

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

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: Adult sixty female (60) Wistar Albino rats 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 and received (250mg, 500mg and 1000mg) 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 drastic reduction 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. Other biochemical parameters such as; Alanine transaminases (ALT), Aspartate transaminase (AST) and Alkaline phosphatase. Lipid profile; Total cholesterol, High density lipoprotein (HDL), Low density lipoprotein (LDL) and Triglycerides (TG), as well as body weight indices were considered.

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

KEYNOTES: Phytochemical screening, serum enzymes and lipid profile

INTRODUCTION

The *Parkia biglobosa* appears as a dicotyledonous angiosperm belonging to the family of Fabaceae-Mimosoideae. It is categorized under spermatophytes, vascular plants (Thiombiano et al., 2012) is a deciduous perennial that grows between 7 to 20metres high and in some exceptional cases 30metres high respectively (tui et al., 2012). 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 seeds 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 (Karou *et al.*, 2011). The tree was also identified in south western Nigeria to have significant properties influencing the proliferation of dermal fibroblasts significantly (Adetutu *et al.*, 2011).

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 (Olaniyan, 2013).

However the 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 (Ntui *et al.*, 2012). Seeds are often made into a nutritious spice or condiment, called dawadawa, which is beloved in large parts of Africa. The seed is first cooked to remove the seed coat and then fermented to produce the desired result. In a study conducted on the fermentation of dawadawa, it was found that *Gmelinaar borea* as well as banana leaves accelerated fermentation of seeds, while also bringing “an increase in protein, crude fat and moisture contents with corresponding decrease in carbohydrate (Gernah *et al.*, 2007).

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 (karou *et al.*, 2011). The tree also one of two plants “listed as having real wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly (Adetutu *et al.*, 2011). 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 (Traore *et al.*, 2013). 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 (Abioye *et al.*, 2013).

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 (60) 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 (NIH,

2008). 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

100g 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 placed in centrifuge 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 by single intraperitoneal dose of 60mg/kg of Streptozotocin (Sigma chemicals, St Louis USA) dissolved in 0.1M fresh cold citrate buffer at a pH of 4.5 into

12 hours overnight fasted rats (Burcelin *et al.*, 1995). After 3 days, fasting blood sugar levels were monitored with a glucometer (Active Accu Check, Roche Diagnostics GmbH, Germany) and the rats having fasting blood glucose levels more than 200mg/dl were isolated, classified diabetic and used for experimentation.

EXPERIMENTAL 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 the experimental period, 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 supernatant, which is the serum, was carefully decanted and was 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 (1957). The method measures spectrophotometrically the intensity of the colored hydrazine formed from the reaction of pyruvate with 2, 4-dinitrophenylhydrazine at 546 nm.

PRINCIPLE: The assay is based on the following reaction of the enzyme;

Glutamate + Pyruvate $\rightleftharpoons$ α -ketoglutarate + Alanine

The keto acid (pyruvate) reacts with 2, 4-dinitrophenyl hydrazine to form hydrazine.

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 (1957). The method measures spectrophotometrically the intensity of the coloured hydrazine formed from the reaction of pyruvate with 2,4-dinitrophenylhydrazine at 546 nm.

PRINCIPLE: The assay is based on the following reaction of the enzyme;

L-aspartate + α -ketoglutarate $\rightleftharpoons$ Oxaloacetate + L-glutamate

Oxaloacetate produced by transamination activities of AST is spontaneously decarboxylated to pyruvate which then react with 2, 4-dinitrophenylhydrazine. The intensity of the red colour formed is a measure of transaminase activity. The reactions stopped by addition of 2.5ml 0.1 NaOH solution.

DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY

Alkaline phosphatase activity was assayed according to the method described by Bassey *et al.* (1946) and modified by Wright and Plummer (1974).

