

BIOCHEMICAL ASSESSMENT OF PARKIA BIGLOBOSA ETHANOLIC ROOT EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Author`s contributions

This work was carried out in collaboration between all authors. Author URU designed the study, wrote the protocol. Author URN wrote the first draft of the manuscript. Author AIA performed the statistical analysis. Author ACO managed the analysis of the study. All authors managed the literature searches and approved the final manuscript.

ABSTRACT

Aim: To investigate the antidiabetic effect of *Parkia biglobosa* ethanolic root extract in streptozotocin induced diabetic rats.

Place and Duration of Study: Department of Biochemistry, College of Natural Sciences, Joseph Ayo Babalola University, Ikeji Arakeji Osun State, Nigeria, between October 2014 and August, 2015.

Methodology: Sixty adult female Wistar rats weighing between 120-190g were divided into six study groups I, II, III, IV, V, VI, (n=8) weighing between 120-190g. Group 1 was designated as positive control and received distilled water, group II was designated as negative control and received (1ml) of streptozotocin, group III was designated as diabetes induced and orthodox and received (1ml) of Insulin, groups IV, V and VI were designated as diabetes induced and extract treated groups and received (250mg/dl, 500mg/dl and 1000mg/dl) respectively.

Results: The phytochemical screening of the ethanolic root extract showed the presence of flavonoids, tannin, saponins, cardiac glycosides, phenolics, terpenoid and oxalate. There was a significant decrease in blood glucose levels of diabetes treated groups VI, V and VI at the end of the experiment which portrays the therapeutic efficacy of *Parkia biglobosa* ethanolic root extract in ameliorating diabetic condition. There was a significant decrease in Alanine transaminases (ALT), Aspartate transaminase (AST) and Alkaline phosphatase in extract treated groups compared with negative control. However, Total cholesterol, High density lipoprotein (HDL), and Triglycerides (TG) shown increase which was not significant compared to the negative control but Low density lipoprotein (LDL) depicted decrease which was significant compared with the negative control.

Conclusion: The results obtained from all these assays justify the therapeutic efficacy of *Parkia biglobosa* root extract to ameliorate diabetic condition.

KEYNOTES: *Phytochemical screening, liver enzymes and lipid profile*

ABBREVIATIONS

ALT=Alanine Transaminases

AST=Aspartate Transaminase

ALP=Alkaline Phosphatase

HDL=High Density Lipoprotein,

TG = Triglycerides

LDL= Low Density Lipoprotein

VLDL= Low Density Lipoproteins

OAU=Obafemi Awolowo University

INTRODUCTION

The *Parkia biglobosa* appears as a dicotyledonous angiosperm belonging to the family of Fabaceae-Mimosoideae. It is categorized under spermatophytes, vascular plants [1]. It is a deciduous perennial that grows between 7 to 20 metres high and in some exceptional cases 30metres high [2]. The tree is a fire-resistant heliophyte characterized by a thick dark gray-brown bark. The pods of the tree, commonly referred to as locust beans, are pink in the beginning and turn dark brown when fully mature. The most valuable part of the entire tree, are the seeds which is high in lipid (29%), protein (35%), carbohydrate (16%).The seed are often a good source of fat and calcium used as a nutritious spice, called dawadawa, which is one of the essential condiments in most African dishes. Indigenous healers in Africa use different plant parts of the *Parkia biglobosa* tree for health benefits. In a survey conducted on ethnotherapy in Togo, *Parkia biglobosa* was among the most cited medicinal plants used for treating hypertension [3]. The tree was also identified in south western Nigeria to have significant properties influencing the proliferation of dermal fibroblasts significantly [4].

