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The effects of the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* on hematological indices and glycosylated haemoglobin of streptozotocin-induced diabetic wistar albino rats.

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ABSTRACT

The effects of ethanol root-extract and fractions of *Sphenocentrum jollyanum* (SJ) on hematological indices and glycosylated haemoglobin of streptozotocin (STZ) induced diabetic Wistar albino rats were carried out with a total of 48 albino rats. 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 hematological indices and glycosylated haemoglobin were determined using standard laboratory procedures. 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 weight of ethylacetate and methanol root fractions of *Sphenocentrum jollyanum* significantly ($p<0.05$) increased the level of Hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), platelets, neutrophil count, eosinophil count, monocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) when compared with the positive control. However, the treatment of STZ-induced diabetic albino rats significantly ($p<0.05$) decreased the level of WBC, lymphocytes, basophil and glycaeted haemoglobin relative to positive control. The results of this study indicated that the crude ethanol root extract and fraction did not exhibit any form of haematological toxicity, as statistical evaluation did not show any significant difference ($P>0.05$) between the values of the haematological indices studied in the rats fed with the crude ethanol root extract and fractions when compared to the rats that were not induced with diabetes.

Keywords: *Sphenocentrum jollyanum,* hematological indices, glycosylated haemoglobin, albino rats

INTRODUCTION

Herbal medicines are the use of plant for therapeutic purposes. They are great body balancers and tonics that help in regulating some body functions. It can be used to support metabolic processes of the body and offer some other nutrients that the body fails to receive due to poor diet or environmental deficiencies in the soil [1, 2, 3].

Sphenocentrum jollyanum (SJ) Pierre (*Menispermaceae*) is a rain forest plant, an under growth of dense forest which grows naturally along the west coast sub region of Africa [4, 5, 6]. The plant is deep rooted

and bears fruit that is yellowish in colour when ripe. SJ has been reported to possess wide spectrum of pharmacological activities. Its medicinal importance was first highlighted by Dalziel (1956) [5] in which it was noted that the leaves decoctions were used as vermifuge. It is widely used in dressing wounds especially chronic wounds, treatment of feverish conditions and cough, as well as being an aphrodisiac [7, 8, 9, 10, 11]. Studies have shown the seed to possess significant antipyretic and analgesic activities [12, 13, 14, 15, 16, 17]. Investigations have also revealed that the seed exhibited significant antioxidant [18, 19, 20] and anti-inflammatory properties [21]. The leaf and the root of SJ have equally been shown to possess haematinic property [22]. In recent years, the popularity of traditional medicine in the area of metabolic disorder has increased due to the number of increasing available scientific data [7]. Diabetes mellitus can be treated with physical exercise, diet and medicinal plants. Also, plant extracts are equally considered to be less toxic and freer from side effects than synthetic drugs hence making the use of herbs to triple over the last 10 years [13].

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 [1] and [19]. 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 [1], [7].

MATERIALS AND METHODS

Collection of Biological Materials

The present study was carried out using the roots of *Sphenocentrumjollyanum* 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. Forty eight albino *wistar* rats 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 *Sphenocentrum jollyanum* 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°C. The concentrated ethyl acetate (EAF) and methanol root fractions of *Sphenocentrum jollyanum* were used for subsequent analyses.

Determination of Hematological Parameters

Hemoglobin concentration, packed cell volume,total erythrocyte, total leucocyte count, white blood cell differential counts, and platelet countwere determined by the method of Ochei and Kolhatkar (2008). Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were determined theoretically using different formulas:

$$MCV = \frac{PCV \times 10}{RBC}$$

$$MCH = \frac{Hemoglobin \times 10}{Red\ blood\ cell\ count}$$

$$MCHC = \frac{Hemoglobin \times 100}{PCV}$$

Glycosylated hemoglobin was determined as described by Bunn (1981) [4].