165 **PRINCIPLE:**

166 Phenol is released by enzymatic hydrolysis from disodium phenylphosphate under defined
167 conditions of time, temperature and pH. This reacts with 4-aminoantipyrine in the presence of
168 alkaline oxidizing agent to give a red colored compound, which is estimated at 405nm against a
169 black reagent. Colour development is rapid and stable for at least an hour in bright light. Sodium
170 hydroxide is added immediately after incubation to raise the pH and stop the reaction. Potassium
171 ferricyanide is the oxidizing agent and sodium bicarbonate is added to provide the alkaline
172 medium.

173 **DETERMINATION OF SERUM ALBUMIN**

174 The method was described by Plummer (1979) was used to determine the albumin concentration



175 **PRINCIPLE:**

176 Cupric ions in alkaline solution form a purple colored complex with any compound containing
177 repeated –CONH links (peptide bonds) such as proteins. The purple colour is due to the
178 coordination between cupric ions and the unshared electron pair of peptide nitrogen and the
179 oxygen of water to form the colored complex. The colour intensity is a measure of the protein
180 content of the sample.

181 **CALCULATION:**

182 The concentration of the protein (Albumin) was calculated by the protein (albumin) content in
183 each sample obtained from the protein calibration curve and multiplied by the dilution factor i.e.
184 Protein concentration= concentration of the sample extrapolated from the calibration curve X
185 Dilution factor.

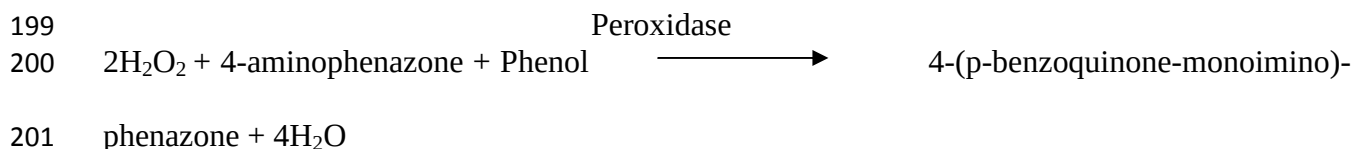
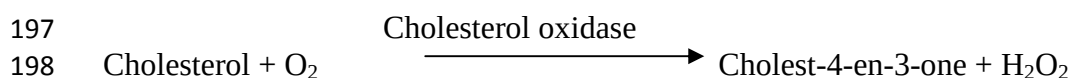
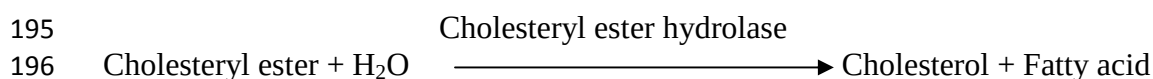
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188 **LIPID PROFILE**

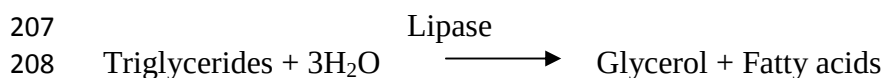
189 **TOTAL CHOLESTEROL DETERMINATION**

190 Cholesterol was measured enzymatically, in serum or plasma, in a series of coupled reactions
 191 that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reactions
 192 by products, H_2O_2 was quantitatively measured in a peroxidase catalyzed reaction that produces a
 193 colour. Absorbance was measured at 500nm. The colour intensity was proportional to cholesterol
 194 concentration. The reaction sequence is as follows:

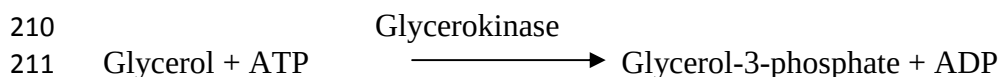


202 **TRIACYLGLYCEROL (TAG) DETERMINATION**

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



209



212

216 $\text{H}_2\text{O}_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \longrightarrow \text{4-(p-benzoquinone-monoimino)-}$
 217 $\text{phenazone} + 2\text{H}_2\text{O} + \text{HCl}$

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

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.

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).

