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Biochemical Effects of N-Hexane and Chloroform Fractions of *Ceiba pentandra* Leaf Used in the Folkloric Treatment of Diabetes

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Authors' contributions

This work was carried out in collaboration between all authors. Author HLM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors MBB and USO managed the literature searches, analyses of the study performed the biochemical and hematological analysis and author ASA managed the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of this study is to evaluate the antidiabetic properties of n-hexane and chloroform fractions of ethanol extract of *Ceiba pentandra* leaf was investigated in alloxan induced diabetic rats.
Study Design: Experimental study design.
Methodology: Twenty eight healthy albino rats weighing between 100-180 g were randomly allotted to seven groups of four rats each. 100 mg/kg bodyweight (b.w) of alloxan monohydrate was administered intraperitonially to rats and rats with blood glucose >200 mg/kg b.w considered diabetic. 200 and 400 mg/kg b.w of both fractions (n-Hexane and Chloroform) were administered to rats in their respective groups orally once daily for twelve days. The blood glucose was checked after every four days and the experiment was terminated on the seventeenth day. The animals

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were anesthetized under chloroform vapor and the blood samples were collected by carotid puncture into heparin and ethylenediaminetetraacetic acid (EDTA) bottles.
Results & Discussion: Both fractions showed significant (P<0.05) hypoglycemic activity when compared to untreated group, and no significant (P>0.05) gain in bodyweights were observed. The fractions possess hematopoietic activity as there was a significant (p<0.05) increase in platelet count in chloroform extract treated group and white blood cell count in all the groups treated with fractions. All the fractions showed significant (P<0.05) decrease in low density lipoprotein, total cholesterol, triglyceride, alkaline phosphatase, alanine aminotransferase, alkaline aminotransferase, potassium, urea and chloride levels as opposed to the untreated group. 200 mg/kg b.w of n- hexane fraction group depicted more lowering effect (p<0.05) on cholesterol and triglyceride while 400 mg/kg b.w of same extract treated group showed a significant (p<0.05) elevation. Generally, there were no variations in total protein and serum albumin among the groups. Sodium, bicarbonate, high density lipoprotein, total bilirubin, creatinine and conjugated bilirubin showed significant (p<0.05) increase in all treated groups when compared with normoglycaemic group.
Conclusion: n-hexane and chloroform fractions contain potent hypoglycemic and hypolipidemic agents which are dose dependent and capable of reversing hyperglycemia and the abnormalities associated with the pathophysiology of diabetes mellitus.

Keywords: Hyperglycaemia; hypoglycaemia; hypolipidemia; Ceiba pantandra.

1. INTRODUCTION

Diabetes, according to American Diabetes Association [1], is defined as an intricate chronic illness resulting from defect in insulin secretion, insulin action, or both and thereby leading to hyperglycemia. This requires continuous medical care alongside with multifactorial risk reduction strategies beyond glycemic control. Hyperglycaemia can increase oxidative stress by enzymatic and non-enzymatic processes [2]. Increased oxidative stress reduces nitric oxide levels, damages cellular proteins, and promotes leukocyte adhesion to the endothelium while inhibiting its barrier function [3]. The islets of Langerhans in the pancreas plays primary role in the metabolism of glucose by secreting the hormone insulin and glucagon. Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells [4]. Early in the course of diabetes, intracellular hyperglycaemia causes abnormalities in blood flow, increased vascular permeability, decreased the activity of vasodialators, and increased the activity of vasoconstrictors such as angiostensin II, and endothelin-I [5]. The long-term effects of diabetes mellitus include progressive development of the specific vascular damages (micorovascular and macrovascular complications) [6]. Several therapies are use in the treatment of diabetes and limitations of such therapies include: high cost, weight gain, gastrointestinal disturbance, hypoglycemia, and liver toxicity. Based on this, *Ceiba pentandra* leaf used by some ethnic groups in Nigeria as a soup supplement with high nutritional value has been selected for this study. *Ceiba pentandra* is a tropical ethnomedicinal plant commonly called kapok tree, ceiba or silk cotton tree and belongs to the order Malvales and the family Malvaceae. It has a spreading crown, huge trunk, large palm shaped leaves and is a drought deciduous plant [7]. In Nigeria it is known as Akpu by the Igbo tribe found in the south east, in the north it is called Rimi by the Hausa tribe, Kuchi by the Nupes tribe whereas the Yorubas in the west call it Araba or Ogungun. *Ceiba pentandra* is rich in nutrients and phytochemicals and this supports its ethno-medicinal usages as a good source of nutrients and medicine [8]. In Africa, *Ceiba pentandra* is widely used to treat headache, hypertension, dizziness, leprosy, fever, psychological disorder, peptic ulcer and anti-hyperglycaemia [9]. Due to its remarkable therapeutic potential supported by various experimental models, the plant extracts possess various pharmacological properties [10]. The existence of modern medicine in conjunction with herbal medicine is required to improve the lives of people affected with diabetes as naturally available compounds from plants can provide treatment remedies to diabetes. This research was therefore carried out to evaluate the antidiabetic properties of n-Hexane and chloroform fractions of *Ceiba pentandra* leaf and their effects on biochemical and hematological parameters. The choice of n-hexane and chloroform as solvents for extraction is based on their closed polarity, and the aim of the research