Geographically, *Parkia biglobosa* can be found in a belt stretching from the Atlantic coast in Senegal to southern Sudan and northern Uganda. The tree currently exists within a wide range of natural communities but is most abundant in anthropic communities – places where cultivation is semi-permanent. Annual production of seeds in northern Nigeria is estimated at around 200 000t. While the products of the tree are not common in international trade, they form

an important part of local and regional trade in West Africa. The yellow pulp, which contains the seeds, is naturally sweet “and is processed into a valuable carbohydrate food known as Sikomu and dawadawa among the Yoruba and Hausa people of Nigeria, respectively [5]. However, most valuable parts of the locust bean, and possibly the entire tree, are the seeds themselves which are high in lipid (29%), protein (35%), carbohydrate (16%), and is a good source of fat and calcium for rural dwellers [2]. In a study conducted on the fermentation of dawadawa, it was found that *Gmelinaar borea* as well as banana leaves accelerated the fermentation of seeds and increased the protein, crude fat and moisture content with corresponding decrease in carbohydrate [6].

Indigenous healers in Africa use different parts of the locust bean tree for health benefits. In a survey conducted on healers in Togo, *Parkia biglobosa* was one of the highest cited plants used for treating hypertension [3]. The tree was also one of two plants “listed as having real wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly [4]. In a similar survey conducted in Guinea Republic on medicinal plants, *Parkia biglobosa* was cited among antimalarial plants that were successfully used for malaria treatment [7]. In further analysis on the antibacterial properties of the plant, studies unveiled that *Parkia biglobosa* competes favorably with streptomycin, making it a potential source of compounds used in the management of bacterial infections [8].

MATERIALS AND METHODS

CHEMICALS

Streptozotocin, chloroform, ethanol and other chemicals were obtained from Fam-lab Nigeria Limited and Lixok-k chemicals, Akure respectively. Alanine transaminases (ALT), Aspartate transaminase (AST), Serum albumin were obtained from Randox Laboratories Limited, UK. Johnson one touch glucometer kit was purchased from De-shalom pharmacy, Ilesha, Osun State, Nigeria. All other chemicals used were of analytical grade. De-ionised and distilled water was also used during the experimental process.

SOURCE OF STANDARD DRUG

Insulin injection (Randox laboratories, UK) marketed by May and Baker, was obtained from a registered pharmacist in De-shalom pharmacy, Ilesha, Osun State, Nigeria and used for the study.

EXPERIMENTAL ANIMALS

Adult sixty female albino rats between 120-190g were purchased from a disease free stock of the University of Ibadan, Nigeria and used for the study. The rats were randomly assigned on the basis of their body weight into six study groups of eight (8) rats each. Normal feeds and tap water were given to the rats ad-libitum and food and water intake were noted. They were kept in plastic cages of 8 rats per cage, placed in a well-ventilated animal room of Joseph Ayo Babalola University at normal temperature of 30-35°C. The cages were cleaned daily and the rats were treated according to the international guidelines for the care and use of laboratory animals. The animals were allowed for two weeks of acclimatization and their weights were measured before treatment commenced.

PLANT MATERIALS AND IDENTIFICATION

Roots of *Parkia biglobosa* were obtained from Locust bean tree in Kabba forest, Kogi State, Nigeria. The plant samples were taken to the department of Botany, Obafemi Awolowo University (O.A.U) for identification and authentication.

PREPARATION OF PLANT EXTRACTS

Parkia biglobosa roots were sorted out washed to remove dust particles and air dried under shade for 4 weeks. The dried materials were homogenized using mechanical grinder and weighed in a weighing balance. The powder form was kept in airtight container and stored at 4°C until when needed for further analysis.

EXTRACTION PROCEDURES

One hundred grams of the powdered extract was soaked in 98% of ethanol in 350ml at room temperature for 72 hours after which the mixture was filtered. The filtrate was centrifuged at 3000 revolution per 30 minutes. The centrifuged sample formed 2 layers; the upper layer was then poured inside another beaker. It was boiled at a very high temperature using a water bath. During the boiling process, it was observed that the sample formed a crystal structure in a foaming form. The resulting filtrate was air-dried at room temperature into petri dishes. The residue obtained was weighed and kept in air-tight container at 4°C. Percentage yield was determined from the weight of the dried sample.

INDUCTION OF DIABETES

Diabetes mellitus was induced on rats by the method of Burcelin *et al.*, [9].