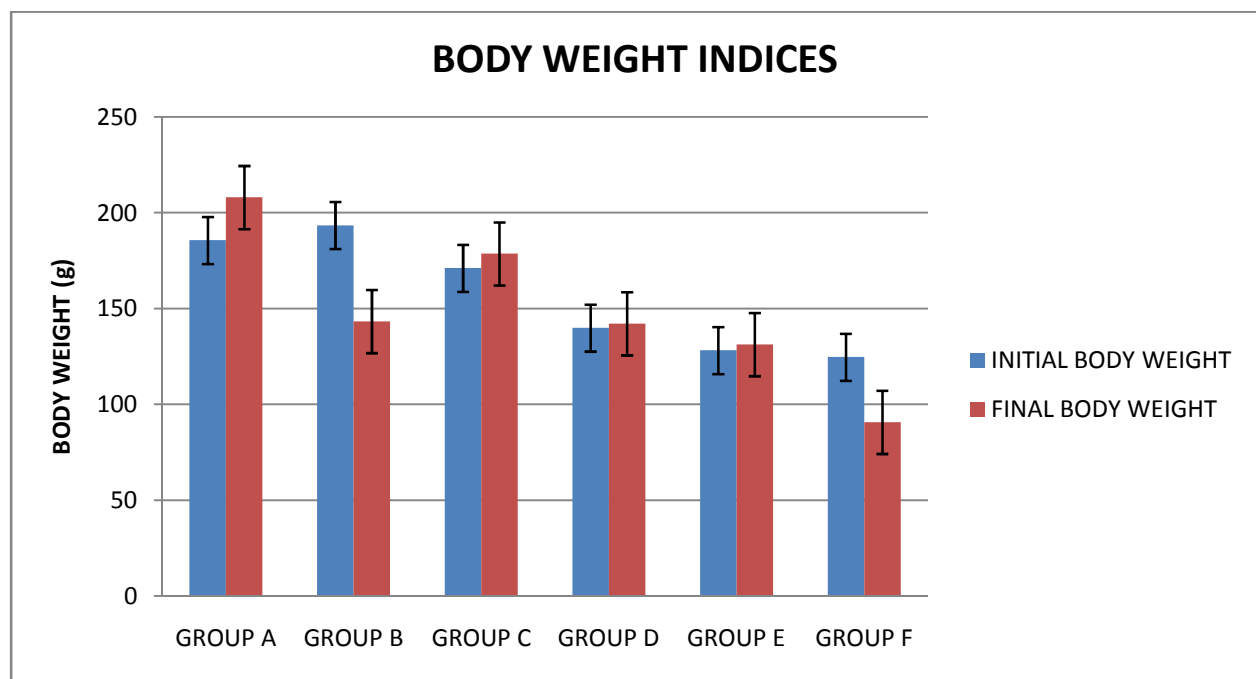
LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

234 $[\text{LDL-cholesterol}] = [\text{Total Cholesterol}] - [\text{HDL-cholesterol}] - [\text{TG}]/5$

