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Ameliorative properties of ethyl acetate fraction of *Ceiba pentandra* on serum glucose, hematological and biochemical parameters of diabetic rats

Muhammad Hadiza Lami[1*], Kabiru Adamu Yusuf[2], Saidu Abubakar Ndaman[2], Busari Musa Bola[3], Babatunde Olajumoke Damilola[1], Abdullah Abubakar Siddique[4]

[1]*Toxicological Unit, Department of Biochemistry, Federal University of Technology, Minna, Nigeria*

[2]*Medical Biochemistry Unit, Department of Biochemistry, Federal University of Technology, Minna, Nigeria*

[3]*Centre for Genetic Engineering and Biotechnology, Global Institute for Bioexploration Unit (GIBEX),Federal University of Technology, Minna, Nigeria*

[4]*Federal Medical Centre, Bida, Niger State, Nigeria*

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ABSTRACT

Objective: To evaluate the antidiabetic potential of *Ceiba pentandra* leaves used by some Nupe speaking community of Niger State, Nigeria in folkloric management of diabetes.
Methods: Fifteen albino rats of both sexes weighing between 100 and 160 g were randomly allotted to five groups of four rats each. Alloxan monohydrate (110 mg/kg body weight) was intraperitoneally administered to rats in their respective groups, and rats with blood glucose (200 mg/kg body weight) were considered diabetic. Diabetic rats in their respective groups received 2.5 mg/kg body weight of standard drug (glibenclamide), 200 and 400 mg/kg body weight of extract once daily for 12 days. Normoglycemic group (reference group I) received 0.5 mL normal saline, while the last group was untreated diabetic (reference group II). The blood glucose was measured by using Accu-Chek Active glucometer every three days and the experiment was terminated at 17th day.
Results: Blood glucose decreased significantly ($P < 0.05$) in all the treated groups during the period of treatment with highest hypoglycaemic activity observed in 200 mg/kg body weight group. The diabetic untreated group showed significant reduction ($P < 0.05$) in body weight as it was a clinical feature of diabetes in reference to normoglycemic, and other treatment groups. Platelets showed a significant decrease and increase respectively in the untreated and treated groups in reference to the normoglycemic group. Decreased packed cell volume, red blood cells and hemoglobin count, and an increase in white blood cell were also observed in the untreated group. Body weight of the treated groups remained stable as against the reference group II. Activities of the serum alanine aminotransferase, aspartate aminotransferase, chloride and potassium increased significantly ($P < 0.05$) in standard drug while carbonate and sodium showed the opposite. The urea, creatinine, total and conjugated bilirubin all increased significantly ($P < 0.05$) in the standard drug and untreated groups. Total cholesterol, triglyceride increased and high density lipoprotein increased in all groups against the untreated group.
Conclusions: Both doses of ethyl acetate fraction of *Ceiba pentandra* were able to reduce the blood glucose and ameliorate the hematological abnormalities associated with diabetes mellitus.

1. Introduction

Ethnobotany is well-known as a useful way to discover future medicines. In 2001, researchers discovered 122 compounds used in modern medicine that were ethnomedical sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant[1]. Many of the pharmaceuticals have long history of use as herbal remedies (*e.g.*, aspirin, digitalis, quinine, and opium). Diabetes mellitus is a metabolic disorder that is characterized by insulin deficiency, insulin resistance or both[2], leading to elevation in blood glucose level. It is one of the widespread metabolic disorders affecting about 2.8% of the world's population and is anticipated to cross 5.4% by the year 2025[3]. Back in the ages, herbal medicines have been a helpful source of medicine and have become a growing part of modern, high-tech medicine. Research through literature sources shows that about 65 species of plants have hypoglycemic properties, and are available in various database with proper categorization according

*Corresponding author: Muhammad Hadiza Lami, Toxicological Unit, Department of Biochemistry, Federal University of Technology, Minna, Nigeria.
Tel: +2348035960300
E-mail: khadijahlami@gmail.com

to the parts used, mode of reduction in blood glucose and active phytoconstituents that have insulin mimetic activity[4]. Amidst the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived[1]. More than two thirds of the world's plant species are estimated to have medicinal value, and these come from the developing countries.

