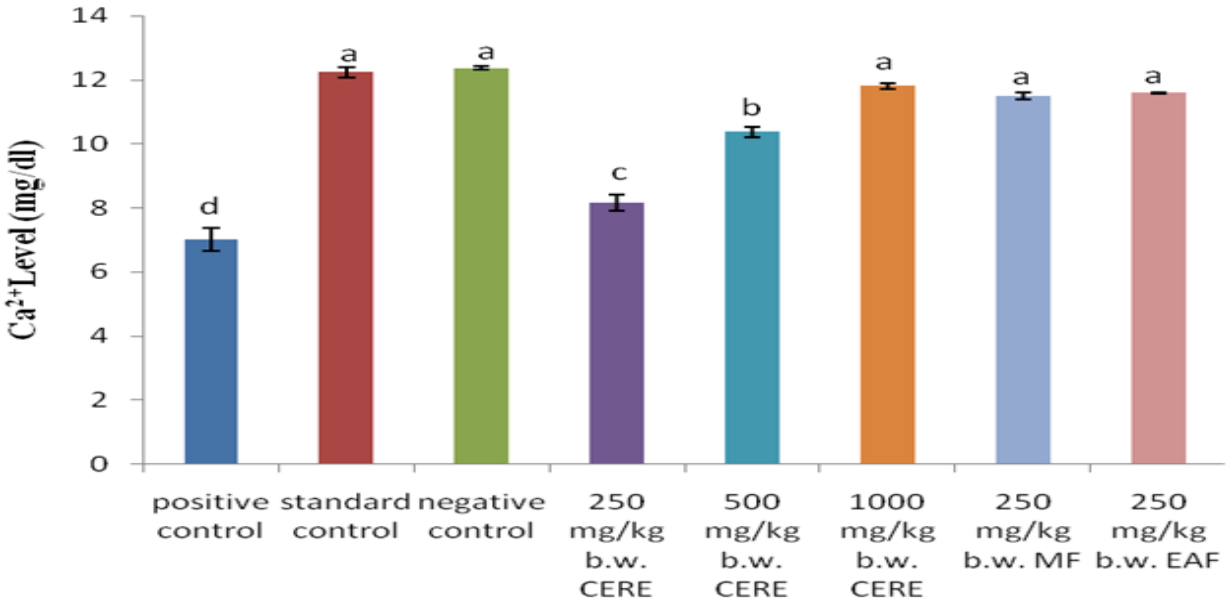

Figure 13: Calcium levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with difFerent alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