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

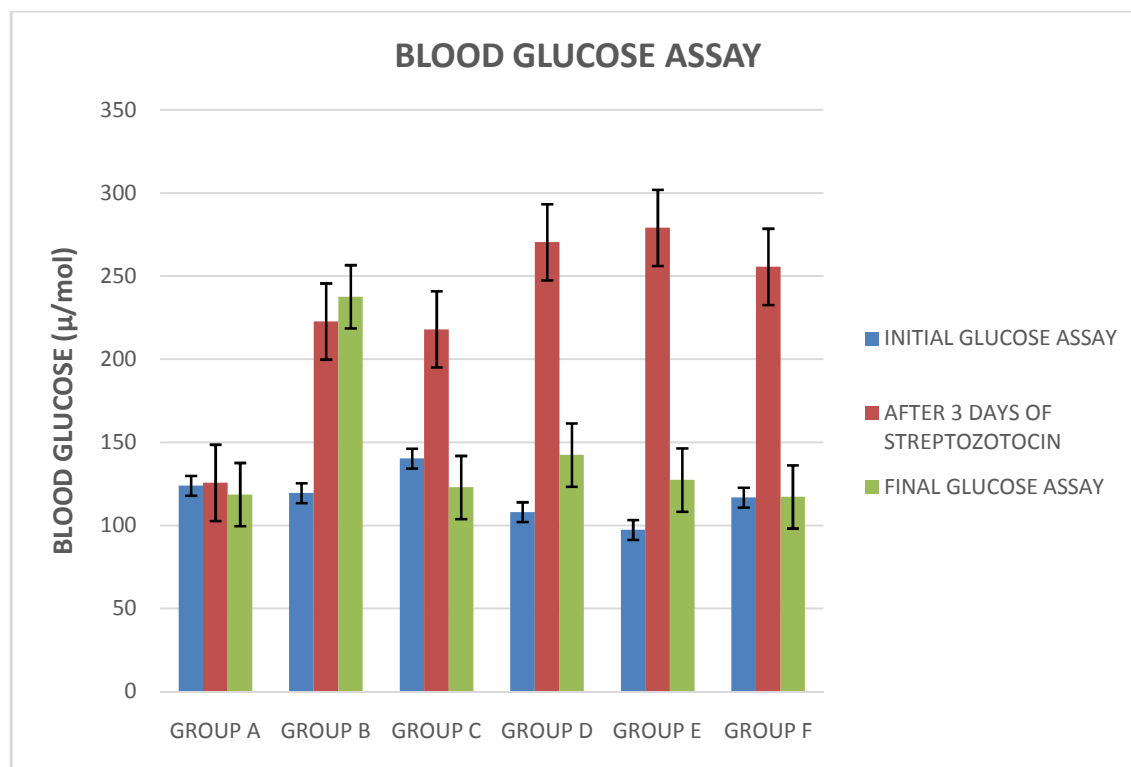
LDL carries most of the circulating cholesterol in man and when elevated contributes to the development of coronary atherosclerosis. LDL-cholesterol is measured to assess risk for CHD and to follow the progress of patients being treated to lower LDL-cholesterol concentrations. Desirable levels of LDL-cholesterol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