STUDY DESIGN

The grouping and treatment given to rats in each group were as follows; Group A: Designated as NDC consisted of non-diabetic control rats administered 1ml of distilled water. Group B: Designated as DC consisted of diabetic control rats administered 1ml of streptozotocin. Group C: Designated as DO consisted of diabetic rats administered 1ml of orthodox drug (insulin). Group D: Designated as DLB1 consisted of diabetic rats administered 250mg of *Parkia biglobosa* roots extract. Group E: Designated as DLB2 consisted of diabetic rats administered 500mg of *Parkia biglobosa* roots extract. Group F: Designated as DLB3 consisted of diabetic rats administered 1000mg of *Parkia biglobosa* roots extract.

SACRIFICE OF THE ANIMALS AND SERUM COLLECTION

At the end of 14days of administration, rats in each study group were fasted overnight and sacrificed under anesthesia by cervical dislocation. After the rats have been sacrificed, 2-4ml of blood was collected from each rat and placed in specific sterile bottles (plain bottles for enzyme

analysis and EDTA bottles for haematological indices). For enzyme analysis, the blood was allowed to stand for 30 minutes to clot and then centrifuge at 3000Rev for 30 minutes. The supernant, which is the serum, was carefully decanted and kept at 4°C for further analysis.

DETERMINATION OF L-ALANINE AMINOTRANSFERASE (EC. 2.6.1.2) ACTIVITY

L-alanine aminotransferase (ALT) activity was estimated by the method of Reitman and Frankel [10].

DETERMINATION OF L-ASPARTATE AMINOTRANSFERASE (EC. 2.6.1.2) ACTIVITY

L-aspartate aminotransferase (AST) activity was estimated by the method of Reitman and Frankel [10].

DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY

Alkaline phosphatase activity was assayed according to the method described by Bassey *et al.* [11] and modified by Wright and Plummer [12].

DETERMINATION OF SERUM ALBUMIN

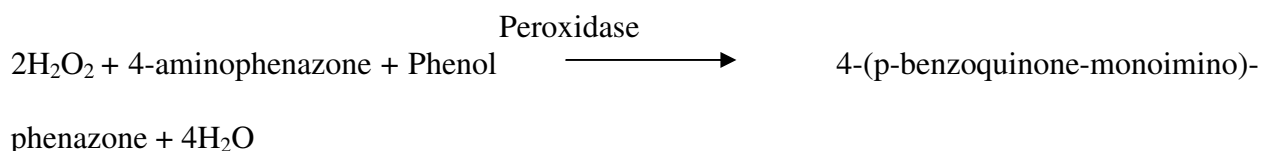
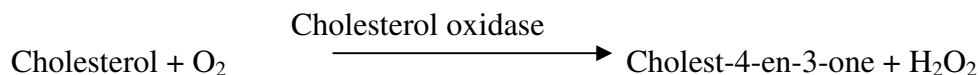
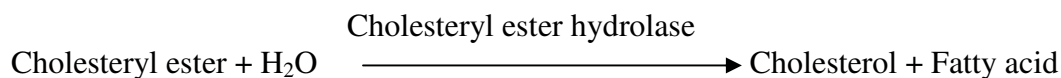
The method described by Wright and Plummer [12] was used to determine the albumin concentration.

LIPID PROFILE

TOTAL CHOLESTEROL DETERMINATION

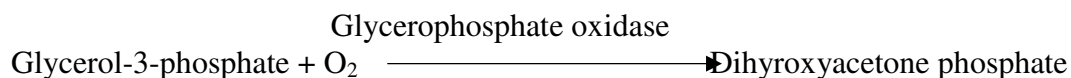
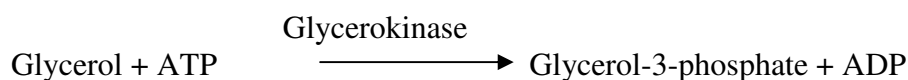
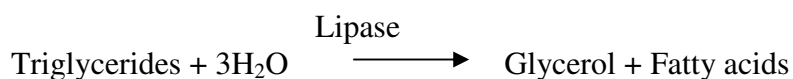
Cholesterol was measured enzymatically, in serum or plasma, in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reactions by products, H₂O₂ was quantitatively measured in a peroxidase catalyzed reaction that produces a

colour. Absorbance was measured at 500nm. The colour intensity was proportional to cholesterol concentration. The reaction sequence is as follows:

