

BIOCHEMICAL STUDIES ON LORANTHUS BENGWENSIS ETHANOLIC LEAF EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

Abstract

Aim: To investigate the biochemical effect of *Loranthus bengwensis* ethanolic leaf 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=10) weighing between 230-250g. Group I was designated as positive control and received distilled water, group II was designated as negative control and received (60mg/kg) 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 (100mg, 200mg and 400mg) respectively.

Results: The phytochemical screening of the ethanolic leaf extract showed the presence of alkaloids, flavonoids, steroids, tannin, saponins, cardiac glycosides, phenolics, terpenoid and quinines. There was a drastic reduction in blood glucose levels of diabetes treated groups VI, V and IV at the end of the experiment which portrays the therapeutic efficacy of *Loranthus bengwensis* ethanolic leaf extract in ameliorating diabetic condition. However, the extract was more efficient at 400mg/kg compared to 100mg/kg and 200mg/kg respectively. This indicates that ethanolic leaf extract of *Loranthus bengwensis* is relatively more efficient at higher concentration. 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 as well as the blood glucose assay justifies the therapeutic efficacy of *Loranthus bengwensis* on ameliorate diabetic condition.

KEYNOTES: *Phytochemical screening, serum enzymes, lipid profile.*

INTRODUCTION

The African mistletoe of the genus *Loranthus* is a semi-parasitic plant widely distributed throughout the Northern and Southern Nigeria is found growing on a variety of evergreen and deciduous trees. Unlike a true parasite which depends on the host for nutrients, mistletoe takes only water and minerals from its host (Hutchinson and Dalziel, 1928). Its green leaves enable it to photosynthesize carbohydrates (Griggs, 1991). It grows all year round along the branches of the host tree when most plants shed their leaves. *Loranthus bengwensis* is also known as 'all healer', mistletoe has been used extensively to treat many complaints (Kafaru, 1993). In some parts of Africa, including Nigeria, it is believed that the aqueous extract of *Loranthus spp*

consumed over a long period will remove the cause of hypertension, diabetes and other metabolic disorders (Dalziel, 1955). *Loranthus bengwensis* is one of the groups in the Loranthaceae family which is specific to Africa and found mostly in the tropics. The chemical composition of mistletoe varies depending on the host tree (Luczkiewicz *et al.*, 2001). *Loranthus bengwensis* grown on apple tree has the strongest pharmacological effect (Hulsen *et al.*, 1986). The constituents of *Loranthus bengwensis* are; flavonoids, lectins, polypeptides, polysaccharides, saponins, tannins, tri-terpenes and viscotoxin. The major constituent is lectins which are carbohydrate binding proteins, which include viscumin, polypeptides known as viscotoxin with a basic chemical structure of thionins and a number of phenolic compounds like digallic acid and o-coumaric acid found in free states or as glycosides (Duong *et al.*, 2003).

Mistletoe plant grows on a wide range of host trees; they commonly reduce their growth and a large plant stunts and commonly kills the distal portion of branch it grows on. A heavy infestation may kill the entire host plant. *Viscum album* successfully parasitizes more than 200 tree and shrub species worldwide. Technically, all mistletoe species are hemiparasites, because they do perform at least a little photosynthesis for at least a short period of their life cycle. However, this is academic in some species whose contribution is very negligible. For example, some species, such as *Viscum minimum*, that parasitize succulents, species of Cactaceae or Euphorbiaceae, grow largely within the host plant, with hardly more than the flower and fruit emerging. Once they have germinated and attached to the circulatory system of the host, their photosynthesis reduces so far that it becomes insignificant (Judd *et al.*, 2002).

Most of the Viscaceae bear evergreen leaves that photosynthesize effectively, and photosynthesis proceeds within their green, fleshy stems as well. Some species, such as *Viscum capense*, are adapted to semi-arid conditions and their leaves are vestigial scales, hardly visible without detailed morphological investigation. Therefore their photosynthesis and transpiration only take place in their stems, limiting their demands on the host's supply of water, but also limiting their intake of carbon dioxide for photosynthesis. Accordingly their contribution to the host's metabolic balance becomes trivial and the idle parasite may become quite yellow as it grows, having practically given up photosynthesis (Visser, 1981).

At another extreme other species have vigorous green leaves. Not only do they photosynthesize actively, but a heavy infestation of mistletoe plants may take over host tree branches, sometimes killing practically the entire crown and replacing it with their own growth. In such a tree the host is relegated purely to the supply of water and mineral nutrients and the physical support of the trunk. Such a tree may survive as a *Viscum* community for years; it resembles a totally unknown species unless one examines it closely, because its foliage does not look like that of any tree. An example of a species that behaves like that is *Viscum continuum*.

A mistletoe seed germinates on the branch of a host tree or shrub, and in its early stages of development it is independent of its host. It commonly has two or even four embryos, each producing its hypocotyl that grows towards the bark of the host under the influence of light and gravity and potentially each forming a mistletoe plant in a clump. Possibly as an adaptation to assist in guiding the process of growing away from the light, the adhesive on the seed tends to darken the bark. On having made contact with the bark, the hypocotyl, with only a rudimentary scrap of root tissue at its tip penetrates it, a process that may take a year or more. In the mean time the plant is dependent on its own photosynthesis. Only after it reaches the host's conductive tissue can it begin to rely on the host for its needs. Later it forms a haustorium that penetrates the host tissue and takes water and nutrients from the host plant (Romer *et al.*, 1977).