is to go further with characterization of the extract that has higher hypoglycaemic and hypolipidemic properties.

2. MATERIALS AND METHODS

Ethical clearance was obtained from Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria.

2.1 Reagents and Chemicals

All the reagents used for the research were of analytical grades and obtained from the Department of Biochemistry, Federal University of Technology, Minna, Nigeria. They include: distilled water, ethanol, n-hexane, chloroform, dimethyl sulfoxide (DMSO), and normal saline.

2.2 Apparatus and Equipments

These include: reflux extractor (SEDI/Minna, Nigeria), milling machine (Gallenhamp/USA), rotary evaporator (Heidolph/Germany), centrifuge (Heidolph/Germany), water bath (Griffin/USA), filter paper (Whatman/UK), measuring cylinders (Minghe/UK), hand gloves (Agary/USA), masking tape (ABRO/UK), beakers (Minghe/UK), syringes (DANA/Nigeria), spatula (Minghe/UK), glucometer (Achu-check), and weighing balance (ADVENTURER/UK).

2.3 Sample Collection

2.3.1 Plant sample

Adequate quantities of fresh leaves of *C. pentandra* were collected from rural area of Gada Oli via wawa New-Bussa, Niger state, Nigeria in the month of April and was identified and authenticated at the Herbarium of Department of Biological, Sciences Federal University of Technology Minna, Nigeria.

2.3.2 Experimental animals

Healthy Swiss Albino rats weighing (100–200 g) were procured from Animal house of Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Nigeria. The animals were accommodated in groups of six in polypropylene cages with stainless steel grill top and a bedding of clean paddy husk. They were housed under standard environmental condition in the Departmental laboratory with free access to commercial grower mesh to feed on *ad libitum*. They were allowed to acclimatize to laboratory conditions for two weeks.

2.4 Sample Preparation

2.4.1 Plant sample

Fresh leaves of *Ceiba pentandra* were washed, blotted and air dried for two weeks in the laboratory to avoid loss of bioactive compounds. The leaves were then milled into powder by milling machine.

2.4.2 Extraction of plant sample

One thousand five hundred (1500 g) of dried powdered sample of *Ceiba pentandra* was weighed and extracted with ethanol using reflux method at temperature 75°C. The ethanol extract was first concentrated using rotary evaporator and then evaporated to dryness in a water bath. The crude extract was stored at 4°C until ready for use. The ethanol extract yielded 130.11 g (9.29%).

2.4.3 Partitioning of crude ethanol extract

40.8 g of crude ethanol extract of *Ceiba pentandra* leaves was fractionated using hexane and chloroform solvent in order of increasing polarity according [11-13]. The crude extract was first homogenized in 100 ml of distilled water and thoroughly extracted with 400 ml of n-hexane and chloroform by consecutive liquid/liquid partition using separating funnel (1000 ml). Both fractions of were dried by evaporating the respective solvent using rotary evaporator. They were stored at 4°C till ready for use. n-Hexane fraction yielded 10.6 g (25.9%) while chloroform fraction yielded 3.1 g (7.5%).