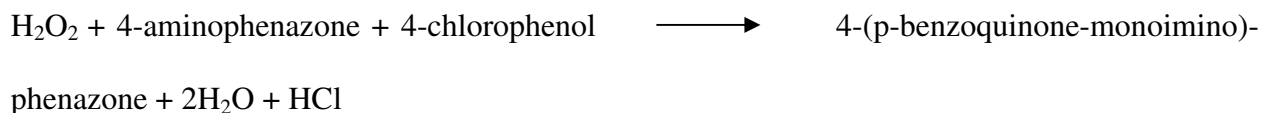


TRIACYLGLYCEROL (TAG) DETERMINATION

Triacylglycerols are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produced glycerol. Glycerol is then oxidized using glycerol oxidase and H_2O_2 , one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500nm. The reaction sequence is as follows;



Peroxidase



HIGH DENSITY CHOLESTEROL DETERMINATION

HDL was measured directly in serum. The basic principle of the method is as follows;

The apo β containing lipoproteins in the specimen reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apo β containing lipoproteins are thus effectively excluded from the assay and only HDL-cholesterol is detected under the assay conditions. The reagents were purchased from Roche/Boehringer-Mannheim Diagnostics. The method uses sulfated alpha-cyclodextrin in the presence of Mg^{2+} , which forms complexes with apo β containing lipoproteins, polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement.

LOW DENSITY CHOLESTEROL DETERMINATION

Most of the circulating cholesterol is found in three major lipoprotein fractions, very low density lipoproteins (VLDL), and low density lipoproteins (LDL), high density cholesterol (HDL).

$$[\text{Total Cholesterol}] = [\text{VLDL-cholesterol}] + [\text{LDL-cholesterol}] + [\text{HDL-cholesterol}]$$

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

$$[\text{LDL-cholesterol}] = [\text{Total Cholesterol}] - [\text{HDL-cholesterol}] - [\text{TG}]/5$$

Where $[\text{TG}]/5$ is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

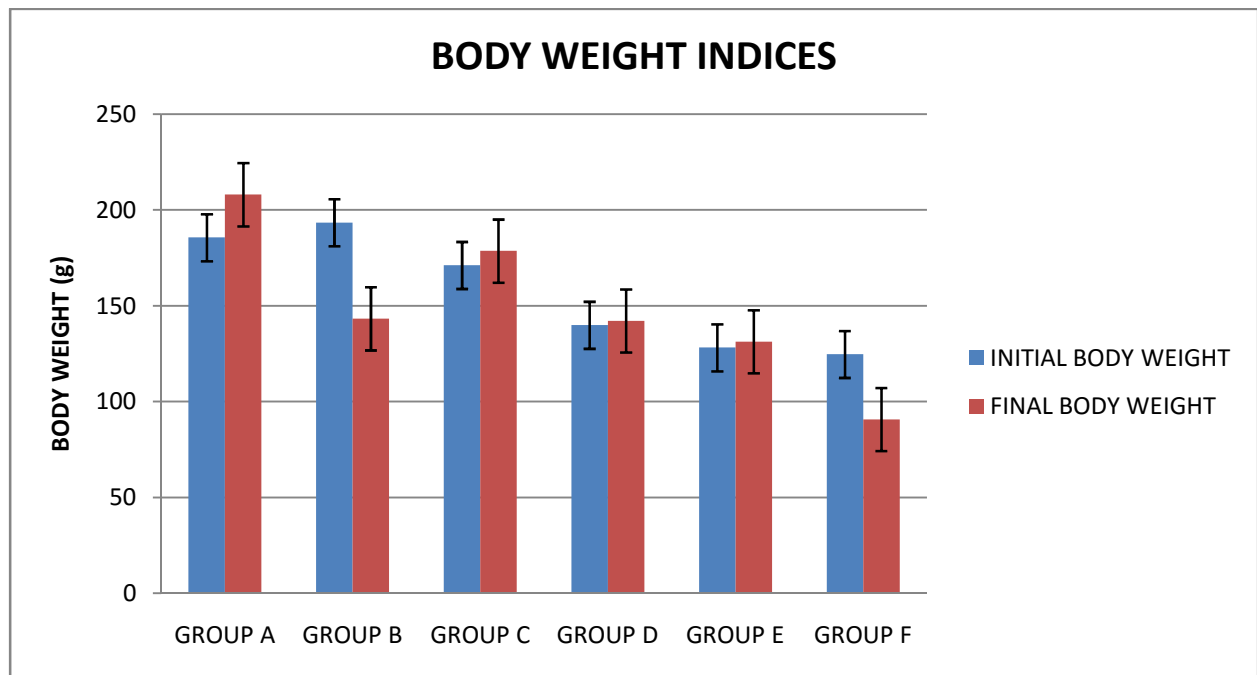
LDL carries most of the circulating cholesterol in man and when elevated contributes to the development of coronary atherosclerosis. LDL-cholesterol is measured to assess risk for CHD and to follow the progress of patients being treated to lower LDL-cholesterol concentrations.