RESULTS

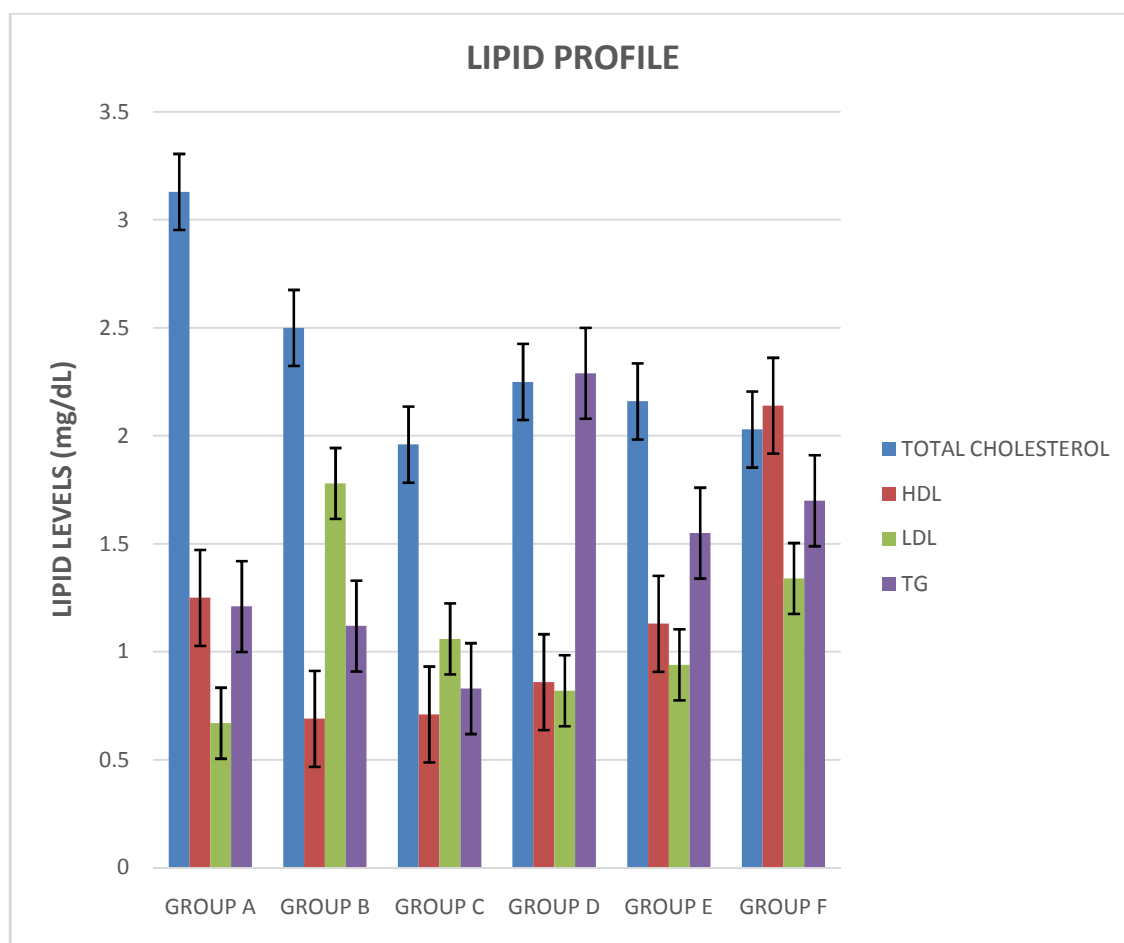


Values expressed in MEANS $\pm$ SEM of 8 determinations.

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*.

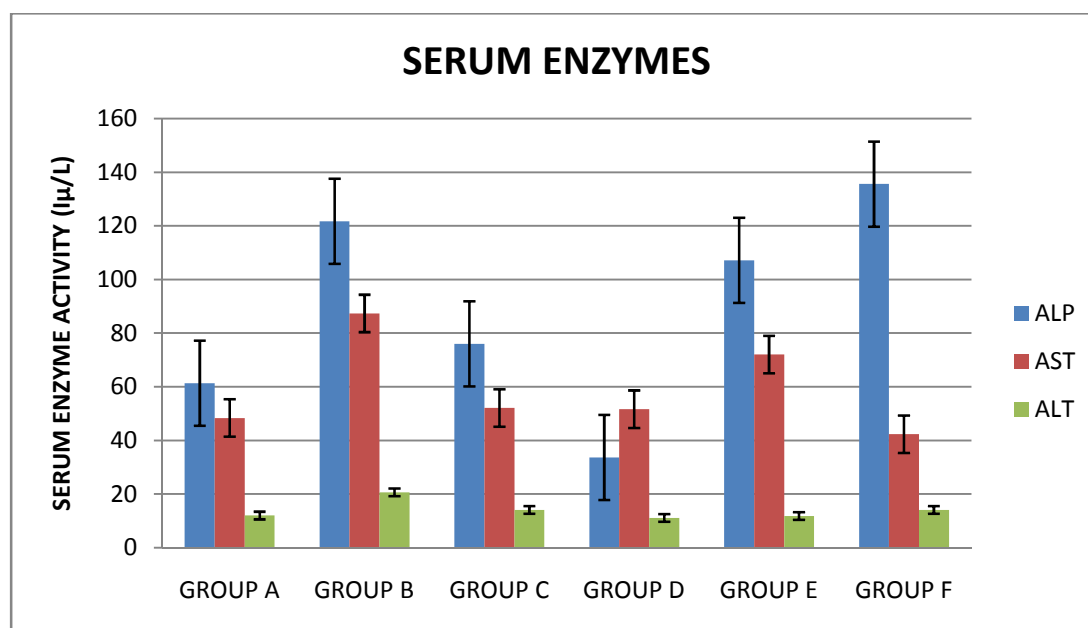


Values expressed in Means $\pm$ SEM of 8 determinations.
Figure 1:2 Percentage reduction in blood glucose between day 0 and 14 days after STZ-induced diabetic rat treated with ethanolic root extract of *Parkia biglobosa*.