Ceiba pentandra (*C. pentandra*) originated in the American tropics. It belongs to the family Malvaceae, and is well known as silk cotton tree[5]. In Africa especially Nigeria, it is used as a soup sauce, and in the traditional medicine it is used to against several infections and disorders. Different morphological parts of the plant have different uses. Folk medicine in Northern Nigeria uses the plant to treat hypertension, headache, dizziness, fever, peptic ulcers, leprosy and diabetes. In India and Malaysia, it is used for the treatment of bowel ailment and diarrhoea[6]. According to Friday *et al.*[7], *C. pentandra* has significant amount of phenolic compounds, alkaloids, flavonoids, tannins, saponins, phytate, oxalate, trypsin inhibitor, and hemaglutinin. Toxicological studies of the plant extracts showed its low toxicity profile and that it was relatively safe as oral medication[8]. Hypoglycemic and hypolipidemic effects of feed prepared with *C. pentandra* leaves were investigated in alloxan induced diabetic rats[9]. Protective activity of ethyl acetate fraction of methanol extract in stem bark of *C. pentandra* against paracetamol-induced liver damage in rats has also been reported by Bairwa *et al.*[10]. This research was therefore designed to evaluate the effectiveness of ethyl acetate fraction of *C. pentandra* leaf extract as a hypoglycemic agent, and the attendant toxicological effects thereof.

2. Materials and methods

2.1. Plant sample

Fresh leaves of *C. pentandra* were collected in July 2014 from Gada Oli, Wawa, in New Bussa Local Government, Niger State, Nigeria. The leaves were authenticated at the herbarium section of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.

2.2. Preparation of plant material

The leaves were air-dried at room temperature in the Departmental Laboratory for 14 days and homogenized into fine powder with a milling machine.

2.3. Ethanol extraction of powdered leaf

Exactly 1400 g of the dried powder was weighed and extracted with ethanol by using the reflux extraction method at temperature of 60 ℃. A rotary evaporator was afterwards used to evaporate the solvent and later concentrated in a water bath at 100 ℃. The extract was kept in a labeled sterile bottle and stored in the refrigerator at -4 ℃ till required for use.

2.4. Partitioning of the fraction

The crude ethanol extract (40.8 g) was subjected to fractionation by solubilization in water and sequential partitioning with 700 mL ethyl acetate[11]. The fraction thus obtained was evaporated to dryness by using rotary evaporator.

2.5. Experimental animals

Swiss albino rats weighing 100-160 g were obtained from the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were kept under standard environmental conditions in the laboratory to acclimatize for two weeks with access to commercial vital grower feed and water *ad libitum*.

2.6. Induction of diabetes with alloxan

Alloxan monohydrate (110 mg/kg body weight) was administered intraperitoneally to each group of rats and thereafter two hours oral administration of 5% glucose solution was administered to overcome death from hypoglycemia[12]. All experimental animals were allowed access to food and water afterwards, and maintained under the same laboratory conditions throughout the experimental period. The diabetic state was confirmed by the measurement of fasting blood glucose concentration 72 h after the induction using Accu-Chek Active glucometer by collecting blood from the tail tip of the rats.

2.7. Grouping of animals

The animals were grouped into five of four rats each. Group 1 received 0.1 mL of normal saline (normoglycemic). Group 2 was diabetes untreated (DUT). Group 3 was diabetic rats treated with standard drug (2.5 mg/kg body weight glibenclamide). Group 4 was diabetic treated rats with 200 mg/kg body weight ethyl acetate fraction. Group 5 was diabetic treated rats with 400 mg/kg body weight ethyl acetate fraction.

2.8. Treatment with extract

Treatment with extract and standard drug lasted for 12 days. Animals in Groups 4 and 5 were orally administered ethyl acetate fraction (200 mg/kg and 400 mg/kg body weight) respectively, while Group 3 rats were orally administered 2.5 mg/kg body weight standard drug once daily for 12 days. The blood glucose was measured with Accu-Chek Active glucometer at every three days with blood obtained from the tail vein of rats. The experimental animals were sacrificed on the 17th day.

2.9. Animal euthanization

At the end of 17 days, the animals were anaesthetized under ether. The blood sample was collected by cardiac puncture into heparinized tubes for biochemical test and into ethylene diamine tetraacetic acid bottles for determination of hematological parameters. The blood samples were centrifuged at 1000 r/min for 5 min and the clear supernatant was used for the estimation of lipid profile, liver and kidney function tests.