3. ACUTE TOXICITY STUDIES

The acute toxicity test of the plant extracts were carried out by the method of [14]. Eighteen albino rats were fasted for 18 hrs but allowed access to water. They were divided into six groups of three rats each and orally administered extract at varied doses of 100, 150, 250, 1000, 2000 and 5000 mg/kg bodyweights. The animals were monitored for 24 hrs to check for convulsion, salivation, diarrhoea, lethargy, sleep, coma, nervousness and/ or mortality.

3.1 Grouping of Animals

The experimental design consisted of 28 rats, twenty four were rendered diabetic, and four were normoglycaemic (positive control) rats. The diabetic untreated rats (negative control) were administered 10 ml/kg bodyweight of normal saline. The animals were grouped into seven as shown below:

Group 1 - Normoglycemic

Group 2- Diabetic untreated (Negative control)

Group 3- Diabetic treated with standard drug – glibenclamide-Positive control

Group 4- Diabetic treated with 200 mg/kg bodyweight of n-hexane fraction

Group 5- Diabetic treated with 200 mg/kg bodyweight of chloroform fraction

Group 6- Diabetic treated with 400mg/kg bodyweight of n-hexane fraction

Group 7- Diabetic treated with 400mg/kg bodyweight of chloroform fraction

3.2 Induction of Diabetic with Alloxan

Rats were made diabetic by the administration of 100 mg/kg bodyweight of alloxan monohydrate intraperitoneally [15]. The animals were then housed in a controlled facility and allowed to drink 5% glucose solution overnight to overcome the hypoglycemia [16]. The diabetic state was confirmed by the measurement of fasting blood glucose concentration using glucometer with blood collected by tail vein puncture. Animals were considered diabetic at blood glucose level of 200 mg/dl after 72 hours of induction.

3.3 Treatment with Extracts

The treatments were carried out for 12 days and the experiment was terminated on the 17^{th} day. Effect of various extract was checked on blood glucose, and serum biomarkers of experimental rats. The n-hexane extract was dissolved in 7ml DMSO and made up to 10 ml with 3 ml normal saline while chloroform extract and glibenclamide (standard drug) were dissolved in 10 ml normal saline (0.9% NaCl). Group three rats were orally administered 2.5 mg/kg bodyweight of standard drug (glibenclamide). Groups four and five were orally administered 200 mg/kg bodyweight extract fractions of hexane and chloroform respectively, Groups six and seven were orally administered 400 mg/kg bodyweight extract fractions of n-hexane and chloroform respectively.

3.4 Serum Biochemical and Hematological Parameters

On the 17^{th} day of the experiments, the rats were fasted for 12 hours and anaesthetized under chloroform vapor and sacrificed. Blood samples was collected by carotid puncture into heparinized tubes, centrifuged at 1000 rpm for 5 minutes and the clear serum supernatant was used freshly for the assessment of lipid profile, liver, and kidney function tests. For the hematological parameters, the blood was collected into EDTA test tubes.

3.4.1 Hematological parameters

Blood samples were collected into EDTA bottles. The full blood count includes; total red blood cell (RBC), hemoglobin concentration (HGB), white blood cell count (WBC), platelet count (PLT) and other hematological parameters were determined using Swelab Auto Hematology Analyzer.

3.4.2 Plasma lipid profiles

The plasma total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density Lipoprotein (LDL) were determined using Randox diagnostic kits. The absorbance was determined and calculated using Stat fax 4500 semi- automated chemistry analyzer. All analyses were carried out in triplicates (n=3), and according to manufacturer's protocol.

3.4.3 Liver and kidney function tests

Plasma enzyme- aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), total protein, total bilirubin (BIL), Creatinine, Serum electrolytes ($K,^{+}Cl,^{-}$ HCO_3^{-} Na^{+}) and other parameters were determined using Randox diagnostic kits. All analyses were carried out in triplicates (n=3), and according to manufacturer's protocol.

3.5 Statistical Analysis

Statistical Product for Social Solution (SPSS) software was used to analyze all the data. Results are expressed as Mean ± Standard error mean (SEM) using one-way analysis of variance (ANOVA), followed by Turkey, Duncan and

Dunnet's multiple comparison test and the values of $p<0.05$ were considered to be statistically significant.

4. RESULTS AND DISCUSSION

4.1 Discussion

Acute toxicity studies extract of Ceiba pentandra leaf observed at varying doses revealed no clinical signs in the treated rats, and no mortality was observed inall the groups that were orally administered *Ceiba pentandra* after 24 hours. The LD_{50} value of *Ceiba pentandra* was estimated therefore to be above 5000 mg/kg body weight.

