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The effects of crude ethanol root extract and fractions of *sphenocentrum jollyanum* on the lipid profile 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 the lipid profile 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. STZ-induced diabetic albino rats treated 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 levels of triacylglycerides, low-density lipoproteins and total cholesterol. The crude ethanol root extract and fractions significantly ($p<0.05$) increased the level of HDL-C in the treated groups. The result also showed a significant ($p<0.05$) difference in the crude ethanol root extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) difference when the crude ethanol root extract was compared with the fractions. The results of this research on the lipid profile of STZ-induced diabetic rats treated with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* revealed that the crude ethanol root extract and fractions have hypo-cholesterolaemic and hypo-triacylglycerolaemic effects by decreasing the LDL-cholesterol and increasing the HDL-cholesterol levels.
Keywords: *Sphenocentrum jollyanum,* lipid profile and albino rats.

INTRODUCTION

Herbal medicines are the use of plant for healing purposes. They are wonderful body balancers and tonics that help in regulating some body functions. It can be used to maintain metabolic processes of the body and proffer some other nutrients that the body fails to receive due to deprived 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].

In both insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM), the concentration of plasma triacylglycerol is elevated [4]. Decreased clearance might be the main cause of elevated triacylglycerol in IDDM whereas triacylyglycerolaemia may be due to increased liver production of triacylglycerol in NIDDM and decreased clearance. Production of triacylglycerol by the liver involves the esterification of fatty acid derived either from adipose tissue reserves or synthesized *de novo* in the liver from dietary carbohydrate and amino acids [15]. The elevated plasma triacylglycerol in diabetes is generally associated with VLDL, although increases in other lipoproteins also occur. In absolute insulin deficiency, the plasma concentration and turnover of fatty acids increases, whereas fatty acid synthesis *de novo* decreases and the proportion of fatty acyl-CoA that is esterified as opposed to oxidized also decreases [12].

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 *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. 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^{0}C. The concentrated ethyl acetate (EAF) and methanol root fractions of *Sphenocentrum jollyanum* were used for subsequent analyses.

Assessment of the Lipid Profile

Plasma Total Cholesterol, Plasma HDL-Cholesterol, Plasma LDL-cholesterol and Plasma Triglycerides were determined using the methods of Trinder (1969) [17].

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 Lipid Profile in STZ-induced Diabetic Albino Rats

STZ-induced diabetic albino rats treated 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 levels of triacylglycerides, low-density lipoproteins and total cholesterol as shown in Figures 1, 3 and 4. The crude ethanol root extract and fractions significantly ($p<0.05$) increased the level of HDL-C in the treated groups as shown in Figure 2. The result also showed a significant ($p<0.05$) difference in the crude ethanol root extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) difference when the crude ethanol root extract was compared with the fractions. The effect of the extract was dose dependent and the values of the standard control were quite similar with that of the negative control as shown in Figures 1-4.