Species more or less completely parasitic include the leafless quintral, *Tristerixaphyllus*, which lives deep inside the sugar-transporting tissue of a spiny cactus, appearing only to show its tubular red flowers and the genus *Arceuthobium* (dwarf mistletoe; Santalaceae) which has reduced photosynthesis; as an adult, it manufactures only a small proportion of the sugars it needs from its own photosynthesis, but as a seedling actively photosynthesizes until a connection to the host is established. Some species of the largest family, Loranthaceae, have small, insect-pollinated flowers as with Santalaceae, but others have spectacularly showy, large, bird-pollinated flowers (Milius, 2002).

Most mistletoe seeds are spread by birds that eat the (drupes). Quite a range of birds feed on them, of which the mistle thrush is the best-known in Europe, the Phainopepla in southwestern North America, and Dicaeum of Asia and Australia. Depending on the species of mistletoe and the species of bird, the seeds are regurgitated from the crop, excreted in their droppings, or stuck to the bill, from which the bird wipes it onto a suitable branch. The seeds are coated with a sticky material called viscin. Some viscin remains on the seed and when it touches a stem, it sticks tenaciously. The viscin soon hardens and attaches the seed firmly to its future host, where it germinates and its haustorium penetrates the sound bark (Duong *et al.*, 2003).

Specialist mistletoe eaters have adaptations that expedite the process; some pass the seeds through their unusually shaped digestive tracts so fast that a pause for defecation of the seeds is part of the feeding routine. Others have adapted patterns of feeding behaviour; the bird grips the fruit in its bill and squeezes the sticky-coated seed out to the side. The seed sticks to the beak and the bird wipes it off onto the branch (Maurice and Robert, 2002). Biochemically, viscin is a complex adhesive mix containing cellulosic strands and mucopolysaccharides.

Once a mistletoe plant is established on its host, it usually is possible to save a valuable branch by pruning and judicious removal of the wood invaded by the haustorium, if the infection is caught early enough. Some species of mistletoe can regenerate if the pruning leaves any of the haustorium alive in the wood (Torngren *et al.*, 1980).

Mistletoe was often considered a pest that kills trees and devalues natural habitats, but was recently recognized as an ecological keystone species, an organism that has a disproportionately pervasive influence over its community. A broad array of animals depend on mistletoe for food, consuming the leaves and young shoots, transferring pollen between plants, and dispersing the sticky seeds. In western North America their juicy berries are eaten and spread by birds (notably Phainopepla, or silky-flycatcher). When eaten, some seeds pass unharmed through their digestive systems; if the birds' droppings happen to land on a suitable branch, the seeds may stick long enough to germinate. As the plants mature, they grow into masses of branching stems which suggest the popular name "witches' brooms". The dense evergreen witches' brooms formed by the dwarf mistletoes (*Arceuthobium* species) of western North America also make excellent locations for roosting and nesting of the northern spotted owl and the marbled murrelet. In Australia the diamond fire tail and painted honeyeater are recorded as nesting in different mistletoes. This behaviour is probably far more widespread than currently recognized; more than 240 species of birds that nest in foliage in Australia have been recorded nesting in mistletoe, representing more than 75% of the resident birds (Gilbert, 2000). A study of mistletoe in junipers concluded that more juniper berries sprout in stands where mistletoe is present, as the mistletoe attracts berry-eating birds which also eat juniper berries. Such interactions lead to dramatic influences on diversity, as areas with greater mistletoe densities support higher diversities of animals. Thus, rather than being a pest, mistletoe can have a positive effect on biodiversity,

providing high quality food and habitat for a broad range of animals in forests and woodlands worldwide (Millus, 2002).

MATERIALS AND METHODS

CHEMICALS

Streptozotocin, chloroform, ethanol and other chemicals were obtained from Fam-lab Nigeria Limited and Lixok-k chemicals, Akure. Alanine transaminases (ALT), Aspartate transaminase (AST), Serum albumin were obtained from Randox Laboratories Limited, UK. Accu-check glucometer kit was purchased from De-shalom pharmacy, Ilesa, 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 (40Iu/ml) marketed by May and Baker, was obtained from a registered pharmacist in De-shalom pharmacy, Ilesa, Osun State, Nigeria and used for the study.

EXPERIMENTAL ANIMALS

Adult sixty (60) female albino rats weighing between 90-250g 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 weight into six study groups of eight (8) rats each. Normal feeds and tap water were given to the rats ad-libitum while food and water intake were noted. They were kept in plastic cages of 8 rats per cage, placed in a well ventilated animal house of Joseph Ayo Babalola University at normal temperature of 30-35°C. The cages were cleaned daily and 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

Fresh leaves of *Loranthusbengwensis*(mistletoe) were obtained from kolanut tree in Ikeji-Arakeji plantation, Osun state, Nigeria. The plant samples were taken to the department of Botany, ObafemiAwolowo University (O.A.U) for identification and authentication.