There was a significant reduction ($p< 0.05$) in blood glucose concentration of diabetic rats (shown in Figs.1 and 2) after administration of both extracts of n-hexane and chloroform. The development of diabetes in rats was confirmed 72 hrs after an intraperitonial administration of 100 mg/kg bodyweight of alloxan monohydrate [17]. The development of hyperglycaemia maybe due to cytotoxic action of alloxan (a glucose analogue)s on pancreatic beta cells involving oxidation of essential sulphydryl (-SH) groups, inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis [18]. Medicinal plants that possess hypoglycemic properties usually contain high concentration of alkaloids and flavonoids [19]. These phytochemicals found in abundance in the crude extract [8] elicited this activity and thus account for the significant hypoglycemic effect of both fractions. The hypoglycaemic activity exhibited by these extracts (Figs. 1 and 2) may be due to the ability of the extracts to inhibit the endogenous glucose production, inhibit insulinase activity, or increase insulin production from the β cells of pancreas [20]. The weight gain observed in diabetic treated groups (Figs. 3 and 4) can be attributed to the glucose utilization, thereby explaining the hypoglycaemic properties of the extracts. This has also been supported by various studies that during diabetes mellitus, the blood sugar increases and results in lack of sugar in the cells; forcing the cells to use amino acids and fatty acids as a source of energy which eventually leads to the reduction of proteins and fats in the body. This defect leads to loss in bodyweight, because the diabetic animals fall back on the stored energy when there is insufficient glucose [21].

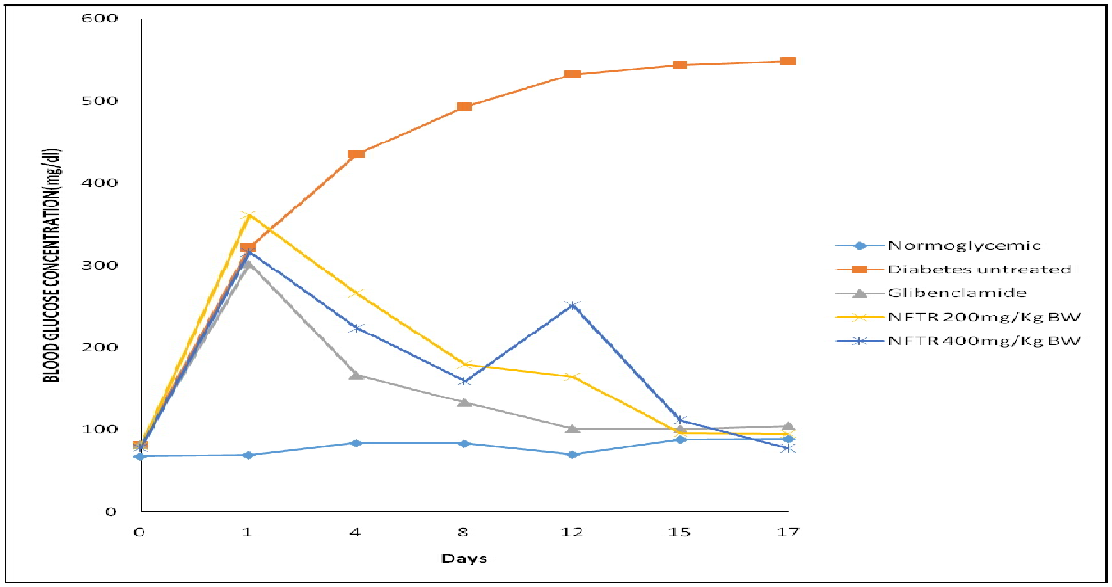

Fig. 1. Fasting blood glucose levels of rats treated with 200 and 400 mg/kg b.w of n-hexane fractions, standard drug (glibenclimide), normoglycaemic, and diabetic untreated