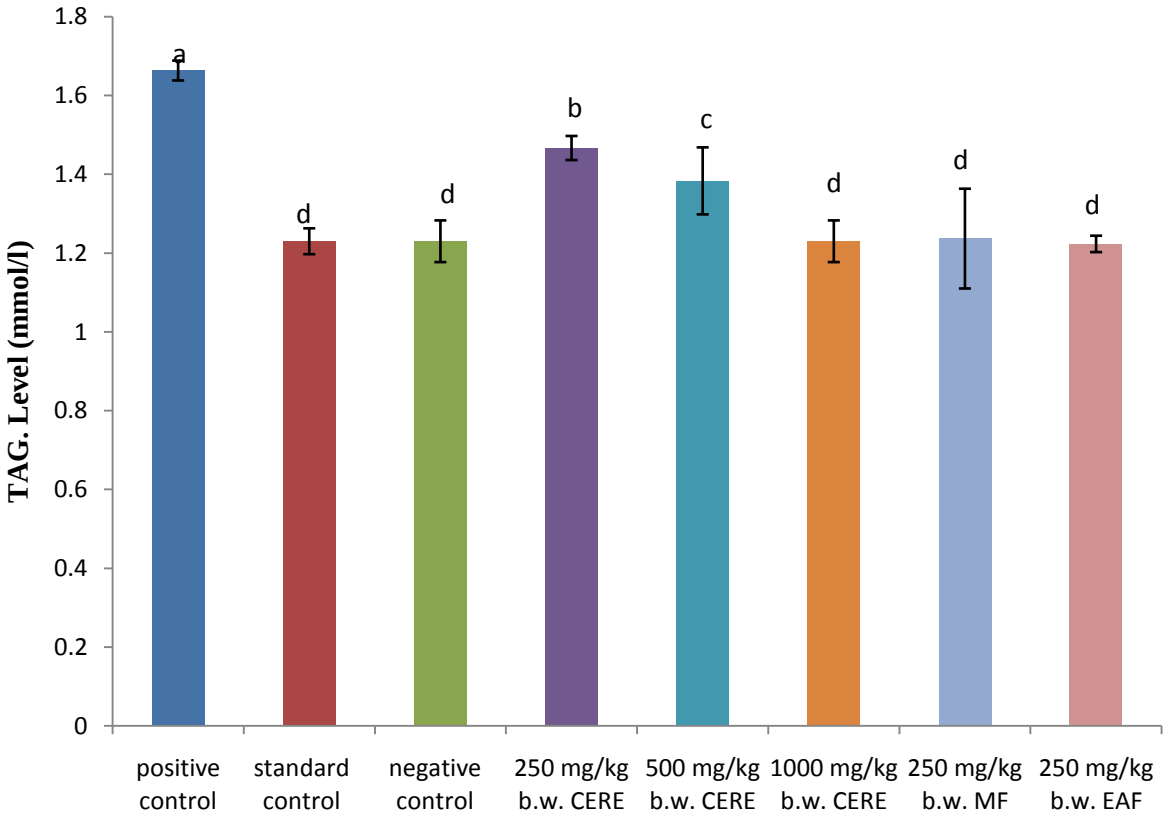

Figure 1: Triacylglycerides levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at $p<0.05$. Key: CERE=Crude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