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 Haematological Indices in STZ-induced Diabetic Albino Rats

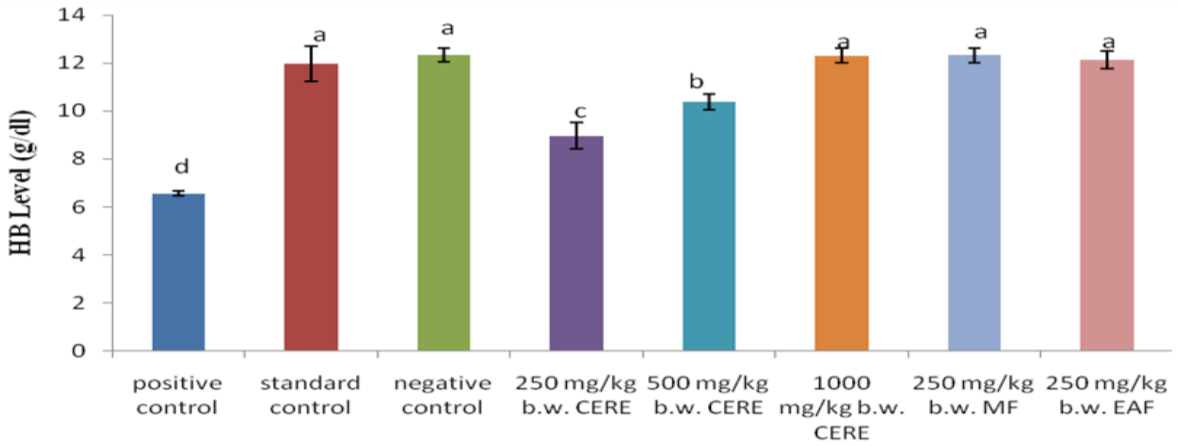

Figure 1: Haemoglobin levels in STZ induced diabetic wistar albino rats Treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as Mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.**Key:** CERE=Crude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

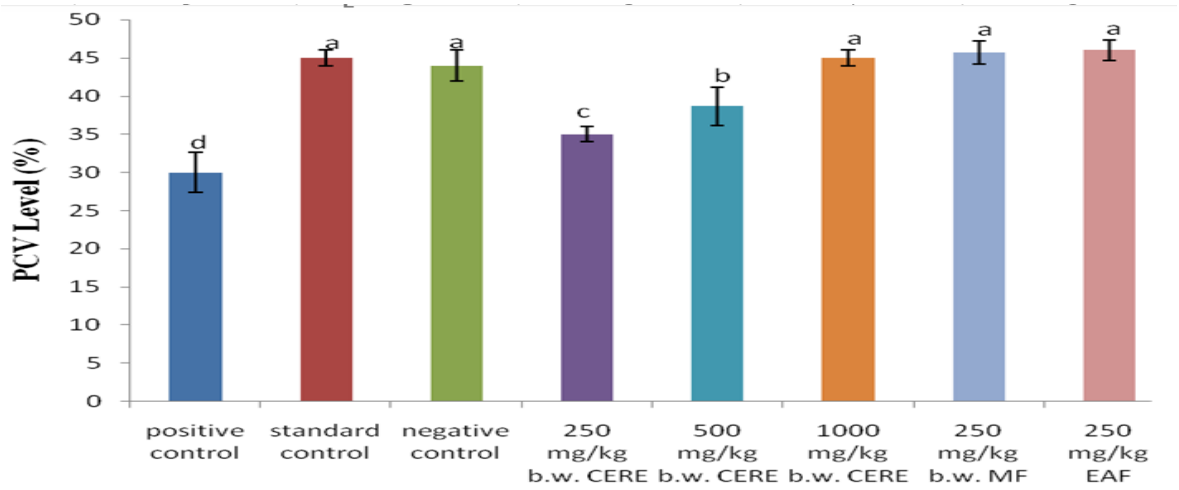

Figure 2: PCV levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as Mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.