NFTR- n-hexane fraction

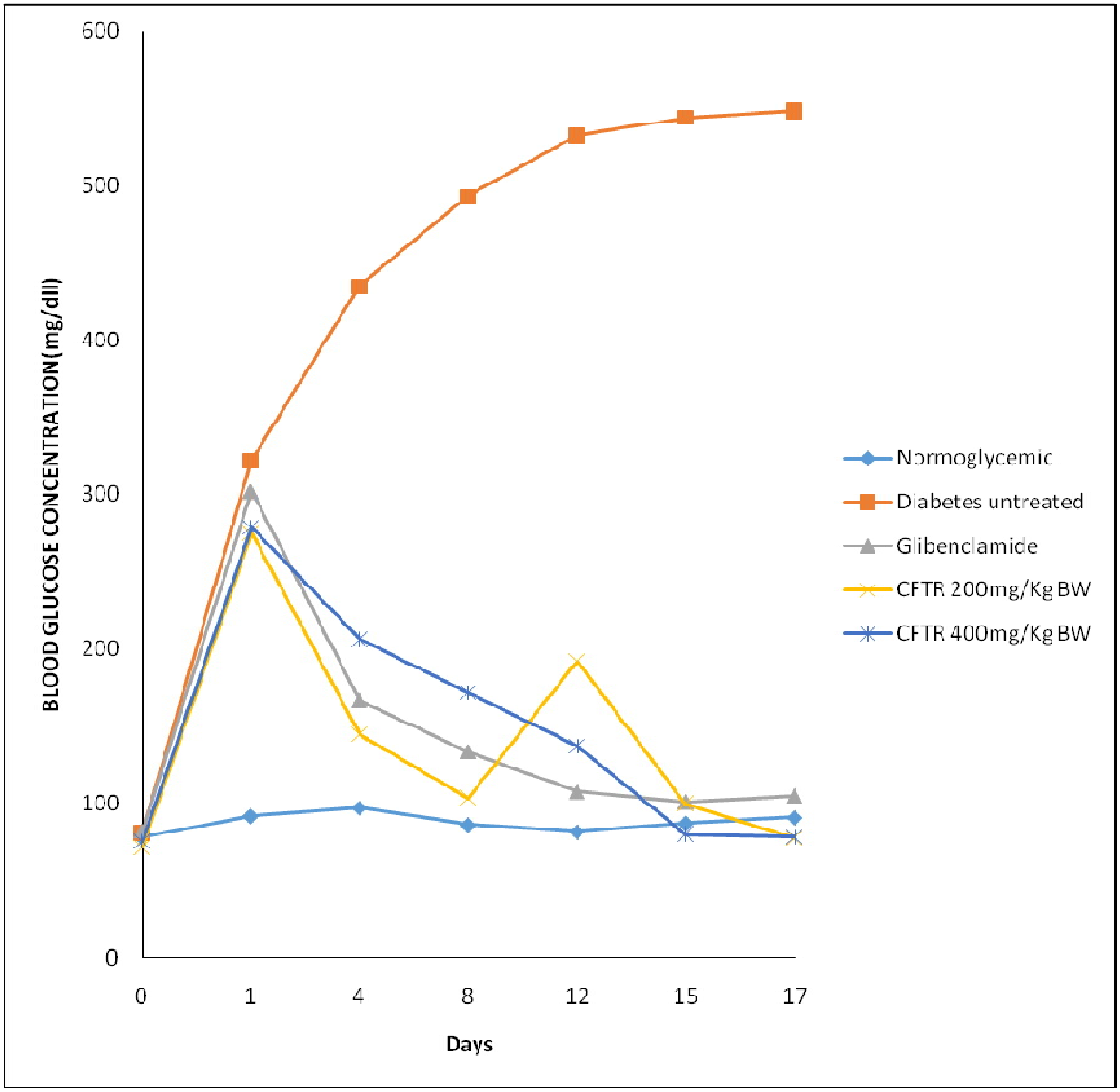

Fig. 2. Fasting blood glucose levels of rats treated with 200 and 400 mg/kg b.w chloroform fractions, standard drug (glibenclimide), normoglycaemic, and diabetic untreated groups

CFTR: Chloroform fraction

Hematological complications consist mainly of abnormalities in the functions, morphology and metabolism of erythrocytes, leukocytes and platelets [22]. The primary reasons for assessing the red blood cell (RBC) is to check anemia and to evaluate normal hematopoiesis [23]. Packed cell volume (PCV) represent the volume of red blood cell in 100 ml of blood and helps to determine, and diagnose states of hydration, polycythemia, and degree of anemia [24]. There was significant decrease ($p>0.05$) in RBC, PCV and HGB levels of diabetic untreated group (Table 1). These may be as a result of anemia or the onset of glycosylation process because the reactive oxygen species (ROS) generated during alloxan metabolism has been implicated in red cell damage [25]. Anemia has also been identified as a common complication of chronic kidney disease (CKD), affecting over half of all patients and the most common cause of CKD in about two-third (2/3) of cases is diabetes mellitus [26]. Both fractions show no damaging effect on RBC, PCV and HGB when compared to normoglycemic.