PREPARATION OF PLANT EXTRACTS

*Loranthusbengwensis*leaves were sorted, washed to remove dirts 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

150g of the powdered extract was soaked in 98% of ethanol in 250ml at room temperature for 72 hours after which the mixture was filtered. The filtrate was placed in centrifuge at 540rev/min for 30 minutes. The centrifuged sample formed 2 layers; the upper layer was then poured inside another beaker. It was boiled at 60°C 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 (Accu Check Active, 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 the rats in each groups are 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 100mg/kg of *Loranthus bengwensis* ethanolic leaf extract. Group E: Designated as DLB2 consisted of diabetic rats administered 200mg/kg of *Loranthus bengwensis* ethanolic leaf extract. Group F: Designated as DLB3 consisted of diabetic rats administered 400mg/kg of *Loranthus bengwensis* ethanolic leaf 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 4000g for 15 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 coloured hydrazine formed from the reaction of pyruvate with 2,4-dinitrophenylhydrazine at 546 nm.

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.

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).

DETERMINATION OF SERUM ALBUMIN

The method was described by Wright and Plummer (1974) and 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, H_2O_2 is quantitatively measure in a peroxidase catalyzed reaction that produces a colour. Absorbance was measured at 500nm. The colour intensity was proportional to cholesterol concentration.

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.

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), low density lipoproteins (LDL), high density cholesterol (HDL).

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

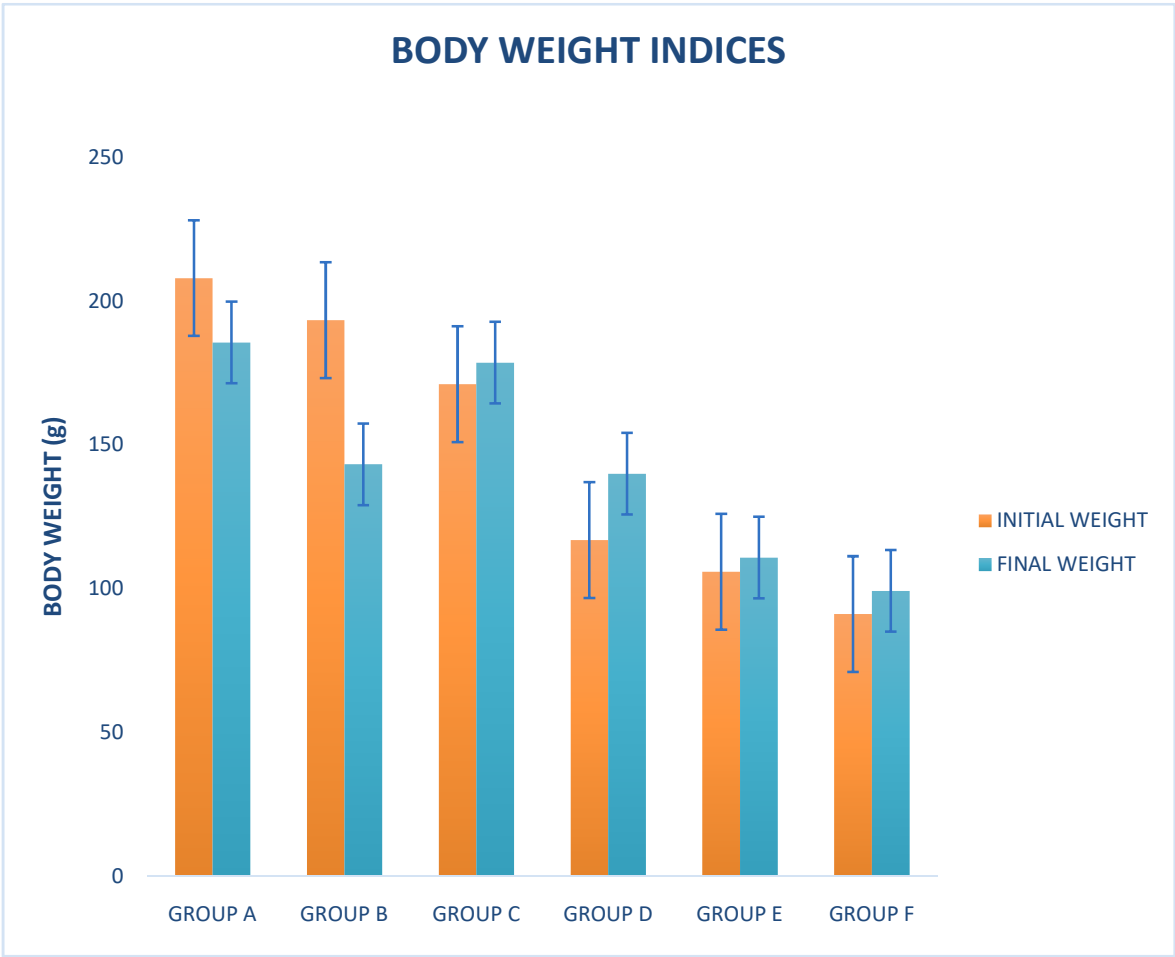
LDL-cholesterol was calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

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

where $[\text{TG}]/5$ was 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 was 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 were 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.