Desirable levels of LDL-cholesterol are those below 130mg/dL in adults and 110mg/dL in children. In NHANES 2001-2002, LDL-cholesterol will be reported only for fasting participants > 5years of age.

Statistical Analyses

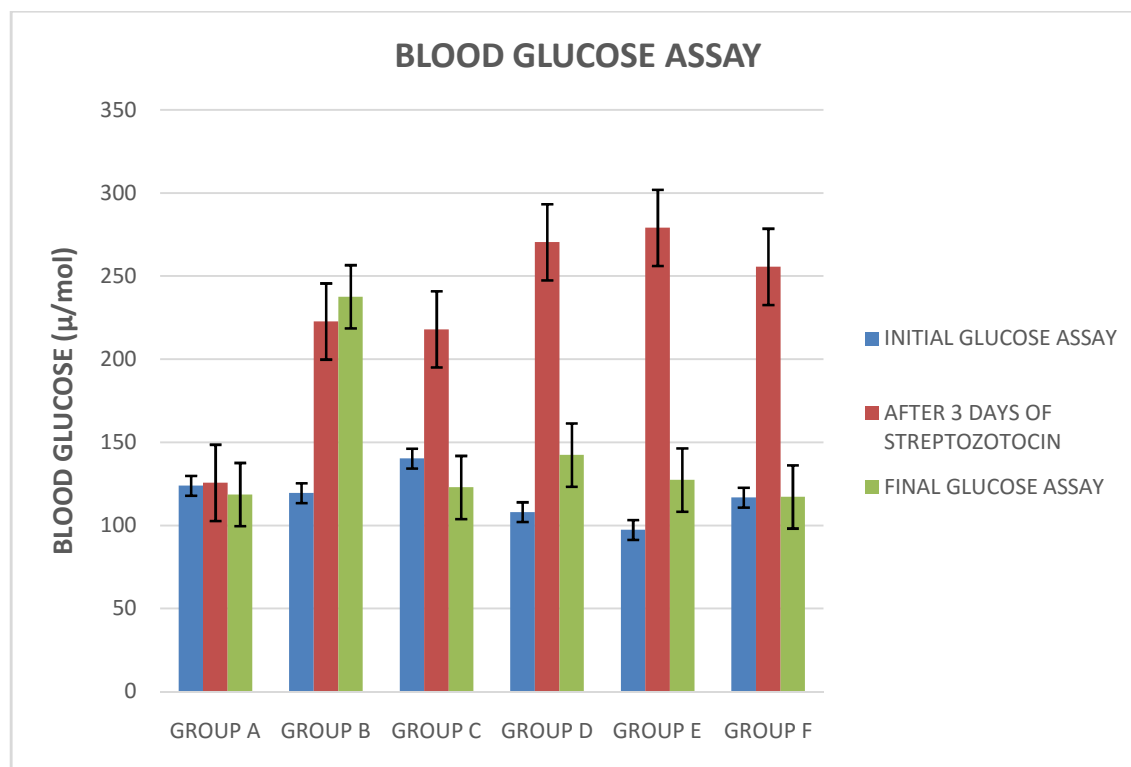
All data obtained were expressed as Mean $\pm$ SEM (standard error of mean) of sample size, n=8. Significant difference between control and experimental groups were obtained by student's t-test using statistical package for social science (SPSS version 16). P-values <0.05 were considered significant.