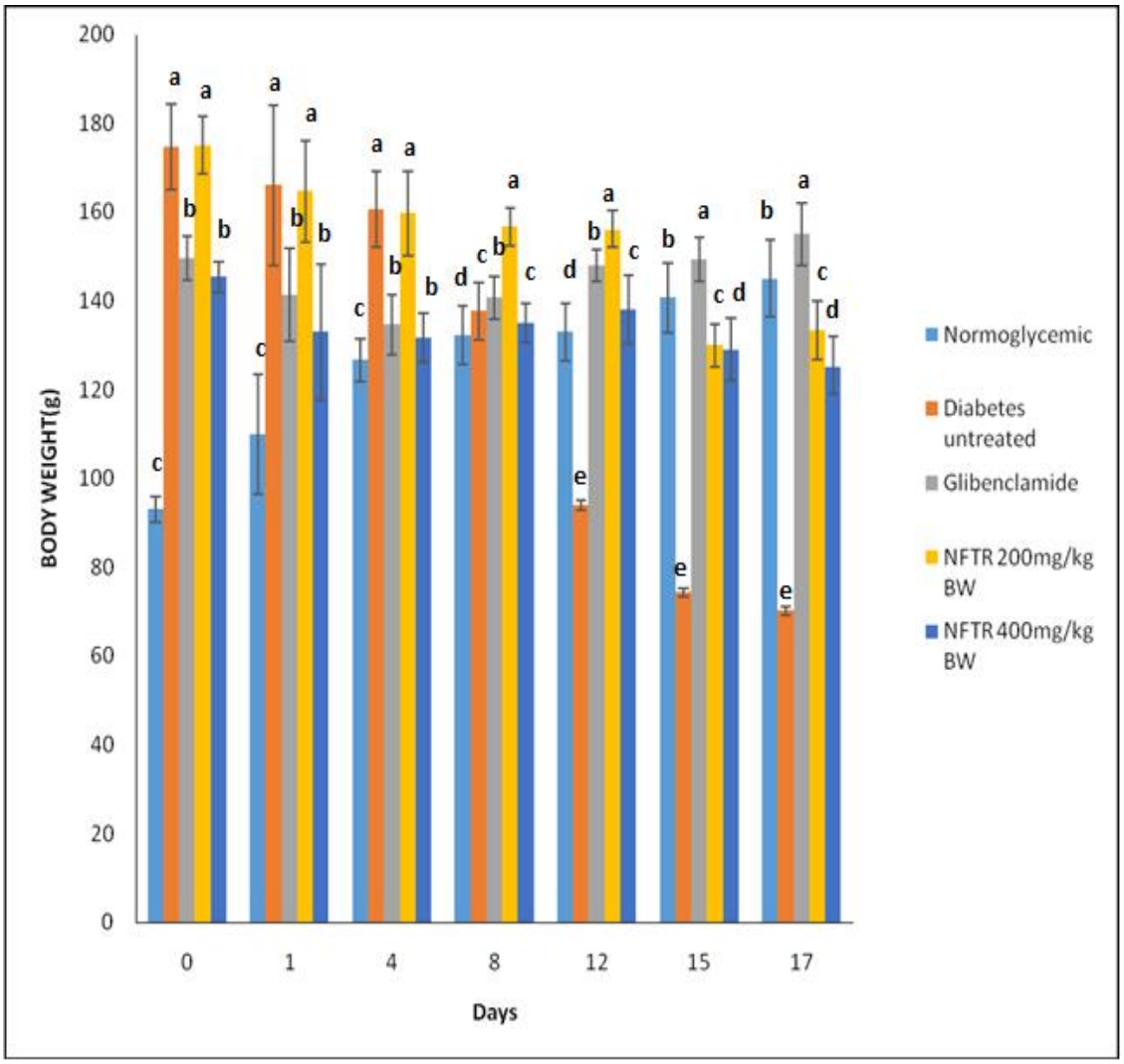

Fig. 3. Mean bodyweight of rats in all groups (n-hexane fractions)

Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3
This chart shows significant p<0.05 decrease in bodyweight of diabetic untreated rats when compared to all other groups
NFTR: n-hexane fraction treated rats; BW: bodyweight

Platelets are fragment of cells that participates in blood clotting, and initiate repair of blood vessels, and are also considered as acute phase reactant to infection or inflammation [27]. Platelet count (PLT) showcases the precise method of determining the degree of acute blood loss while white blood cell count (WBC) measures the total number of white blood cells which defend the blood against opportunistic infection. Diabetic untreated rats indicated a significant (P>0.05) reduction in PLT and increase in WBC when compared to normoglycemic. This is in line with the studies carried out by [28] that alloxan diabetogenesis may cause perturbation in the bone marrow stem cells. Significant (p<0.05) increase in platelet count and white blood cell count was observed in groups treated with 200 mg and 400 mg of chloroform fraction when compared to normoglycemic and other treated groups (glibenclamide and n-hexane). Generally, a reactive thrombocytosis due to abnormal increase in platelet is associated with an increased thrombotic risk when it is accompanied with overproduced red blood cells and white blood cells to some degree [29]. Mean platelet volume (MPV) is used for investigating the ability of a drug to enhance blood clotting [24] and to determine platelet function, as its increase is a

newly emerging indicator for atherothrombosis [30]. In respect to this findings, the diabetic groups treated with chloroform fraction pose no threat since there was no significant ($p<0.05$) difference in MPV value obtained compared to normoglycemic (Table 2). Therefore the increase in PLT and WBC observed in rats treated with chloroform fraction may be due to potential of medicinal compounds or drugs in altering the normal range of hematological parameters [31] and it is seen as the varying doses of chloroform fraction increases. Mean cell volume (MCV), platelet distribution width (PDW), mean corpuscular hemoglobin (MCH), red blood cell distribution width coefficient of variation (RDW-CV), red blood cell distribution width standard deviation(RDW-SD), and mean corpuscular hemoglobin concentration (MCHC) in all groups were normal. And this result is similar with that reported by [32].