2.10. Determination of hematological parameters

The total red blood count (RBC), hemoglobin concentration (HGB), white blood count (WBC), platelet count (PLT), mean platelet volume

(MPV), platelet distribution width (PDW), mean cell volume (MCV), red cell distribution width-coefficient of variation (RDW-CV), red cell distribution width-standard deviation (RDW-SD), packed cell volume (PCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were determined by using Auto Hematology Analyzer.

2.11. Determination of plasma lipid profiles

The plasma total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined by using Randox diagnostic kits.

2.12. Determination of the activities of liver and kidney indices

Activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were determined by Randox diagnostic kits. Same kits were also used to determine total protein (TP), total bilirubin, creatinine, urea albumin, and serum electrolytes.

2.13. Statistic analyses of data

The results were analyzed by using *t*-test of SPSS version 19.0. All the data were expressed as mean ± SEM of triplicate readings and the difference between groups was considered significantly different at 95% confidence level ($P < 0.05$).

3. Results

Significant increase in MCV was observed in both extract treated groups in reference to the normoglycemic group (Table 1). However, there was a significant decrease ($P < 0.05$) in MCH, PLT, HGB, PCV, and RBC of the untreated group in reference to the normoglycemic group.

Table 1

Variation in hematological parameters among treated groups.

Sample	NMG	DUT	STD	200 mg/kg body weight	400 mg/kg body weight
MCV (fL)	57.33 ± 2.29^a	56.76 ± 1.41^a	57.90 ± 1.21^a	58.86 ± 0.72^a	58.86 ± 0.72^a
MCH (pg)	20.53 ± 0.84^a	19.60 ± 0.28^a	19.80 ± 0.23^a	20.76 ± 0.49^a	20.93 ± 0.13^a
MCHC (g/dL)	35.86 ± 0.56^a	36.83 ± 0.49^a	36.06 ± 0.40^a	35.86 ± 0.77^a	35.26 ± 0.27^a
RDW-SD (fL)	42.26 ± 2.54^a	43.90 ± 2.09^a	42.26 ± 1.76^a	43.56 ± 3.83^a	42.36 ± 0.86^a
RDW-CV (%)	17.70 ± 0.72^a	17.60 ± 1.02^a	17.90 ± 0.56^a	18.67 ± 0.77^a	18.26 ± 0.23^a
PDW (fL)	10.03 ± 0.38^a	10.80 ± 0.28^a	10.03 ± 0.37^a	9.86 ± 0.12^a	10.90 ± 0.20^a
PLT (× 10^9/L)	173.66 ± 8.41^a	71.00 ± 3.78^b	186.00 ± 5.90^a	194.66 ± 9.14^a	190.66 ± 8.89^a
MPV (fL)	6.46 ± 0.27^a	6.50 ± 0.28^a	6.53 ± 0.02^a	6.56 ± 0.14^a	6.57 ± 0.23^a
WBC (× 10^9/L)	7.50 ± 2.25^a	12.26 ± 5.33^b	7.46 ± 1.41^a	8.23 ± 0.88^a	9.70 ± 2.11^a
HGB (g/dL)	13.43 ± 3.18^a	6.66 ± 0.31^b	9.06 ± 0.99^a	10.20 ± 0.10^a	11.50 ± 1.35^a
PCV (%)	37.83 ± 9.60^a	17.70 ± 1.38^b	35.70 ± 5.38^a	36.86 ± 4.60^a	35.03 ± 2.61^a
RBC (× 10^{12}/L)	6.65 ± 1.76^a	1.60 ± 0.28^b	4.58 ± 0.45^a	4.98 ± 0.12^a	5.56 ± 0.72^a

Values with the same superscript are not significantly different ($P > 0.05$) along the row. NMG: Normoglycemic; STD: Standard drug.

There was a significant reduction ($P < 0.05$) in fasting blood sugar of animals in the treatment groups with 200 mg/kg body weight having the highest (49.00 ± 1.56) hypoglycaemic activity on the 12th day of the treatment (Figure 1).

There was appreciable gain in body weight of experimental rats 8 days after the commencement of the treatment except for the untreated group which showed considerable weight loss that was a clinical feature of diabetes (Figure 2).