275 **RESULTS**



279 **Values expressed in Mean $\pm$ S.E.M of 8 determinations**

280 **Fig 1.1:** Body weight indices of the experimental rats before and after the experiment.

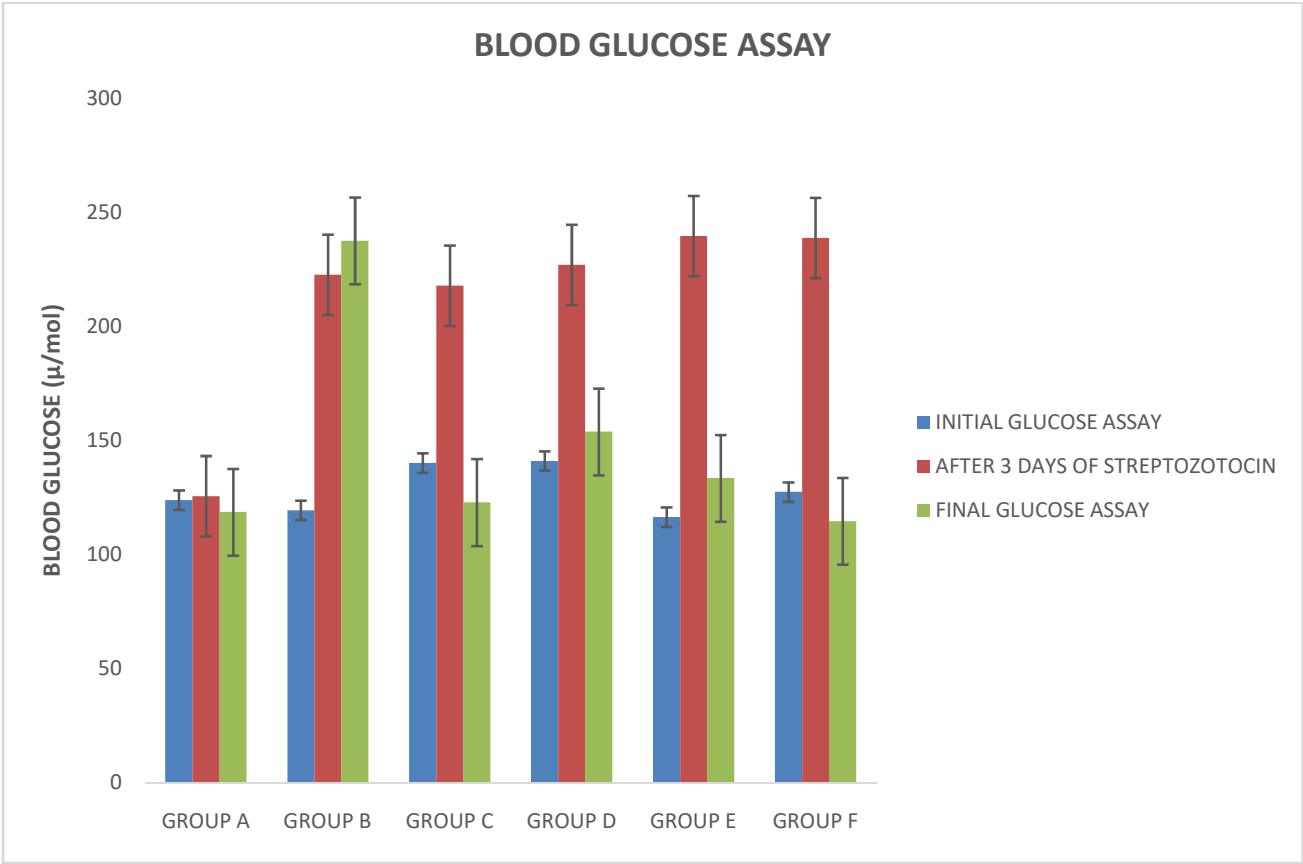
281 **KEY:**

282 INITIAL WEIGHT – Weight of the experimental rats before the commencement of the experiment.

283 FINAL WEIGHT– Weight of the experimental rats at the end of the experiment.