RESULTS



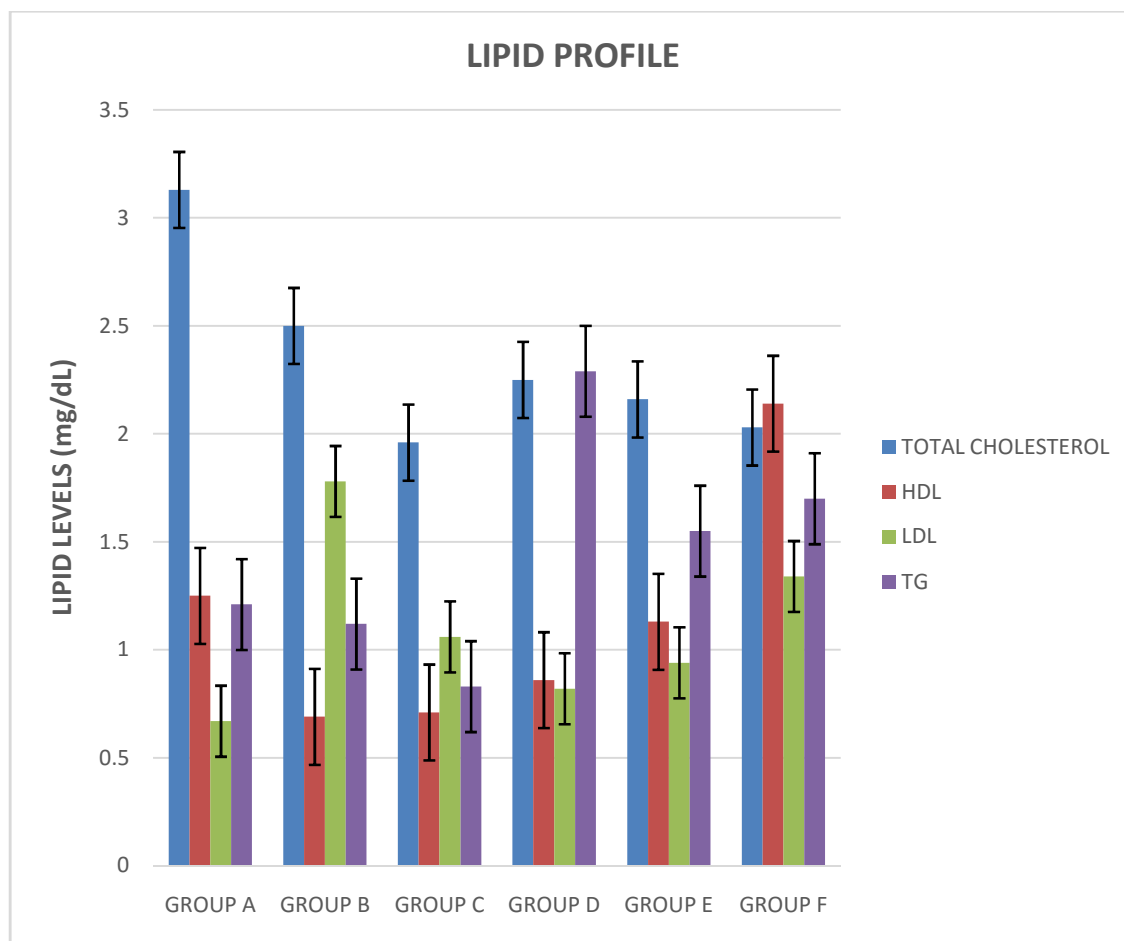
Values expressed in MEANS $\pm$ SEM of 8 determinations.