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

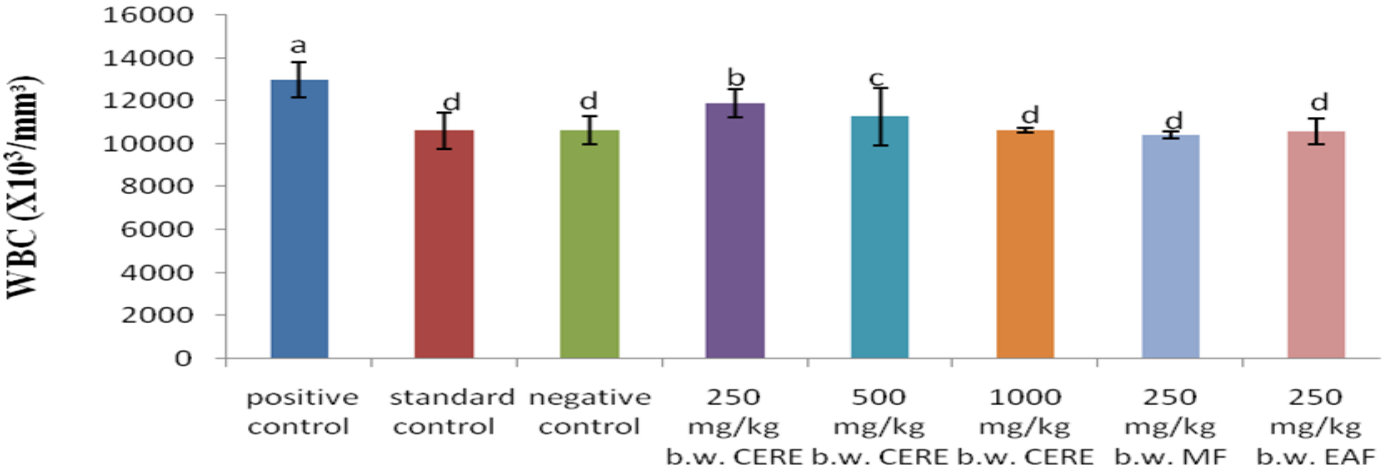

Figure 3: WBC count in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05. **Key**: CERE=Crude ethanol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

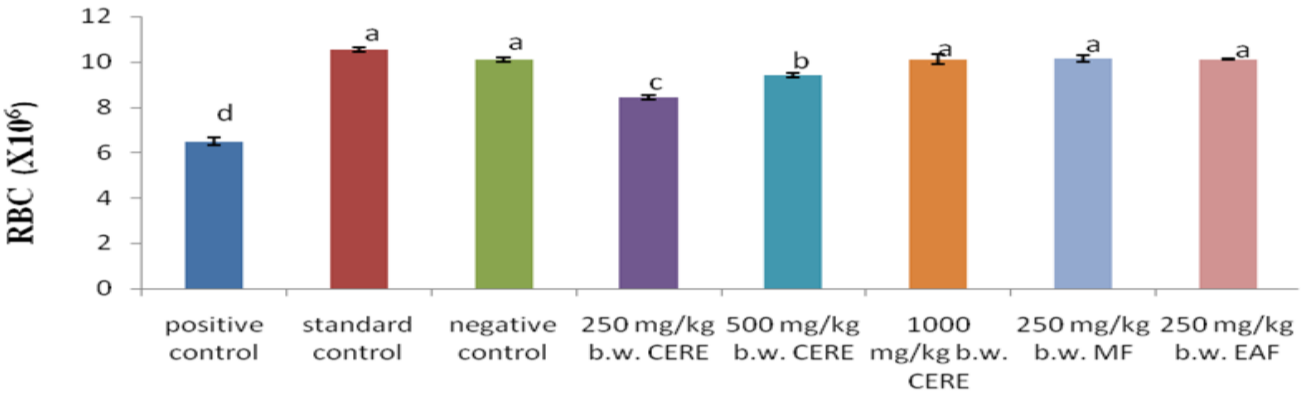

Figure 4: RBC levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.

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

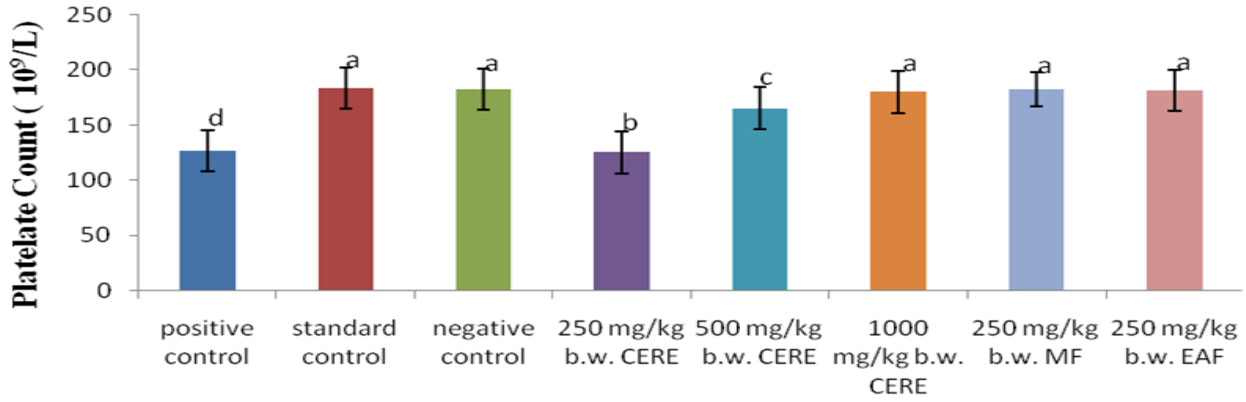

Figure 5: Platelate levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.**Key**:CERE=Crude ethanol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