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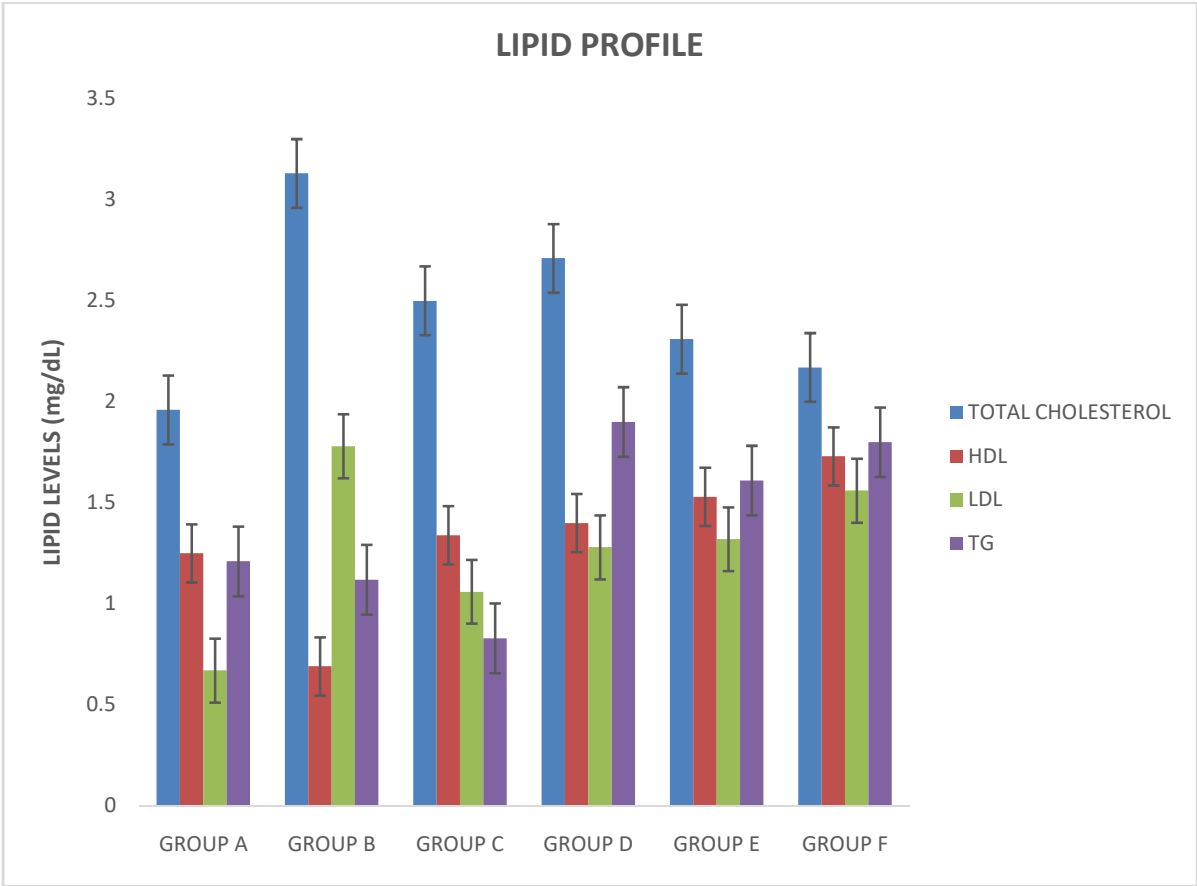
Values expressed in Mean $\pm$ S.E.M of 8 determinations

Fig 1.2: Blood glucose assay of the experimental rats before the experiment, after 3 days of Streptozotocin administration and at the end of the experiment.

KEY:

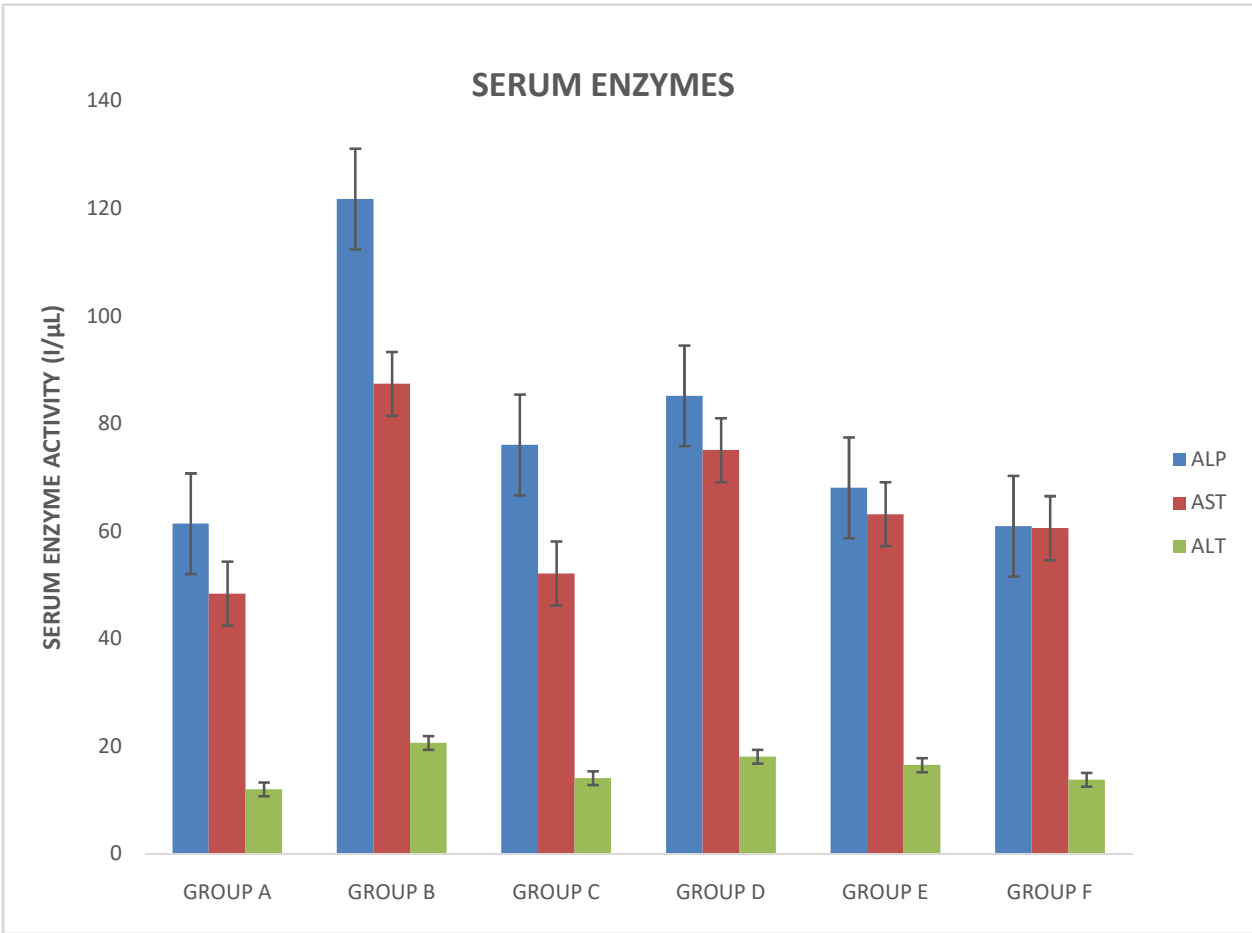
INITIAL GLUCOSE ASSAY – Glucose assay of the experimental rats at the commencement of the experiment.