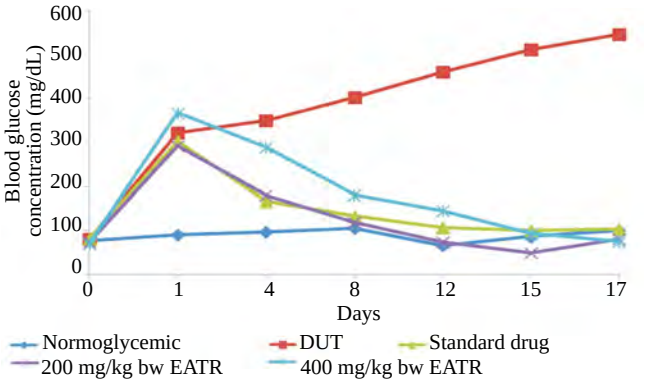

Figure 1. Effect of ethyl acetate fraction of *C. pentandra* leaf on fasting blood glucose of rats in all groups.

EATR: Ethyl acetate fraction treated rats; bw: Body weight.

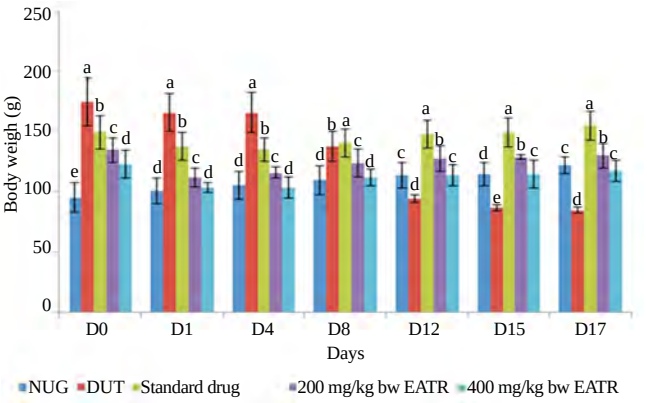

Figure 2. Mean body weight of rats in all groups.

NUG: Normoglycaemic. Values are mean ± SEM ($n = 5$). Values with different alphabet on the chart are significantly different at $P < 0.05$.

There was a significant elevation in the activity of all enzymes in the extract treated group as compared to significant elevation in its activity in the untreated group (Figure 3).

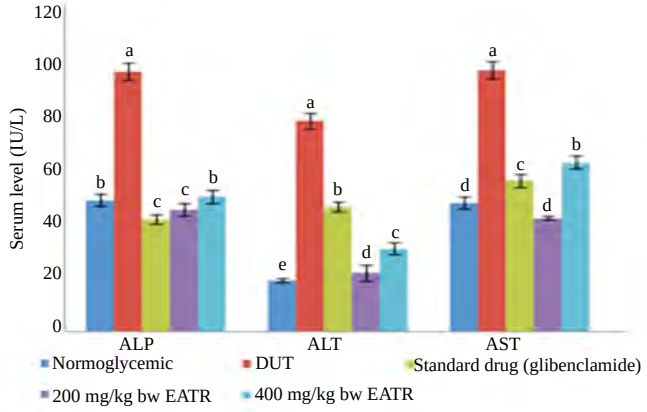

Figure 3. Comparative effects of ethyl acetate fraction of *C. pentandra* leaf on the activities of ALT, ALP and AST.

Values are mean ± SEM ($n = 5$). Values with different alphabet on the chart are significantly different at $P < 0.05$.

TP was significantly elevated ($P < 0.05$) in the extract treated (the highest value was seen in 200 mg/kg body weight) groups as opposed to the reduction seen in the untreated group (Figure 4). Significant increase ($P < 0.05$) in urea and creatinine was also observed in the diabetic untreated group as compared to other treated groups (Figure 4).

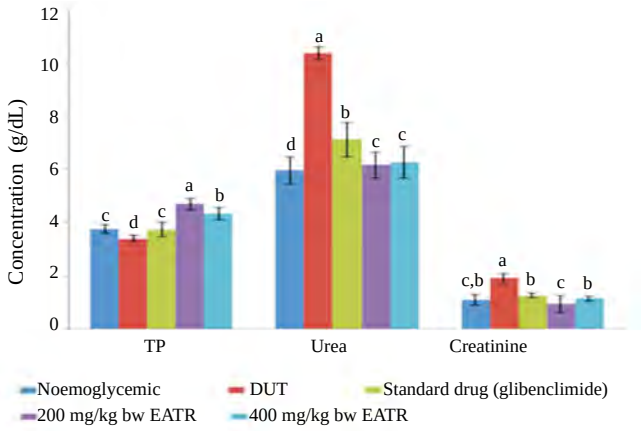

Figure 4. Comparative effects of ethyl acetate fraction of *C. pentandra* leaf on TP, urea, and creatinine.
Values are mean ± SEM (n = 5). Values with different alphabet on the chart are significantly different at $P < 0.05$.