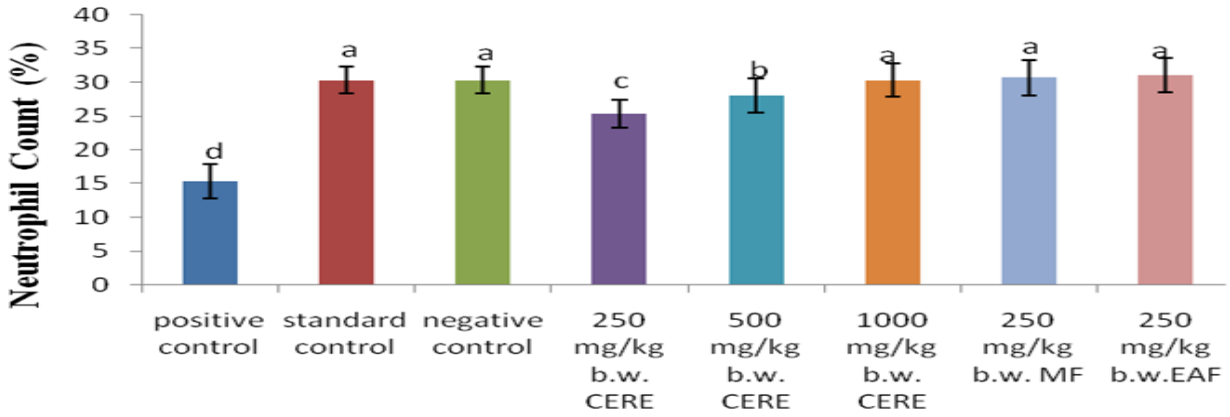

Figure 6: Neutrophil levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and Fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05. **Key**: CERE=Crude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

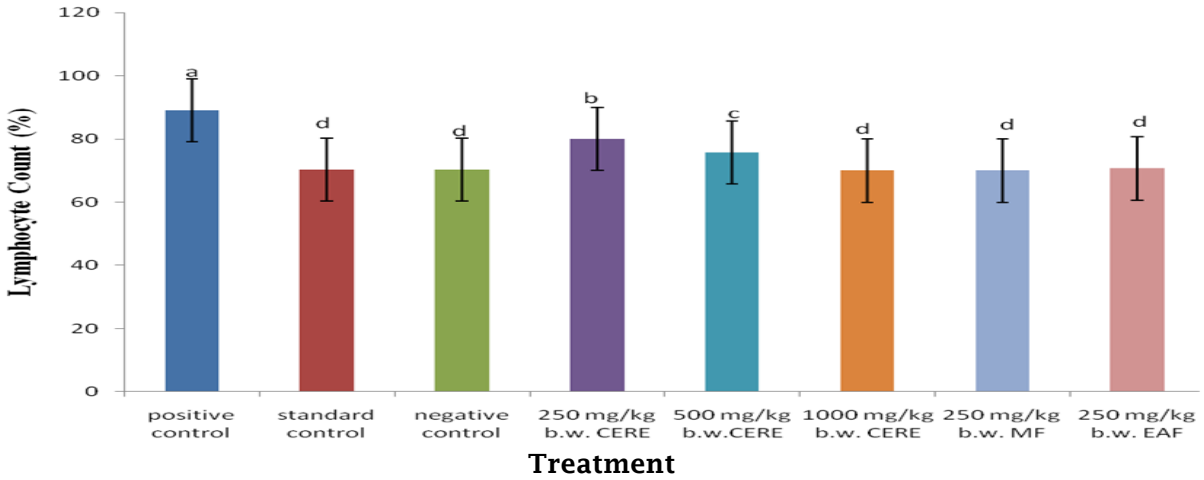

Figure 7: Lymphocyte levels in STZ-induced wistar diabetic albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05. **Key**: CERE=Crude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