AFTER 3 DAYS OF STREPTOZOTOCIN – Glucose assay of the experimental rats after administration of 1ml of streptozotocin.



Values expressed in Mean $\pm$ S.E.M of 8 determinations

Fig 1.3: The levels of various lipids in the serum of the rats at the end of the experiment.
KEY:
 TOTAL CHOLESTEROL - Total cholesterol levels in the serum of the rats at the end of the experimental period.
 HDL – High Density Lipoprotein levels in the serum of the rats at the end of the experimental period.
 LDL – Low Density Lipoprotein levels in the serum of the rats at the end of the experimental period.
 TG – Triglyceride levels in the serum of the rats at the end of the experimental period.



Values expressed in Mean $\pm$ S.E.M of 8 determinations

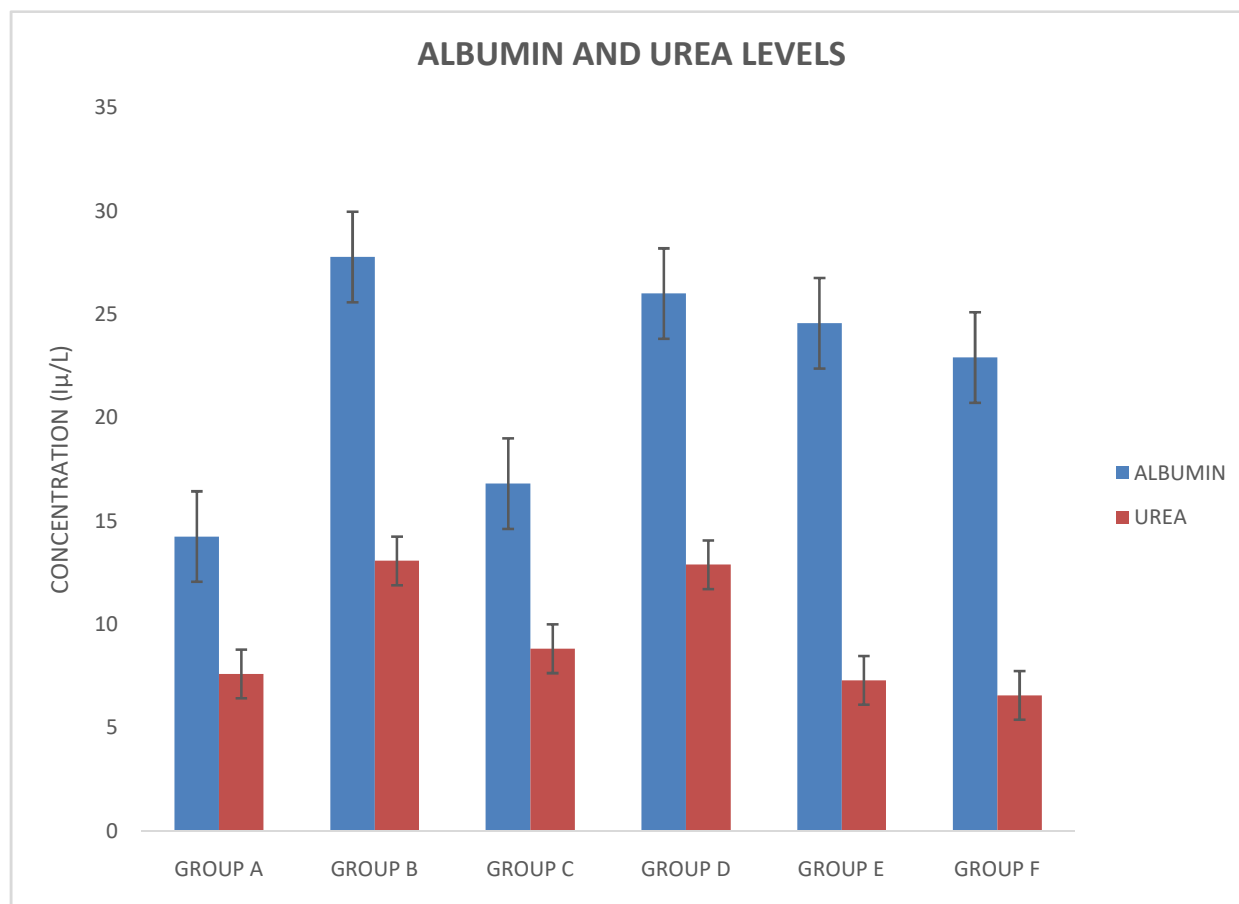
Fig 1.4: Serum enzyme activity at the end of the experiment.