Bicarbonate levels were elevated in the treated groups as opposed to reduction seen in the untreated group, while chloride, sodium, and potassium levels did not show any significant differences in their values in comparison with the normoglycemic group. But a significant increase was observed in the chloride and potassium levels of the diabetic untreated groups in comparison with the normoglycemic and treated groups (Figure 5).

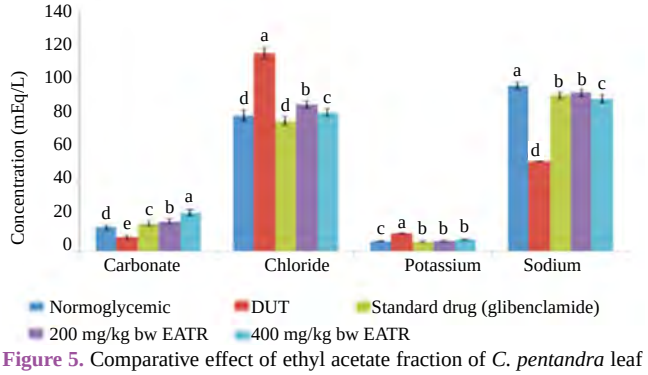

Figure 5. Comparative effect of ethyl acetate fraction of *C. pentandra* leaf on serum electrolytes in all groups.
Values are mean ± SEM (n = 5). Values with different alphabet on the chart are significantly different at $P < 0.05$.

There was significant ($P < 0.05$) increase in total and conjugated bilirubin in untreated, standard drug and 400 mg/kg body weight groups as opposed to the normoglycemic group. Albumin decreased only in untreated group (Figure 6).

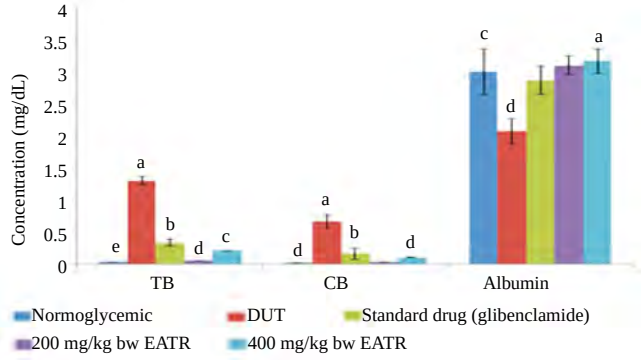

Figure 6. Effect of ethylacetate fraction of *C. pentandra* albumin, total bilirubin, and conjugated bilirubin.
TB: Total bilirubin; CB: Conjugated bilirubin. Values are mean ± SEM (n = 5). Values with different alphabet on the chart are significantly different at $P < 0.05$.

Significant ($P < 0.05$) increase was also observed in TC, TG and LDL of diabetic control group compared to other treated groups while the reverse occurred in HDL (Figure 7).

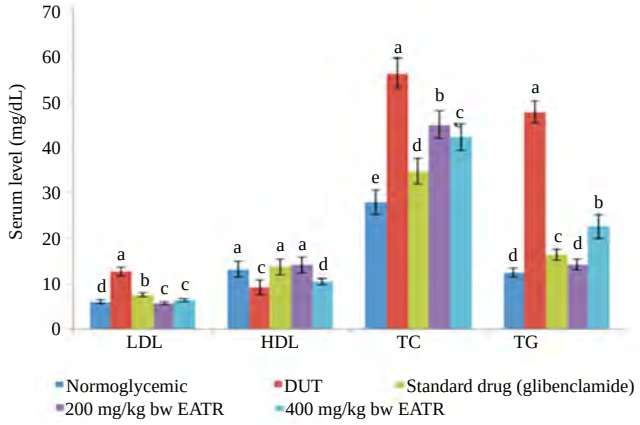

Figure 7. Comparative effect of ethyl acetate fraction of *C. pentandra* leaf on lipid profile.
Values are mean ± SEM (n = 5). Values with different alphabet on the chart are significantly different at $P < 0.05$.