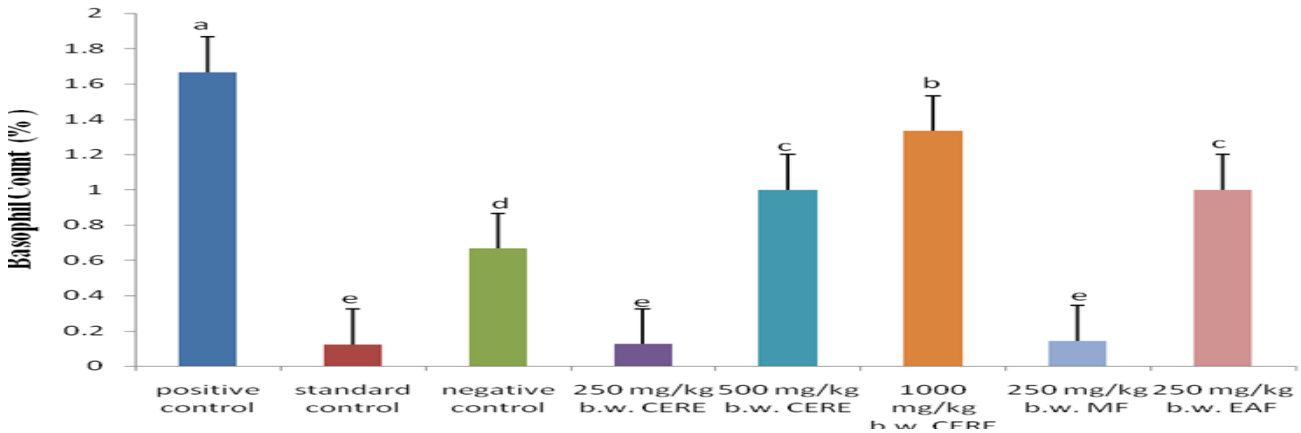

Figure 8: Basophil levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± Standard deviation (n=4). Mean values with different alphabet showed significant difFerence at p<0.05. **Key**:CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

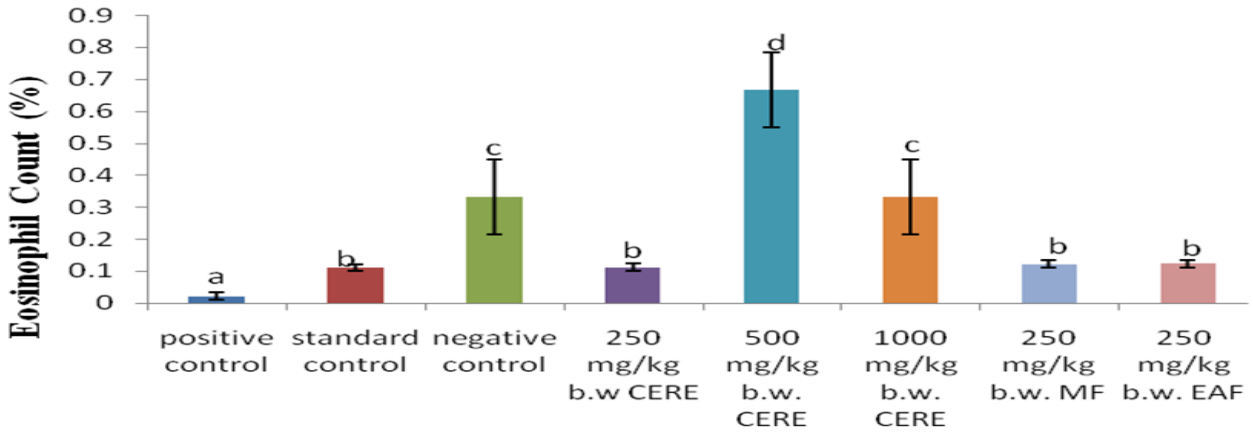

Figure 9: Eosinophil levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05.**Key**:CERE=Crude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