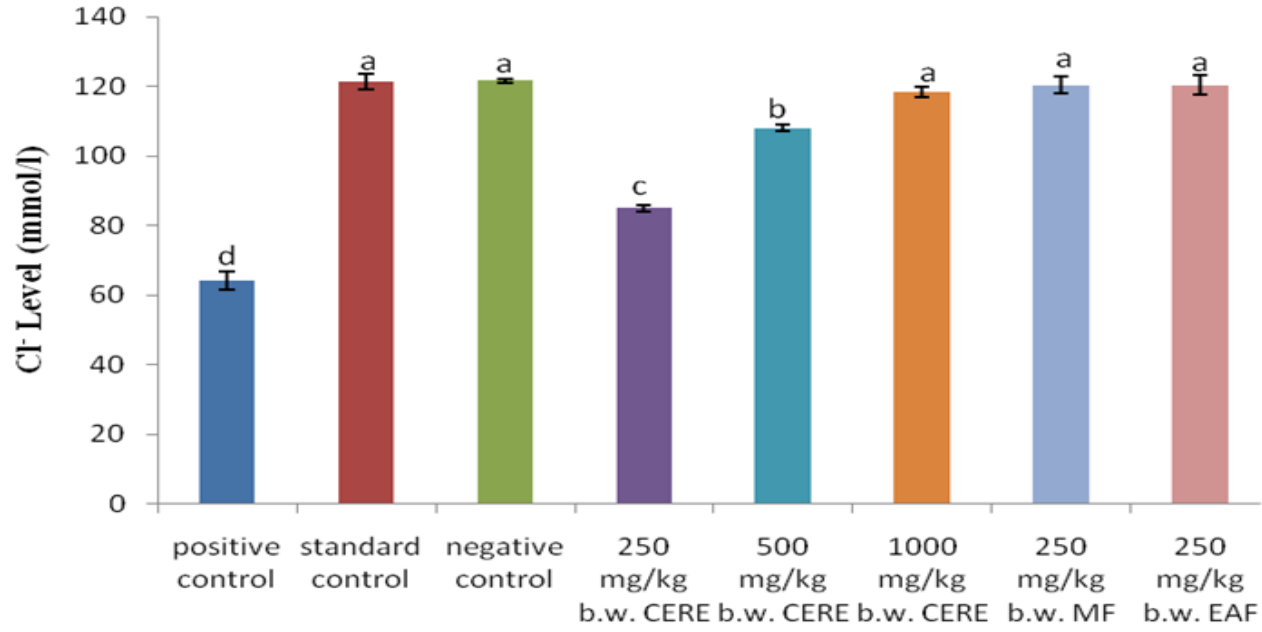

Figure 14: Chloride levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as Mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at p<0.05.
Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

DISCUSSION

Effect of Crude Ethanol Root-Extract and Fractions of *Sphenocentrum jollyanum* on Liver Function Parameters in STZ-induced Diabetic Albino Rats

The treatment of STZ induced diabetic albino with crude ethanol root-extract of *Sphenocentrum jollyanum* at doses of 250, 500 and 1000 mg/kg body weight and 250 mg/kg body weight of ethylacetate and methanol root fractions of *Sphenocentrum jollyanum* significantly ($p<0.05$) increased the levels of total protein and albumin relative to positive control as shown in Figures 6 and 7. The result also showed a significant ($p<0.05$) decrease in the levels of these parameters in the crude ethanol extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) changes relative to the fractions. The treatment of STZ-induced diabetic albino rats with crude ethanol root-extract at doses of 250, 500 and 1000 mg/kg body weight and 250 mg/kg body weight of ethylacetate and methanol root fractions significantly ($p<0.05$) decreased the level of bilirubin relative to positive control as shown in Figure 5. The result equally showed no significant ($p>0.05$) changes in the fractions relative to negative control and standard control. The effect on the crude extract were dose dependent and the value of the standard control showed no significant ($p>0.05$) difference relative to the negative control.

STZ-induced diabetic albino rats treated with crude ethanol root-extract at doses of 250, 500 and 1000 mg/kg body weights and 250 mg/kg body weights of ethylacetate and methanol root fractions significantly ($p<0.05$) decreased the activities of ALT, ALP, AST and GGT relative to the positive control as shown in Figures 1, 2, 3, and 4. The result also showed a significant ($p<0.05$) increase in the crude extract relative to fractions except at the dose of 1000 mg/kg body weight of crude extract which showed no significant ($p>0.05$) changes with the fractions. The result too, showed no significant ($p<0.05$) differences in the treated group relative to the negative and standard control except at the doses of 250 and 500 mg/kg body weights which showed significant ($p<0.05$) increase relative to other groups. The effects on the crude extract was dose dependence and the value for the standard drug was quite similar with that of the negative control as shown in Figures 1-7.

Reduction in plasma total protein and albumin levels were observed in diabetic rats in this study and this is consistent with the results obtained by [40], [41] which showed that total protein decreased significantly ($p<0.05$) in children suffering from insulin dependent diabetes. The significant decrease ($p<0.05$) in protein and albumin levels may be due to microproteinuria and albuminuria which are important clinical markers of diabetic nephropathy [42] and/or may be due to increased protein catabolism [43]. The results of the present study demonstrated that the treatment of diabetic rats with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* caused a significant increase in the plasma total protein and albumin levels as compared with the rats that were induced with diabetes without treatment (positive control) and this is in agreement with the work of [44] who observed that on the treatment of STZ-induced diabetic rats with 60 mg/kg body weight of *Equisetum arvense* siginificantly increased ($p<0.05$) albumin of male albino rats. Such improvement of serum protein and albumin were equally previously observed after the oral administration of *Balanites aegyptiaca* to experimental diabetic rats [45]; [46].