KEY:

ALP – Alkaline phosphatase levels in the serum of the rats at the end of the experimental period.

AST – Aspartate transaminase levels in the serum of the rats at the end of the experimental period.

ALT – Alanine transaminase levels in the serum of the rats at the end of the experimental period.



Values expressed in Mean $\pm$ S.E.M of 8 determinations

Fig 1.5: Albumin and urea activity in the blood of the experimental rats at the end of the experiment.

KEY:

ALBUMIN – Albumin levels in the serum of the rats at the end of the experimental period.

UREA – Urea levels in the serum of the rats at the end of the experimental period.

Statistical analysis

Data obtained were analysed using microsoft excel 2007 and expressed as mean SEM. The statistical package SPSS version 16.0 was used to establish statistical significance at $p < 0.05$

RESULTS AND DISCUSSION

The lipid profiles of the serum of the experimental rats were investigated for HDL, LDL, TG and total cholesterol levels respectively. A low total cholesterol level was observed in the serum of the rats in the treated groups at the end of the experiment compared to the positive control group. In Groups IV and V, there was a decrease in the total cholesterol level as compared to the negative control. This decrease was however statistically insignificant at $P < 0.05$. At a higher

concentration in Group VI (400mg/kg), there was an increase in the cholesterol level compared to the negative control, which was significant at $P < 0.05$. According to Bhatnagar *et al.*, (2008), high cholesterol levels could be due to a combination of genetic and environmental factors. It also has secondary causes which includes type 2 diabetes mellitus. Hence, the administration of the extract at 100mg/kg (Group I) and 200mg/kg (Group V) is effective enough to reduce the cholesterol levels in the serum as observed in the experimental rats. This decrease was more noteworthy, at $P < 0.05$, in the orthodox drug- treated group (Group III), when compared to the negative control group (Group B), than in the groups treated with the ethanolic leaf extract of *Loranthus bengwensis* (Group IV, V and VI).

HDL (Good cholesterol) is an essential lipoprotein which is required to transport fat molecules (including cholesterol) out of the artery walls, reduce macrophage accumulation and thus help prevent cardiovascular diseases etc. while LDL (bad cholesterol) also a lipoprotein but it transports fat molecules into the artery walls, attract macrophages and thus could initiate atherosclerosis and other cardiovascular diseases. The HDL levels in the treated groups was on the increase compared to the positive control group and negative control group, hence illustrating the ability of the plant extract at varying concentrations to help increase HDL levels. LDL levels in the treated groups, (Group IV, V and VI) decreased significantly at $P < 0.05$ compared to the negative control group but however increased when compared to the positive control group. This ultimately shows the ability of ethanolic extract of *Loranthus bengwensis* to reduce LDL levels and increase HDL levels for healthy living in diabetic patients as observed in the treated groups (Groups IV, V and VI) when compared to the diabetic control group (Group II). Also, in the standard drug treated group (Group III), there was an increase in HDL levels and decrease in LDL levels when compared to the negative control group (Group II), this increase and decrease in HDL and LDL levels respectively, was however less notable compared to the increase and decrease observed in the groups treated with the ethanolic leaf extract of *Loranthus bengwensis* (Group IV, V and VI). This is in agreement with Syed *et al.*, (2005) who carried out a research on the anti-diabetic activity of *Terminaliacatappa* linn. Leaf extracts in alloxan-induced diabetic rats and observed that HDL and LDL levels increase and decrease respectively when compared to the diabetic control group. Triglyceride as a blood lipid helps in the bidirectional transference of adipose fat and blood glucose from the liver. TG levels in the extract treated groups are higher than the levels in the negative and positive control groups. Hence, it can be said that the ethanolic leaf extract of *Loranthus bengwensis* initiates an increase in TG levels in the serum. However, there was a decrease observed in the orthodox treated group (Group III), when compared to the negative control group (Group II), than in the groups treated with ethanolic leaf extract of *Loranthus bengwensis* (Group IV, V and VI). This is in line with a research carried out by Syed *et al.*, (2005) on the anti-diabetic activity of *Terminaliacatappa* linn. leaf extracts in alloxan-induced diabetic rats and observed an increase in TG levels of the extract treated group compared to the normal control group.