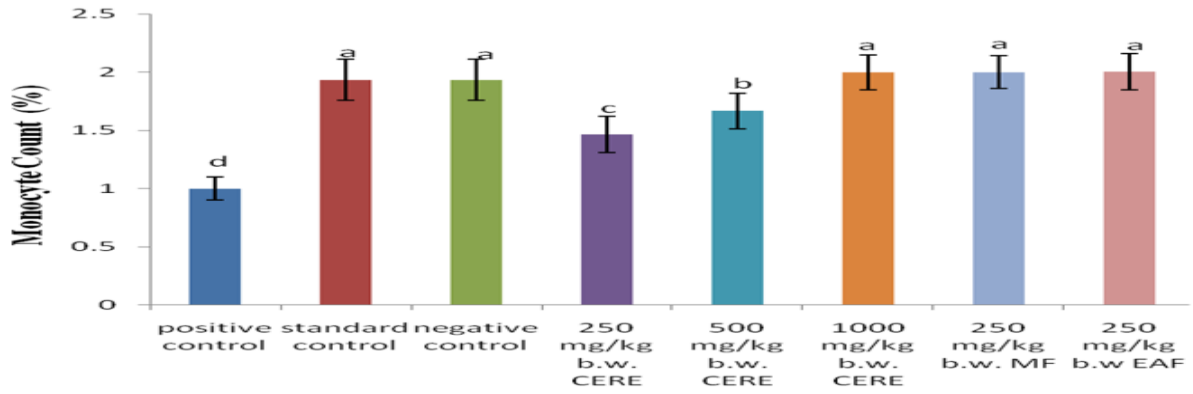

Figure 10: Monocyte levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean Values with different alphabet showed significant difference at p<0.05.**Key**: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

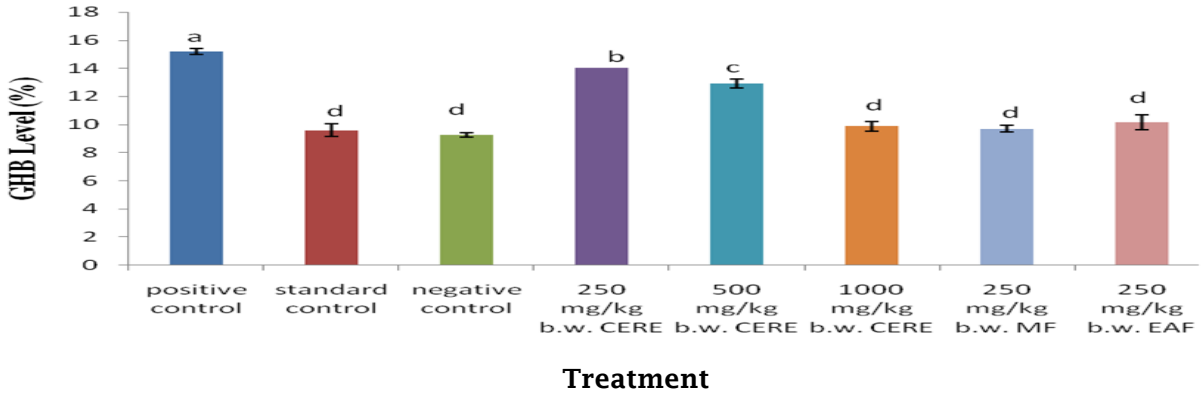

Figure 11: Glycaeted haemoglobin Levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05. **Key**: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

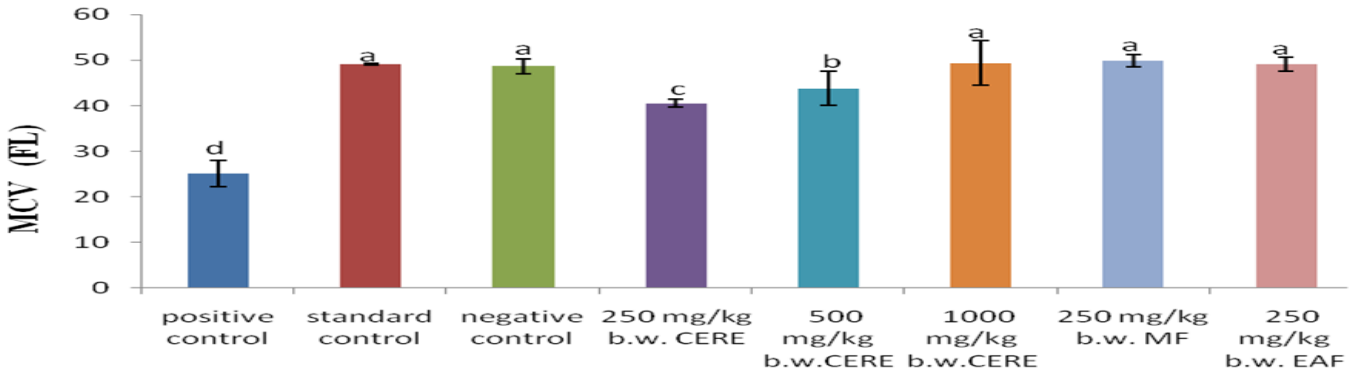

Figure 12: MCV levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as Mean ± standard deviation (n=4). Mean values with different alphabet showed significant difference at p<0.05. **Key**: CERE=Crude ethanol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