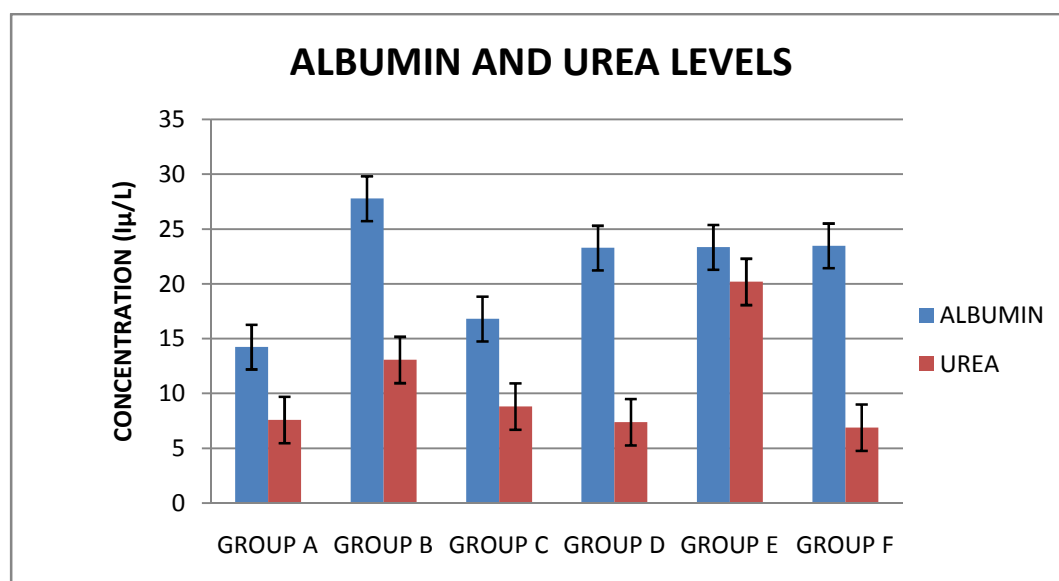
Values expressed in MEANS $\pm$ SEM of 8 determinations.

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



Values expressed in MEANS $\pm$ 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 $\pm$ 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.

272 **DISCUSSION**

273 The lipid profile obtained in the present study showed a significant ($P<0.05$) decrease in total
274 cholesterol and high density lipoprotein in extract treated groups when compared with diabetic
275 group but with attendant increase when compared with negative control. And for low density
276 lipoprotein and triacylglycerol, there was a significant increase in extract treated groups when
277 compared to the non-diabetic group but with a significant decrease when compared to diabetic
278 control. This finding is in agreement with the reports of Fernandes *et al.*, (2010) who
279 demonstrated increased in serum lipids as a result of diabetes in animals. In the same vein,
280 (Nimenibo-uadia, 2003) reported that diabetes-induced hyperlipidemia due to excess of fat from
281 the adipose tissue during underutilization of glucose. But lack of insulin and elevations of the
282 glucagon may lead to activation of lipase that stimulate lipolysis and enhanced the release of
283 free fatty acids from adipose tissue (Rotimi *et al.*, 2011).

284 The hepatic serum enzymes are valuable tool in clinical diagnosis that provides information on
285 the effect and nature of pathological damage to any tissue (Daisy and Saipriya, 2012). AST,
286 ALT, ALP are biomarkers which are often used to assess the integrity of the plasma membrane
287 and tissues after being exposed to certain pharmacological agents (Rathod *et al.*, 2009). Result
288 obtained in the present study showed that the activities of serum liver enzymes; aspartate
289 transaminase (AST) and alkaline phosphatase (ALP) extract treated groups, were significantly
290 increased when compared with the non-diabetic control but with a decrease when compared to
291 diabetic control. ALT was significantly decreased in the extract treated diabetes group compared
292 to the control. This report is consistent with the studies of Abolfathi *et al.*, 2011 who reported that
293 the elevation in markers of liver injury such as ALT, AST and ALP indicate hepatocyte damage
294 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 (Concepcion *et al.*, 1993). 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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