At $P < 0.05$, there was a statistically significant increase in the ALP activity in the serum of the rats in the treated groups as compared to the non-diabetic control group (Group I) but a decrease was observed compared to the diabetic control group (Group II). An increase in ALP levels in the serum indicates large bile duct obstruction, intrahepatic cholestasis or other diseases of the liver (Berg *et al.*, 2006). However, in Group VI (400mg/kg), there was a decrease in ALP levels as compared to diabetic control group, hence, providing optimum efficiency to eradicate other complementary diseases associated with diabetes mellitus in relation to ALP level. ALP levels in

the diabetic control group is high compared to all other experimental groups because of the diabetic condition of the rats in this group and hence, hepatocellular damage results causing an inevitable increase in the serum enzyme, ALP. This decrease was more noteworthy, at $P < 0.05$, in the groups treated with the ethanolic leaf extract of *Loranthusbengwensis* (Group IV, V and VI) when compared to the negative control group (Group II) than in the orthodox drug-treated group (Group III). This observation is in agreement with Oluwatosin *et al.*, (2012) who carried out an experiment to show the action of *Viscum album* in promoting carbohydrate metabolism and observed that ALP levels increase in the extract treated group when compared to the normal control group. Serum AST and ALT is commonly used as indicators of hepatocellular damage. These enzymes are present in the liver in high concentrations and in conditions of hepatic intoxications leak into circulation, thus increasing the serum level of the enzymes (Goel and Sodd, 1989). The significant increase in the concentrations of these enzymes may be due to acute hepatocellular damage which may terminate into circulation. The extent of aminotransferase elevations in the serum depends on the severity of the damage, which will invariably cause an increase in cell permeability. Comparing the ALT and AST levels of the non-diabetic control group (Group I) with the treated groups (Group IV, V and VI), a sharp increase was observed statistically insignificant at $P < 0.05$. In the negative control, there was an increase in the levels of ALT and AST when compared to the positive control. This is due to the diabetic condition of the rats in this group resulting in hepatocellular damage and hence, amount to increase in serum enzymes (ALT and AST) levels. The treated groups, when compared to the negative control group showed a significant decrease at $P < 0.05$. This can be attributed to the ability of ethanolic leaf extract of *Loranthus bengwensis* to ameliorate the diabetic condition in these rats and hence, restoring the body system of the rats back to normal as compared to the positive control group. A more considerable decrease was observed in Group VI (400mg/kg) when compared to the negative control group (Group II). However, there was also a decrease observed in ALT and AST levels of the rats in the orthodox treated group (Group III) when compared to the negative control, this decrease was relatively less evident when compared to the groups treated with ethanolic leaf extract of *Loranthus bengwensis* (Groups IV, V and VI). The significant increase in serum enzyme activity of ALT and AST in the treated groups IV, V and VI compared to the positive control group at $P < 0.05$ level is in agreement with Oluwatosin *et al.*, (2012) who carried out an experiment to show the action of *Viscum album* in promoting carbohydrate metabolism and also observed that ALT and AST levels increase in the extract treated groups when compared to the normal control group.

There was an increase in the albumin levels of the rats in all treated groups when compared to the non-diabetic control group (Group I) but a decrease was observed when compared to the negative control group (Group II). This, therefore, according to Green *et al.*, (2012), indicates liver or kidney disease associated with diabetes. In these treated groups, it is observed that administration of the ethanolic extract of *Loranthus bengwensis* orchestrated a sharp decrease in the albumin levels compared to the diabetic control group (Group II) and hence, returning the body system of these rats back to normal as observed when compared to the positive control group. However, this decrease was significant at $P < 0.05$ when compared to the negative control group (Group II), in the groups treated with the ethanolic leaf extract of *Loranthus bengwensis* (Group IV, V and VI) than in the orthodox drug-treated group (Group III).

Urea is an important metabolite in the urine which is the major nitrogen containing compound in the urine. It plays a role as a major carrier of nitrogen. There was a statistically insignificant

decrease ($p < 0.05$) in the urea levels of the extract treated groups, IV, V and VI compared to the negative control group. This may be due to the ability of the plant extract to reduce urea levels in the blood serum of the rats, at varying concentrations, maybe by facilitating their excretion through the urine. Moreover, the effect of the ethanolic extract of *Loranthus bengwensis* on the experimental rats considering the various parameters illustrated a better activity than the effect of the standard drug as can be deduced from the results so far obtained.

CONCLUSION

This research work has shown that the ethanolic leaf extract of *Loranthus bengwensis* has antidiabetic activity on the streptozotocin induced diabetic rats. However, its antidiabetic activity is dose-dependent, i.e. its efficacy is more prominent at a high concentration as observed in the results obtained from the various assessments. It can therefore be said that the higher the concentration of *Loranthus bengwensis*, the more efficient its antidiabetic activity on diabetic patients.

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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