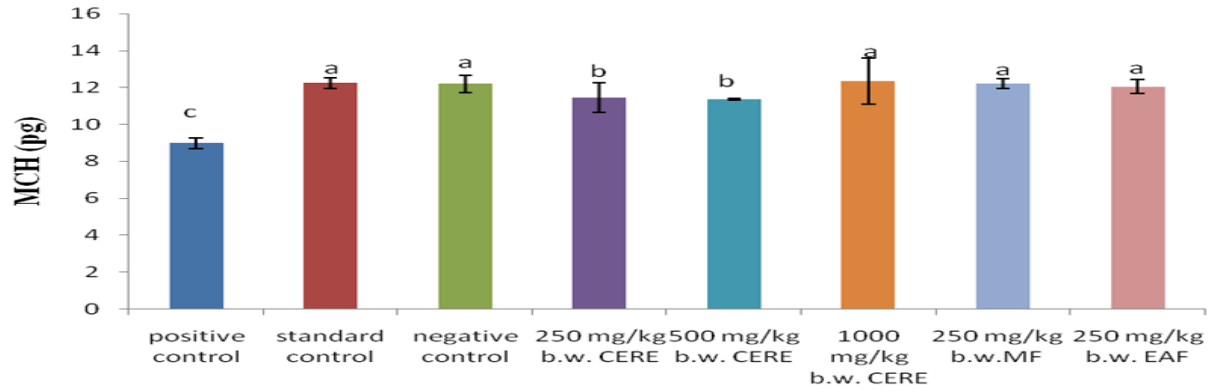

Figure 13: MCH in STZ-induced diabetic albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant different at p<0.05. **Key**: CERE=Crude ethanol root extract,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

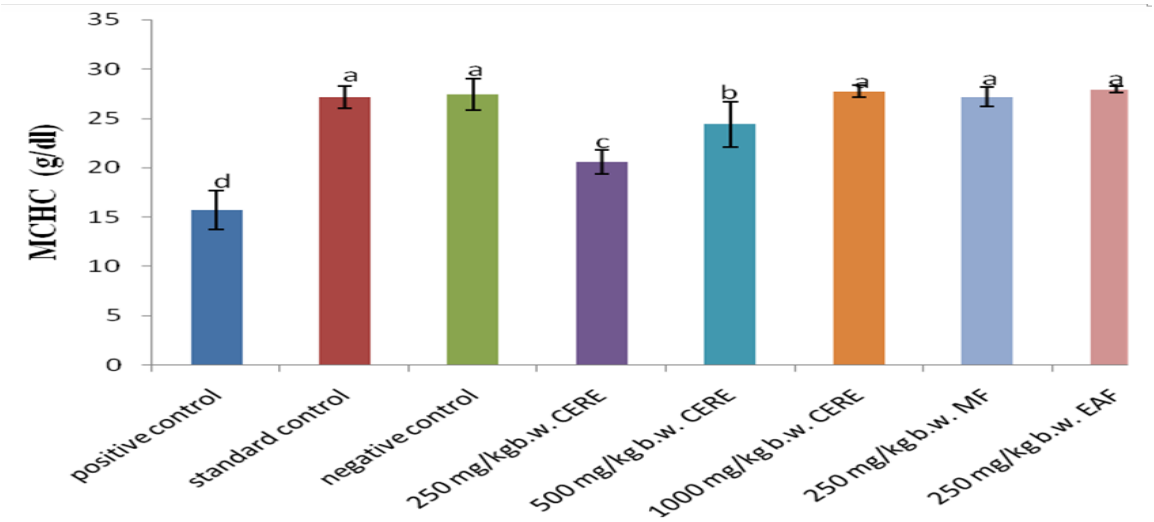

Figure14: MCHC level in STZ induced diabetic albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). Mean values with different alphabet showed significant different at p<0.05. **Key**: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

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 weight of ethylacetate and methanol root fractions of *Sphenocentrum jollyanum* significantly ($p<0.05$) increased the level of Hb, PCV, RBC, platelates, neutrophil, eosinophil and monocyte counts, MCV, MCH and MCHC relative to positive control as shown in Figures 1, 2, 4, 5, 6, 9, 10, 12, 13 and 14 respectively. The result also showed a significant ($p<0.05$) changes in the fractions relative to the extract except at the dose of 1000 mg/kg body weight which showed no significant ($p<0.05$) differences relative to the fractions. The result equally showed no significant ($p<0.05$) changes in the fractions and extract relative to negative and standard group except at the dose of 250 mg/kg and 500 mg/kg body weights which showed significant ($p<0.05$) changes relative to the negative and standard control group. The effects of these parameters were dose dependence and the values on the standard controls were quite similar with the value of the negative control. The treatment of STZ-induced diabetic albino rats with crude ethanol root-extract at doses of 250, 500 and 1000 mg/kg body weights and 250 mg/kg body weight of ethylacetate and methanol root fractions significantly ($p<0.05$) decreased the level of WBC, lymphocytes, basophil and glycaeted haemoglobin relative to positive control as shown in Figures 3, 7, 8 and 11 respectively.