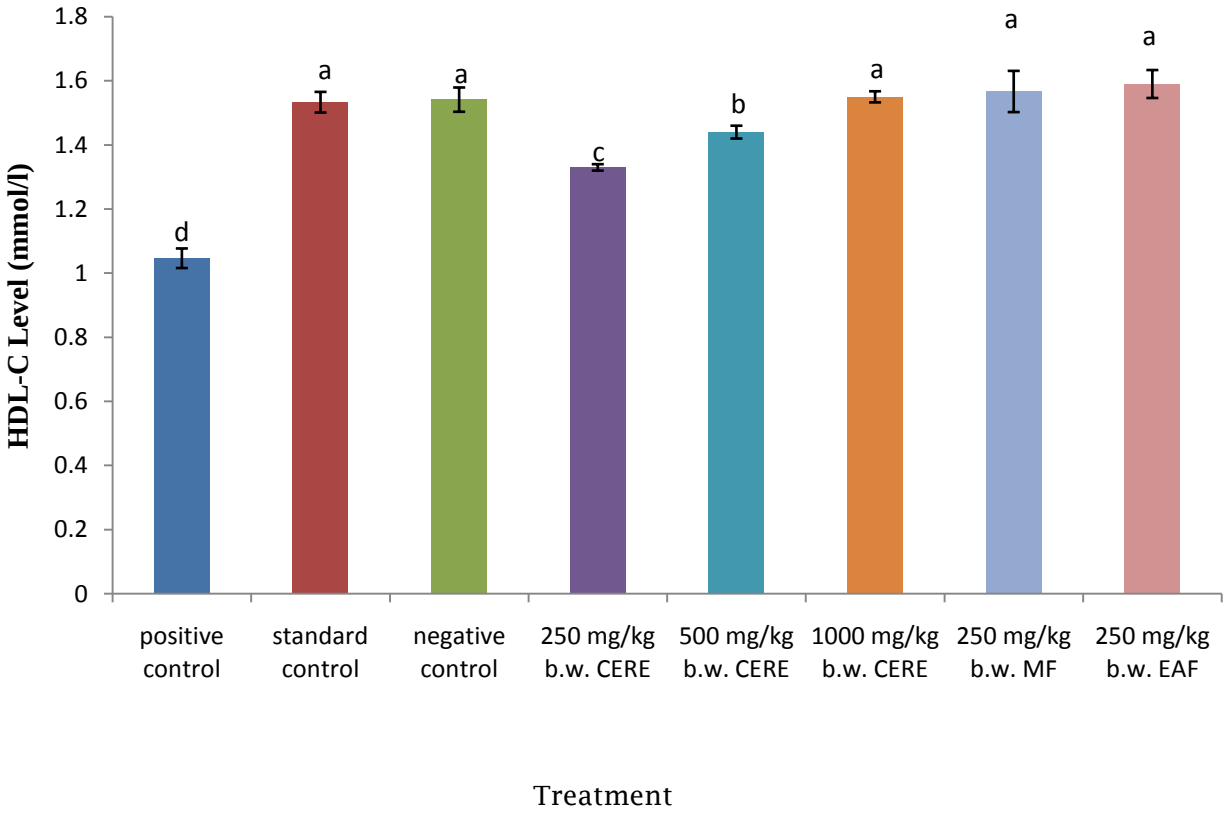

Figure 2: HDL-C levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at $p<0.05$. Key: CERE=Crude ethanol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

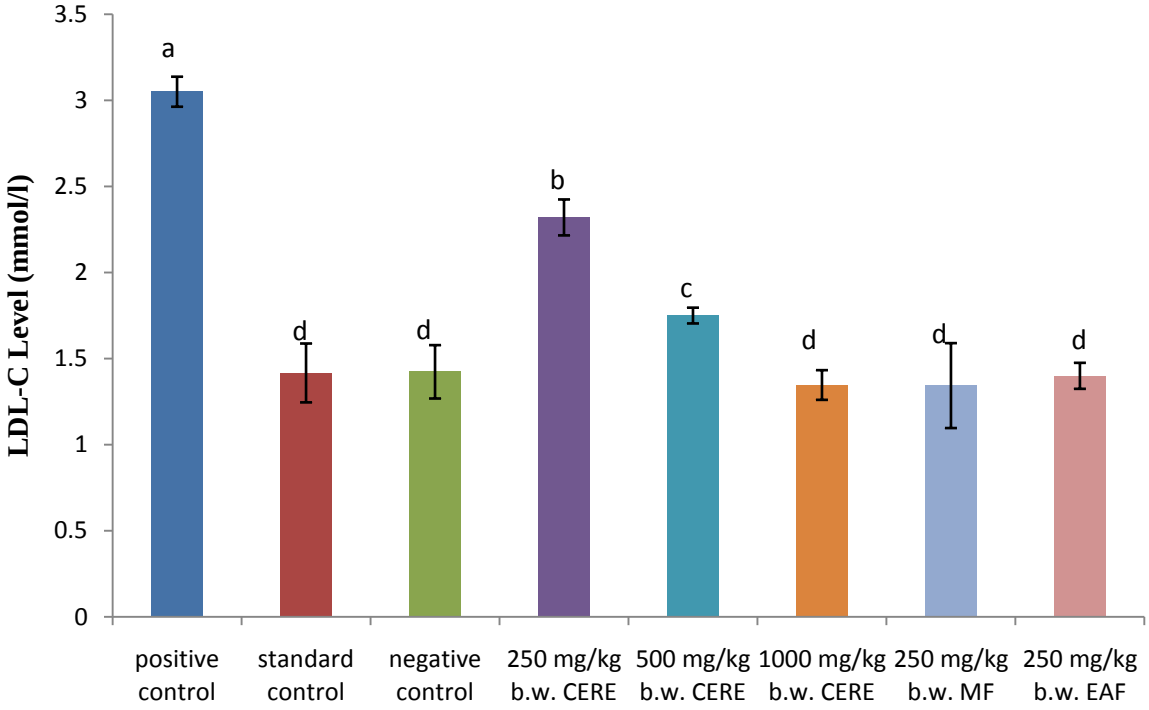

Figure 3: LDL-C levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at $p<0.05$. Key: CERE=Crude ethanol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