Elevated levels of serum enzymes and indices, such as ALT, AST, ALP, GGT and bilirubin are well known markers of hepatic damage; the activities of these enzymes (ALT, AST, ALP, GGT) are high in the blood stream as a consequence of damage to hepatic tissue [12]. In the present study, the treatment of the STZ-induced diabetic rats with *Sphenocentrum jollyanum* crude ethanol root extract and fractions significantly ($p<0.05$) reduced the activity of these enzymes and bilirubin levels compared to the values obtained from untreated diabetic group

(positive control). This is consistent with the work of [22] who treated diabetic rats with aqueous ethanolic extract of *Hibiscus rosasinensis* and showed that the extract and fractions were able to decrease the activities of serum liver enzymes when compared to the diabetic untreated group (positive control).

Bilirubin is formed by the breakdown of RBC's in the spleen, liver and bone marrow [27]. Small amount of bilirubin circulates in the plasma loosely bound to albumin which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid which forms a soluble compound. This is referred to a direct bilirubin. Elevated levels are found in hepatitis, cirrhosis, haemolytic jaundice, obstruction of biliary tract and drug induced reactions [29]. Bilirubin is produced by the liver, therefore, interference with the normal liver function affects its rate of conjugation and excretion. Thus levels of bilirubin is used as one of the indices of liver function [34]. The present study showed a significant increase ($p<0.05$) in total bilirubin in diabetic untreated group (positive control). These levels are however, reduced on treatment of STZ-induced diabetic rats with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* suggesting the enhancement of liver functions by the crude ethanol root extract and fractions and this was comparable to standard drug, glibenclamide. This is consistent with similar findings on the liver by [37].

Effect of ethanol crude root-extract and fractions of *Sphenocentrum jollyanum* on kidney function indices in STZ-induced diabetic albino rats

The treatment of STZ-induced diabetic albino rats with crude ethanol root-extract of *Sphenocentrum jollyanum* at doses of 250, 500 and 1000 mg/kg body weights and 250 mg/kg body weights of methanol and ethylacetate root fractions of *Sphenocentrum jollyanum* significantly ($p<0.05$) decreased the level of urea, creatinine, Na^+ and HCO_3^- as shown in Figures 8, 9, 10 and 11. The crude ethanol root extract and fractions significantly ($p<0.05$) increased the level of K^+, Ca^{2+} and Cl^- relative to positive control as shown in Figures 12, 13 and 14. The result also showed no significant ($p>0.05$) changes in the values of urea, creatinine, Na^+ and HCO_3^- in the crude extract relative to fractions except at the dose of 250 and 500 mg/kg body weights as shown in Figures 8, 9, 10 and 11. The result equally showed a significant ($p<0.05$) decrease in the levels of Ca^{2+} and Cl^- in crude extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) changes relative to the fractions. The effect on the crude extract was dose dependent and the value for standard control showed no significant ($p<0.05$) changes relative to the negative control as shown in Figures 8-14.

The kidney is an important organ in glucose homeostasis, which relies on the adequate production of insulin from pancreatic beta cells and action of insulin in peripheral tissues [40]. Both production of insulin and tissue sensitivity to insulin are impaired in the setting of chronic kidney diseases caused by diabetes [42]. Elevated levels of urea and creatinine are usually seen in renal damage and injury [44]. The treatment of STZ-induced diabetic albino rats with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* decreased the plasma levels of urea and creatinine compared with that of diabetic untreated group (positive control). This is consistent with the work of [45], [46] who treated diabetic rats with 100mg/kg body weight of ethanol extract of *Momordica dioica* fruits and 100mg/kg body weight of ethanol extract of *Merremia emarginata Burm* F. respectively and reported a decrease in the plasma levels of urea and creatinine in diabetic rats treated with the fruit and root extract compared with the diabetic untreated group. The results from this present study showed that the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* have ameliorated the effect of diabetes on the renal indices of urea and creatinine and decreased these levels in diabetic rats treated with the extract and fractions which is comparable with that of the non-diabetic rats (negative control)

and rats treated with glibenclamide (standard control) indicating nephroprotective effects of the crude ethanol root extract and fractions of *Sphenocentrum jollyanum*.