Figure 1:1 Percentage (%) Change in weight of rats on day 0 and day 14 of STZ-induced diabetic rats treated with ethanolic root extract of *Parkia biglobosa*.



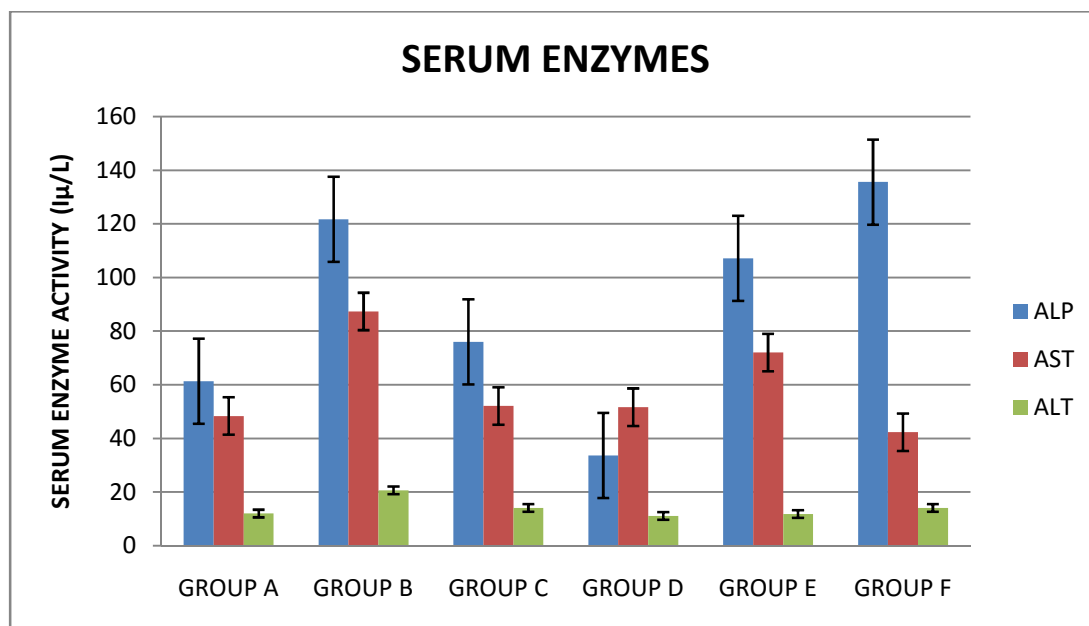
Values expressed in Means $\pm$ SEM of 8 determinations.

Figure 1:2 Percentage reduction in blood glucose between day 0 and 14 days after STZ-induced diabetic rat treated with ethanolic root extract of *Parkia biglobosa*.