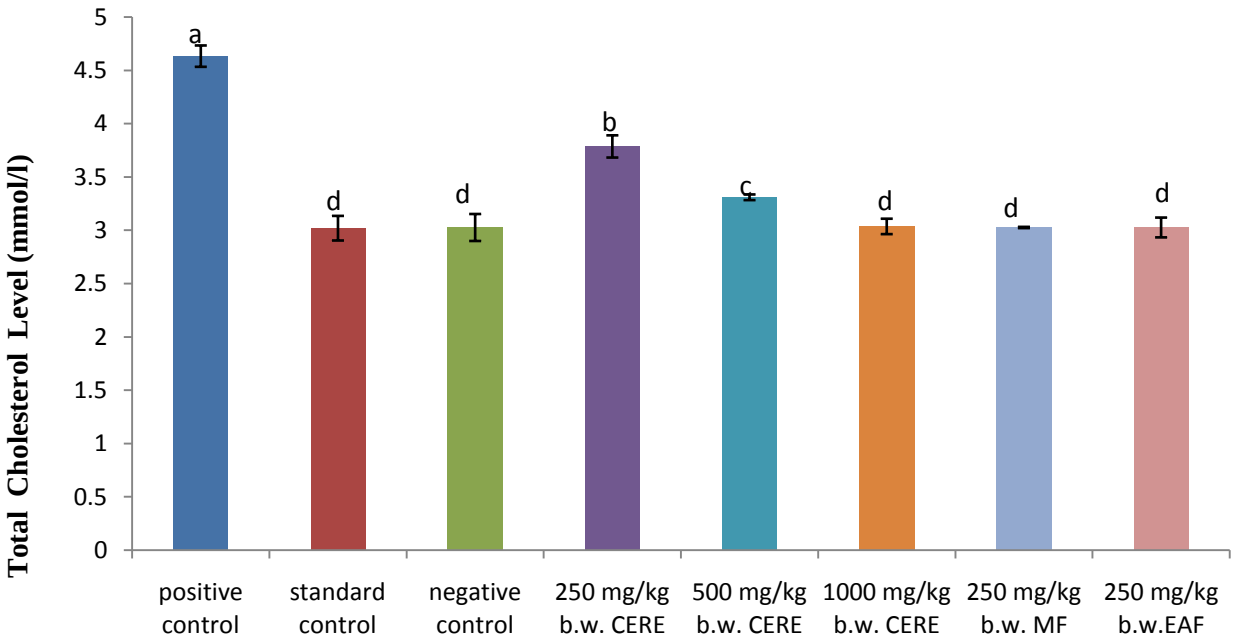

Figure 4: Total cholesterol levels in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=6). Mean values with different alphabet showed significant difference at $p<0.05$. Key: CERE=Crude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

Effect of crude ethanol root-extract and fractions of *Sphenocentrum jollyanum* on lipid profile in STZ-induced diabetic albino rats

Coronary heart disease and acute myocardial infarction are leading causes of death due to weakening of the muscle of the heart [1, 2]. Cardiac dystrophy is the reduced blood (oxygen) transport to the heart muscle due to the narrowing (stenosis) of the blood vessels of the arteries of the heart [13]. The ubiquity of dyslipidaemia, obesity, diabetes and hypertension has been gradually escalating and is thought to be the driving influence behind the epidemic of heart disease faced today[6]. Of the risk factors, diabetes and its predominant form, type 2 diabetes mellitus, has a distinctive association with coronary heart disease. Those with diabetes have two to four-fold higher risk of developing coronary heart disease than people without diabetes and cardiovascular disease accounts for an overwhelming 65-75 per cent of deaths in people with diabetes [6].

The results of the study on the lipid profile of STZ-induced diabetic rats treated with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* compared with that of the diabetic untreated rats (positive control) reveal that generally, the crude ethanol root extract and fractions relatively have hypo-cholesterolaemic and hypo-triacylglycerolaemic effects, while decreasing the LDL-cholesterol and increasing the HDL-cholesterol levels. This result seems to give credence to the claim by herbalists that *Sphenocentrum jollyanum* have hypo-lipidaemic effect. Medicinal plants act on both the pancreas and liver/gall bladder, helping to promote the production and release of the pancreatic enzyme lipase and bile, which ensure good digestion of fats and oils and proper functioning of the excretory functions of the liver thereby confering on it hypolipidaemic properties [1]. The results of this research on the lipid profile give positive evidence that *Sphenocentrum jollyanum* have the potential of being a lipid-lowering supplement/drug in mixed hyperlipidaemic states. There is evidence that a salient relationship exists between high serum cholesterol levels and the incidence of atherosclerosis and cardiovascular diseases [2]; the observed hypocholesterolaemic effect of the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* is therefore a desired positive effect.

CONCLUSION

The results of this research on the lipid profile of STZ-induced diabetic rats treated with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* revealed that the crude ethanol root extract and fractions have hypo-cholesterolaemic and hypo-triacylglycerolaemic effects by decreasing the LDL-cholesterol and increasing the HDL-cholesterol levels.

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