Electrolyte imbalance in diabetes is primarily as a result of elevated blood glucose [2]. During hyperglycemia, the body tries to get rid of the excess blood glucose by increasing urinary output [5]. Increased urination lead to electrolytes loss, that upsets the body's balance of electrolytes. The balance is especially disturbed between sodium and potassium [9]. Treatment of STZ-induced diabetic rats with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* decreased the plasma levels of Na^+ and HCO_3^- when compared with diabetic untreated group (positive control). Also, the result of the present research shows that the crude ethanol root extract and fractions have ameliorating effect on diabetes induced reduction on Na^+ and HCO_3^- levels and as a result restored them to the same level comparable with that of the non diabetic rats (negative control) and diabetic rats treated with glibenclamide (standard control). This is consistent with the work of [15] who reported that treating diabetic rats with *Allium cepa* supplemented diet restored the plasma electrolytes. Also treatment of STZ-induced diabetic rats with the extract and fractions of *Sphenocentrum jollyanum* increased the plasma levels of potassium, calcium and chloride when compared to the diabetic untreated rats (positive control). The result also shows that the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* has ameliorating effect on diabetes induced reduction on potassium, calcium and chloride levels thereby restoring them to the same level comparable with that of the non diabetic rats (negative control) and diabetic rats treated with glibenclamide (standard control). This is in agreement with the work of [19] who reported that treating diabetic rats with *Allium cepa* supplemented diet restored the plasma electrolytes.

REFERENCES

1. Akintobi, O. A., Adejuwon, A. O., Bamkefa, B. A.,Daniels, O. V. C and Ojo, V. O. (2013). Antimicrobial potency of *Sphenocentrum jollyanum* on some human pathogenic bacteria. *Academia Arena,* **5**(5): 1 -7.
2. Alese, M. O., Adewole, O. S., Ijomone, O. M., Ajayi, S. A. and Alese, O. O. (2014). Hypoglycemic and hypolipidemic activities of rnethanolic extract of *Sphenocentrum jollyanum* on streptozotocin-induced diabetic wistar rats, *European Journal of Medicinal Plants,* **4**(3):353-364.
3. Almdal, T. P. and Vilstrup, H. (1988). Strict insulin therapy normalises organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. *Diabetlogia*, **31:** 114-118.
4. Amidu, N., Woode, E., Owiredu, K. B. A., W. and Asare, A. G. (2008). An evaluation of toxicity and multagenicity *of Sphenocentrum jollyanum. International Journal of Pharmacology,* **4:** 67-77.
5. Anandakirouchenane, E., Chandiran, I. S., Kanimozhi, V. and Kadalmani, B. (2013). Antioxidant and protective effect of *Curculigo orchioides* on liver, pancreas and kidney tissue in alloxan-induced diabetic experimental rats. *Drug Invention Today*, **5:** 192-200.
6. Bakris, G. L. (1993). Diabetic nephropathy. What you need to know to preserve kidney function. *Postgraduate Medicals*, **93:** 89-100.
7. Bartels, H. and Bohmer, M. (1972). Serum creatinine determination without protein precipitation. *Clinica Chimica Acta*.**37:**193-197.
8. Davidson, I. and Henry, J. B. (1979). Clinical Diagnosis by laboratory method, 1st edition. ELBS New York. Pp: 340-500.
9. Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971). Albumin standards and determination of serum albumin with bromocresol