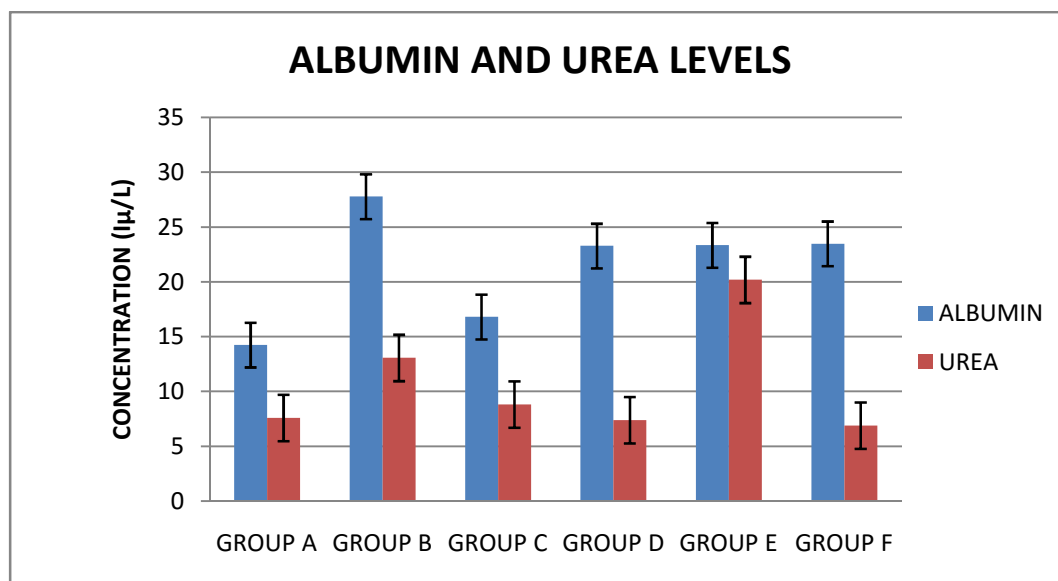
Values expressed in MEANS $\pm$ SEM of 8 determinations.

Figure 1.3: Effect of ethanolic root extract of *Parkia biglobosa* on lipid profile levels in streptozotocin-induced diabetic rats.



Values expressed in MEANS ± SEM of 8 determinations.

Figure 1.4: Effect of ethanolic root extract of *Parkia biglobosa* on serum enzymes in streptozotocin-induced diabetic rats.



Values express in MEANS ± SEM of 8 determinations.

Figure 1.5: Effect of ethanolic root extract of *Parkia biglobosa* on albumin and urea levels in streptozotocin-induced diabetic rats.

DISCUSSION

The lipid profile obtained in the present study showed a significant ($P < 0.05$) decrease in total cholesterol and high density lipoprotein in extract treated groups when compared with non diabetic group but with corresponding increase when compared with negative control. However, for low density lipoprotein and triacylglycerol, there was a significant increase in extract treated groups when compared to the non-diabetic group but with a significant decrease when compared to diabetic control. This finding is in agreement with the reports of [13] who demonstrated increased in serum lipids as a result of diabetes in animals. In the same manner, [14] reported that diabetes-induced hyperlipidemia due to excess of fat from the adipose tissue during underutilization of glucose. But lack of insulin and elevations of the glucagon may leads to activation of lipase that stimulate lipolysis and enhanced the release of free fatty acids from adipose tissue [15].

The hepatic serum enzymes are valuable tool in clinical diagnosis that provides information on the effect and nature of pathological damage to any tissue [16]. AST, ALT, ALP are biomarkers which are often used to assess the integrity of the plasma membrane and tissues after being exposed to certain pharmacological agents [17]. Result obtained in the present study showed that the activities of serum liver enzymes; aspartate transaminase (AST) and alkaline phosphatase (ALP) extract treated groups, were significantly increased when compared with the non-diabetic control but with a decrease when compared to diabetic control. ALT was significantly decreased in the extract treated diabetes group compared to the control. This report is consistent with the studies of [18] who reported that the elevation in markers of liver injury such as ALT, AST and ALP indicate hepatocyte damage in experimental diabetes. And the increase in the level of these

enzymes in diabetes may be as a result of leaking out of these enzymes from the compromised tissue into the blood stream [19]. The ability of *Parkia biglobosa* root extract to ameliorate diabetic condition in animals with significantly decrease the ALT, AST and ALP serum levels suggest their hepato-cellular protective function and this can be attributed to its antioxidant effects.

CONCLUSION

The significant antidiabetic effect of *Parkia biglobosa* root extract could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone or in synergism can impart therapeutic effect. It is therefore, concluded from the data obtained that *Parkia biglobosa* possesses potent antihyperglycemic activity and it may prove to be effective treatment of both types of diabetes.

ETHICAL APPROVAL

All authors hereby declare that principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the College of Natural Sciences, Joseph Ayo Babalola University (JABU) Research and Ethics Committee.

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COMPETING INTERESTS: Authors have declared that no completing interest exist.

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