4. Discussoion

All types of diabetic conditions are characterized by hyperglycemia, and excess glycogen accumulation in the liver of 80% diabetic patients. Glycogen synthesis in the liver is impaired in diabetes due to defective activation of glycogen synthase. Hyperglycemia was established in rats three days (termed Day 1) after induction, and oral administration of the extracts and standard drug (glibenclamide) commenced immediately.

The hypoglycemic activity according to Frederick *et al.*[13] may be due to the ability of the plant extract to inhibit endogenous glucose production or interfere with gastrointestinal glucose absorption[14]. It is postulated that insulin is metabolized by insulinase in the liver, kidney, and placenta and about 50% of insulin secreted by the pancreas is removed by first-pass extraction in the liver. Insulin promotes glycogen synthesis (glycogenesis) in the liver and inhibits its breakdown (glycogenolysis). It promotes protein, cholesterol, and TG synthesis and stimulates formation of very-low-density lipoprotein (VLDL) cholesterol. It also inhibits hepatic gluconeogenesis, stimulates glycolysis, and inhibits ketogenesis. The liver is the primary target organ for glucagon action, where it promotes glycogenolysis, gluconeogenesis, and ketogenesis[15]. According to Etuk *et al.*[16], shunting of excess intracellular glucose into the hexosamine pathway might also cause manifestation of diabetic complications. The islets of Langerhans secrete insulin, and its deficiency or insensitivity of its receptors plays a role in all forms of diabetes mellitus. Many cells in the body, including fat, liver, and muscle cells, have specific cell membrane insulin receptors, and insulin facilitate the uptake and utilization of glucose by these cells. Glucose rapidly equilibrates between the liver cytosol and the extracellular fluid. Transport into certain cells, such as resting muscle, is tightly regulated by insulin, whereas uptake into the nervous system is not insulin-dependent. The hypoglycemic activity of stem bark decoction of *C. pentandra* has been previously reported by Friday *et al.*[7], while Saif-ur-Rehman *et al.* reported that its root bark extract has hypoglycemic effect in normal and alloxan-induced

diabetic rats[17]. The result of this research agrees with them.

The weight gain in the treated groups is thus an indication of the ameliorating effect of the extract. Weight gain at 200 mg/kg body weight may be attributed to glucose utilization and correlates with glucose reduction at the same dose.

ALP is a maker enzyme for the plasma membrane and endoplasmic reticulum, and is often employed to assess the integrity of the plasma membrane[18]. The increase in ALT can also be a clinical feature of diabetic condition[19]. This result is also in line with the earlier work of Jamil *et al.* where ethyl acetate fraction of *C. pentandra* stem bark was administered to rats against paracetamol-induced liver damage[11]. ALT and AST are enzymes found mostly in the cells of the liver and kidney. Much smaller amounts are also found in the heart and muscles. In healthy individuals, ALT and AST levels in the blood are low. When the liver is damaged, they are released into the blood stream, usually before more obvious signs of liver damage occur, such as jaundice. Activity of ALT increased in all groups with the highest and mild elevation respectively in untreated and 200 mg/kg body weight treated groups. Activity of AST increased in all groups except the 200 mg/kg treated group. Elevation in the activities of the enzymes may be attributed to their greater need for gluconeogenic substrates. However, ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST in rats. The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury, in parts because of the longer half-life of ALT and the greater proportion of AST that is bound to mitochondria[20].

Diabetes mellitus has been associated with altered protein metabolism as much of the structural proteins are used in gluconeogenesis. TP was significantly elevated ($P < 0.05$) in the extract treated (highest value was seen in 200 mg/kg body weight) groups as opposed to the reduction seen in the untreated group as a result of insulin which is an important factor that plays a key role in the maintenance of protein balance. It may stimulate the uptake of aminoacids and protein synthesis, but also inhibit protein degradation[21]. In contrast to TP, urea was elevated in the standard drug treated and diabetic untreated groups but not in the extract treated groups in reference to the normoglycemic group. Urea is the major waste product of protein metabolism, and urea levels are used to detect diseases and disorders that affect the kidney *e.g.*, acute kidney failure. Ammonia released during deamination is removed from the blood by its conversion into urea. In serious liver disease, ammonia accumulates in the blood, and the glomerular filtration of urea is low. Same result is also applicable to creatinine but the 200 mg/kg body weight showed a significant reduction. This agrees with the work of Adesokan *et al.*[22], where administration of *Aloe barbadensis* in juice extract showed a significant decrease in urea and creatinine levels.