green. *Clinica Chimica Acta*.**31:**87-96.
10. Faulker, W. R and Meister, S. (1982). Selected Methods for the Small Clinical Chemistry, Washington D.C. p. 125.
11. Iwu, M. M. (1993). Handbook of African Medicinal Plants. CRC Press Incorporation. Pp. 239-245.
12. Jack, D. (2003). Overview of the anti-diabetic agents. *Endocrine Pharmacotherapy Module, Spring*,**7:** 1-33.
13. Jendrassik, L. and Grof, P. (1938). Colorimetric measurement of bilirubin. *Biochemistry,* **297:** 81-83.
14. Koleosho, A. T., Jose, A. R., Oyibo, P. G., Roland, M. A. and Uloko, M. E. (2013). Antimicrobial activity of *Sphenocentrum jollyanum* and *Mangifera indica linn* on *Salmonella typhi*, *IOSR Journal of Pharmacy and Biological Sciences,* **5** (3): 50-54.
15. Koppe, L., Pelletier, C. C. and Alix, P. M. (2014). Insulin resistance in chronic kidney disease: new lessons from experimental models. *Nephrol Dial Transplant*,1666-1674.
16. Kremer, A. E., Elferink, R. P. and Beuers, U. (2011). Pathophysiology and current management of pruritus in liver disease. *Clinical Research Hepatology Gastroenterology*, **35:** 89-97.
17. Lenzen, S. (2008). The mechanisms of alloxan and streptozotocin-induced diabetes. *Diabetologia,* **51:** 216-226.
18. Lorenzo, C. , Nath, S. D. and Hanley, A.J. (2009). Risk of type 2 diabetes among individuals with high and low glomerular filtration rates. *Diabetologia,* **52:** 1290-1297.
19. Mansour, H. A. and Newairy, A. A. (2000). Amelioration of impaired renal function associated with diabetes by *Balanites aegyptiaca* fruits in streptozotocin-induced diabetic rats. *Journal of Medical Research Institute,***21:** 115-125.
20. Maruthappan, V. G. and Shree, K. S. (2010). Blood cholesterol lowering effect of *Adenanthera pavonia* seed extraction atherogenic diet induced hyperlipidemia rats. *International Journal of Pharmaceutical Science Research*, **1:** 87-94.
21. Matsuada, H., Tomolino, N. M., Yasuko, G. and Kubo, M. (2011). Anti-allerdgic effects of *Crudii monnieri. Osthology of Biological Phytochemistry,* **25** (6): 809-812.
22. Mauer, S. M., Steffes, M. W. and Brown, D. M. (1981). The kidney in diabetes. *American Journal of Medicals*, **70:** 603-612.
23. Mayes, P. A. (2000). The Pentose Phosphate Pathway and other Pathway of Hexose Metabolism. In: Herper's Biochemistry, Murray, R.K., D.K. Granner and V.W. Mayes (Edition.). McGraw-Hill, USA., Pp: 219-237.
24. Mbaka, G. O., Adeyemi, O. O. and Adesina, S. A. (2010). Anti-diabetic activity of the seed extract of *Sphenocentrum jollyanum* and morphological changes on pancreatic beta cells in alloxan-induced diabetic rabbits. *Journal of Medicine and Medical Science,* **1:** 550-556.
25. Muko, K. N., Ohiri, P. C. and Ezegwu, C. O. (1998). Antipryetic and analgesic activities of *Sphenocentrum jollyanum. NigerianJournal of Natural Product Medicine,* **2:**52-53.
26. Nia, R., Paper, D. H., Essien, E. E., Lyadi, K. C., Bassey, A. I. L., Anti, A. B. and Franz, G. (2004). Evaluation of the anti-oxidant and anti-angiogenic effects *of Sphenocentrum jollyanum* Pierre. *African Journal of Biomedical Research,* **7:** 129-132.
27. Olubunmi, G. A. and Adeyemi, S. A. (2015). Effect of Allium cepa supplemented diet on plasma glucose electrolytes and renal histology of streptozotocin-induced diabetic rats. *IOSR Journal of Pharmacy and Biological Sciences*, **10**(4):25-32.
28. Österreicher, C. H. and Trauner, M. (2012). Xenobiotic-induced liver