The treatment of STZ-induced diabetic albino rats with the crude ethanol root extract and fractions of *Sphenocentrumjollyanum* significantly ($p<0.05$) increased the level of Hb, PCV, RBC, platelates, neutrophil, eosinophil and monocyte counts, MCV, MCH and MCHC relative to rats that were induced with diabetes without treatment (positive control). The findings of this study is in agreement with the findings of Oyedemi *et al*. (2011) [18], that showed the same trend in streptozotocin-induced diabetic rats treated with stem bark of *Afzelia africana.*

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals [18].

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins [3]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [3]. If a herb or medicinal plant is toxic, this can be reflected in a reduction in some or all of the haematological parameters measured in a full/complete blood count because of direct toxicty to or lysis of the cells in the blood [3]. If however it is non toxic or actually nourishing and immunity boosting, this will reflect in the maintainance or increase in levels of some of the haematological parameters and cells especially those implicated as imparting immunity, though this increase will not be as high as the increase seen in a pathological state [2].

The cells implicated as contributing especially to natural immunity are maintained at normal levels or raised to normal levels or a little above normal levels by herbs and medicinal plants. Medicinal plants have been shown to be more involved in imparting natural immunity than acquired immunity, though it can enhance acquired immunity when necessary [2, 7, 8].

The results of this study indicates that the crude ethanol root extract and fractions without exception did not exhibit any form of haematological toxicity, as statistical evaluation did not show any significant difference ($P>0.05$) between the values of the haematological indices studied in the rats fed with the crude ethanol root extract and fractions when compared to the rats that were not induced with diabetes (negative control).

The treatment of STZ-induced diabetic albino rats with the crude ethanol root extract and fractions significantly ($p<0.05$) decreased the level of WBC, lymphocytes, basophil and glycaeted haemoglobin relative to the rats that were induced with diabetes without treatment (positive control). This result is not in agreement with the findings of Oyedemi *et al.* (2011) [18] that reported an increase in WBC after oral administration of 60mg/kg body weight of *Afzelia africana* on STZ-induced diabetic wistar rats were compared with the diabetic control.

White blood cells fight infection and elevated levels may indicate other problems, such as: infection, stress, inflammation, trauma, allergy, or certain diseases [22]. A high white blood cell count (leukocytosis) usually requires further investigation. The increase in the white blood cell, lymphocyte and basophil counts as seen in the rats that were induced with diabetes without treatment (positive control) could be as a result of the inflammation of the pancreatic islet of langerhans by diabetogenic agent, streptozotocin [22]. STZ causes a massive degeneration of beta-cells of the islets of langerhans and induces hyperglycemia through several processes such as oxidation of essential-sulfhydryl groups, inhibition of glucokinase, disturbances in intracellular calcium homeostasis, or generation of free radicals [19]. Treatment with crude ethanol root extract and fractions decreased the levels of WBC, lymphocyte and basophil counts and were comparable with that of the rats that were not induced with diabetes (negative control), indicating an ameliorative effect of the crude ethanol root extract and fractions on inflammation caused by streptozotocin in the pancreatic islets of langerhans.

The results from this study showed that treatment with the crude ethanol root extract and fractions decreased the glycated hemoglobin when compared to the rats that were induced with diabetes without treatment (positive control). This is in agreement with the work of Diana *et al.* (2010) [6], which showed that oral administration of antidiabetic agents decreases the glycated hemoglobin. When blood glucose levels are high, glucose molecules attach to the hemoglobin in red blood cells. The longer hyperglycemia occurs in blood, the more glucose binds to hemoglobin in the red blood cells and the higher the glycated hemoglobin. Once a hemoglobin molecule is glycated, it remains that way [20]. A buildup of glycated hemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation [20].

CONCLUSION

The results of this study indicates that the crude ethanol root extract and fractions without exception did not exhibit any form of haematological toxicity, as statistical evaluation did not show any significant difference ($P>0.05$) between the values of the haematological indices studied in the rats fed with the crude ethanol root extract and fractions when compared to the rats that were not induced with diabetes.

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