Chloride ions work with other electrolytes (potassium, sodium, and bicarbonate) to regulate the amount of fluid in the body and maintain the acid-base balance. The result obtained agreed with the work of Sheriff *et al.* where administration of honey or glucophage alone or their combination showed a significant increase in the diabetic untreated group[23]. The reason for this increase may be a result of hyperglycemia that causes polyuria, and hence dehydration.

Bilirubin is the main bile pigment that is formed from the breakdown of heme in red blood cells. Conjugated bilirubin also known as indirect bilirubin is water soluble and it is synthesized in the liver. Albumin is produced in the liver, and is one of the most abundant proteins in body fluid or plasma. A proper balance of albumin is required to keep fluid from leaking out of blood vessels.

Total and conjugated bilirubin increased in untreated, standard drug and 400 mg/kg body weight groups as opposed to the normoglycemic group. Albumin decreased only in untreated group. Elevation may occur due to excessive hemolysis or destruction of red blood cells as seen in obstruction of biliary tract disease (gallstone), liver diseases such as hepatitis and cirrhosis[24]. Decreased albumin in the untreated group may be a result of increased utilization of proteins for gluconeogenesis in a diabetic situation.

The significant increase in TG seen in diabetic group reflects the abnormalities in lipid metabolism associated with diabetes. Insulin can affect the adipocytes by inhibiting lipolysis and promoting the storage of TG in adipocytes. Lack of it or inadequate utilization (as in diabetic condition) therefore enhances the hydrolysis of TGs to diglycerides, unesterified fattyacids and free glycerol. These fatty acids may diffuse out of the cells or may be re-esterified into TGs for storage or secretion of VLDL. Deficiency of insulin suppressing VLDL leads to hyperlipidemia. High concentration of TGs in circulation causes an increase in the hydrolysis of VLDL to LDL and a concurrent increase in HDL of the treated groups. LDLs carry most of the cholesterol in the blood and are cleared mainly by the hepatic LDL receptors which are increased by insulin[25]. Due to the reduction in the rate of cholesterol removal as a result of insulin deficiency in diabetic state, hypercholesterolemia ensued. This is in line with the work of Bairwa *et al.*[10], where dried powdered leaf of *C. pentandra* was administered orally to rats and the diabetic untreated showed elevated level of LDL and TG and a decrease in HDL.

Hematological complications consist of abnormalities in functions, morphology, and metabolism of erythrocytes, leucocytes, and platelets[26]. Significant increase in MCV was observed in both extract treated groups in reference to the normoglycemic group. However, there was a significant decrease ($P < 0.05$) in MCH, PLT, HGB, PCV, and RBC of the untreated group in reference to the normoglycemic group. This may be as a result of anemia or onset of glycosylation process because the reactive oxygen species generated during alloxan metabolism has being implicated in red cell damage[27]. Anemia is also identified as a common complication of chronic kidney disease, affecting many patients and most common cause of chronic kidney disease in two-thirds of cases is diabetes mellitus[28]. There was a significant increase ($P < 0.05$) in WBC and a corresponding decrease in platelets levels of diabetic untreated group when compared to the normoglycemic and treated groups. Platelets are fragment of cells that participate in blood clotting and initiate repair of blood vessels. They are considered as acute phase reactant to infection and inflammation. The WBC on the other hand measures the total number of white blood cells which defend the blood against the opportunistic infections. The result obtained in this study corresponds with the work of Edet *et al.*[29]. The study shows no significant difference in MCV and MCH and MCHC values indicating absence of microcytic and macrocytic anemia. Significant increase was observed in the PLT of normoglycemic and treated groups compared with diabetic untreated. RDW-CV, RDW-SD, MPV, and PDW showed no significant differences in the entire groups

and this showed a similar result with the earlier work of Johnson *et al*.[30].

Conflict of interest statement

We declare that we have no conflict of interest.

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