injury and fibrosis. *Expert Opinion on Drug Metabolism and Toxicology,***8**(5): 571-80.
29. Owiredu, W. K. B. A., Nafiv, A., Amissah, F. and Woode, E. (2007). The effects of the ethanolic extract of root *of Sphenocentrum jollyanum* Pierre on sexual behaviour-and hormonal levels in rodents. *Journal of Science and Technology,* **27**(2): 9-20.
30. Rajesh, M. and Sreenivas, S. A. (2011). Anti-Diabetic effects of aqueous ethanolic extract of *Hibiscus rosasinensis L.*on Streptozotocin-induced diabetic rats and the possible morphologic changes in the liver and kidney. *International Journal of Pharmacology*, **7:** 363-369.
31. Rajiv, G. G. and Sasikumar, P. (2012). Antidiabetic effect of *Merremia emarginata* Burm F. in Streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicals*, **2**(4): 281-286.
32. Rambir, S. and Aarti, S. and Poonam, S. (2011). Hypoglycemic, antidiabetic and toxicological evaluation of Momordica dioica fruit extracts in alloxan induced diabetic rats. *Journal of Pharmacology and Toxicology*, **6:** 454-467.
33. Reitman, S. and Frankel, S. (1957). Alanine aminotransferase measurement. *American Journal of Clinical Pathology,* **28:** 56-58.
34. Safiyeh, S., Fathallah, F., Vahid, N., Habib, S. S. and Nabat, N. (2007). Effect of *Equisetum arvense L.* (*Equisetaceae*) in microalbuminuria and creatinine excretion in streptozotocin-induced diabetes in male rats. *International Journal of Pharmacology*, **3:** 155-159.
35. Schales, O. and Schales, S. S. (1941). A simple and accurate method for the determination of chloride in biological fluids. *The Journal of Biological Chemistry,***140**: 879-884.
36. Shin, H. J., Kim, H., Yi, J. H., Han, S. W. and Kim, H. J. (2012). Polyuria with the concurrent manifestation of central diabetes insipidus (CDI) and type 2 diabetes mellitus. *Electrolyte and Blood Pressure*, **10** (1): 26-30.
37. Skyler, J. S. (2004). Diabetesmellitus: pathogenesis and treatment strategies. *Journal of Medical Chemistry,* **47:** 4311-4115.
38. Szasz, G. (1969). A kinetic photometric method for serum gamma-glutamyl transpeptidase. *Clinical Chemistry,***22:** 124-136.
39. Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in the β-cells of the Rat pancreas. *Physiological Research,* **50:** 536-546.
40. Tietz, N.W., Pruden, L.E. and Andersen, S. (1996).Electrolytes In: Tietz Fundamentals of Clinical Chemistry, Tietz N.W. (ed.) (2nd edn). W.B. Saunders Co. Philadelphia USA: 721-738.
41. Tuvemo, T., Ewald, U., Kobbah, M. and Proos, L. A. (1997). Serum magnesium and protein concentrations during the first five years of insulin-dependent diabetes in children. *Acta Paediatrica*, **86:** 7-10.
42. Usha, K., Kasturi, G. M. and Hemalatha, P. (2007). Hepatoprotec tive effect of *Hygrophila spinosa* and *Cassia occidentalis* on carbon tetrachloride induced liver damage in experimental rats. *Indian Journal of Clinical Biochemistry*, **22:** 132-135.
43. Weatherburn, M. W. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry,* **39**(8): 971-974.
44. Weiss, R. B. (1982), Streptozotocin; A review of its pharmacology, efficacy and toxicity. *Cancer Treatment and Reproduction,* **66:** 427-438.
45. Woode, E., Amidu, N., William, K. B. A., Ansah, C. and Duwiejva, M. (2009). Anxiogenic-like effects of

root extract of *Sphenocentrum jollyanum* Pierre in Murine behavioral models. *Journal of Pharmacology and Toxicology,* **4**(3): 91-106.

.

46. Yusuf, A., Olusola, A., Emmanuel, O., Bernard, U. and Olusegun, D. (2012). *Allium cepa* protects renal functions in diabetic rabbit, *World Journal of Life Science and Medical Research*, **2**(2): 86-90