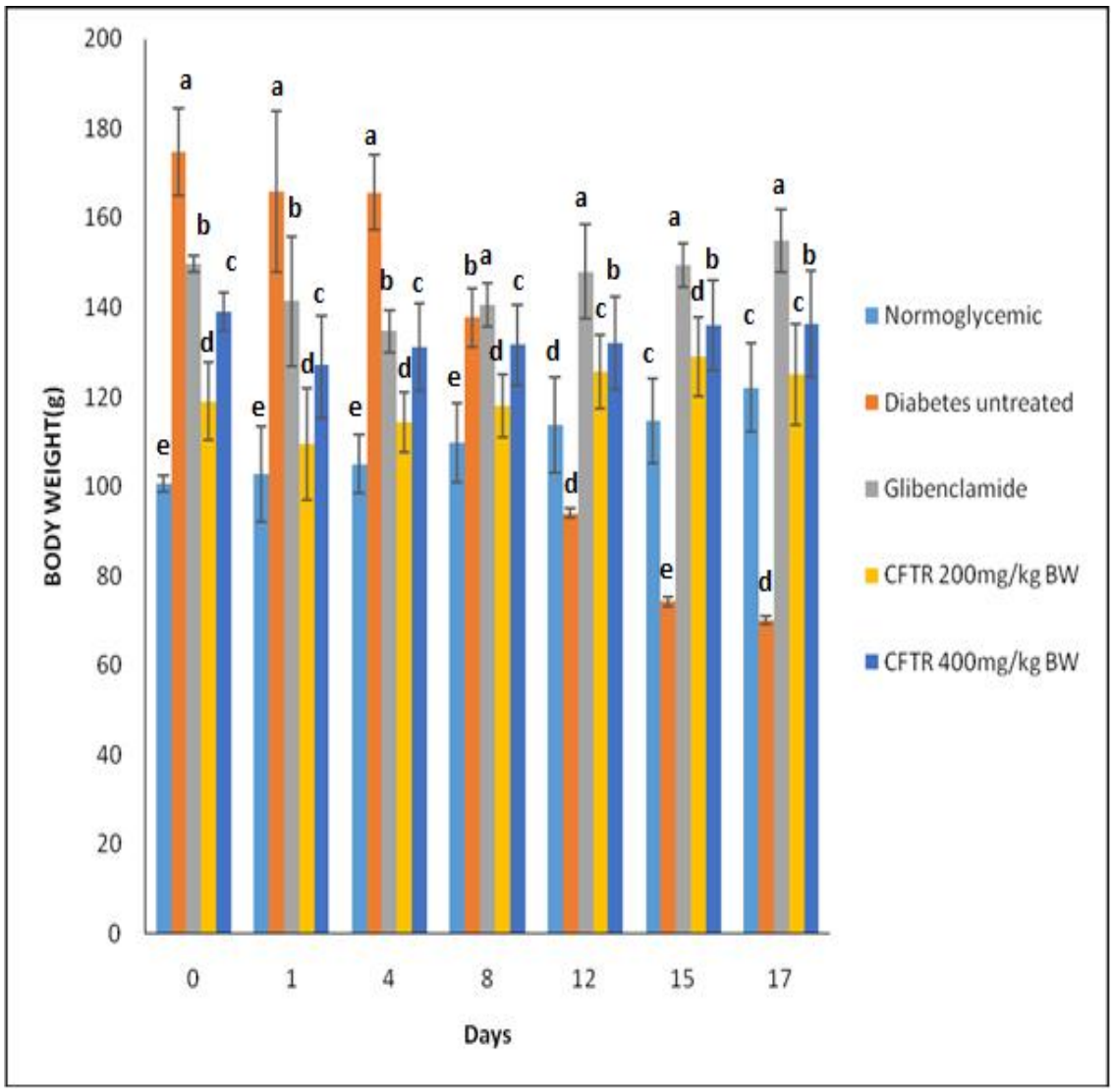

Fig. 4. Mean bodyweights of rats in all groups (chloroform fractions)

Values in each parameter with the same alphabet are not significantly different ($p<0.05$). Mean ± SEM, n = 3; This chart shows significant ($p<0.05$) decrease in body weight of diabetic untreated rats when compared to all other groups

CFTR: chloroform fractions; B.W: bodyweight

Table 1. Effect of dose variation of n-Hexane fraction of *Ceiba pentandra* leaf on hematological parameters of treated and untreated rats

Parameter	Normoglycemic	Diabetic untreated	Standard drug	n-Hexane (200 mg/kg b.w)	n-Hexane (400 mg/kg b.w)
RBC(10^{12}/l)	4.87±0.18[a]	1.60±0.18[b]	4.58±0.28[a]	4.06±0.43[a]	4.05±0.24[a]
MCV (f l)	56.73±1.18[a]	52.76±0.89[b]	54.90±0.76[a]	54.56±1.42[a]	55.37±1.06[a]
RDWCV(%)	17.00±0.47[a]	17.60±0.64[a]	17.90±0.35[a]	17.11±0.62[a]	17.16±0.46[a]
RDW-SD(f l)	38.51±1.46[a]	33.90±0.69[a]	38.26±1.11[a]	39.10±1.60[a]	38.05±1.18[a]
HCT (%)	28.96±1.94 [a]	17.70±0.87[b]	25.26±1.79[a]	22.30±2.25[a]	22.57±1.59[a]
PLT (10^9/L)	165.16±10.9[a]	71.00±2.39[b]	109.33±2.37[a]	138.33±12.32[a]	144.89±10.25[a]
MPV(f l)	6.61±0.15[a]	6.50±0.18[a]	6.53±0.12[a]	6.61±0.17[a]	6.73±0.13[a]
PDW (f l)	10.16±0.23[a]	10.80±0.18[a]	10.03±0.23[a]	10.03±0.29[a]	10.21±0.22[a]
WBC (10^9/L)	5.01±1.09[a]	3.60±1.27[b]	4.93±0.82[a]	4.91±1.76[a]	5.11±1.46[a]
HGB(g/dl)	11.53±1.68[a]	6.66±0.20[b]	9.06±0.62[a]	8.25±0.80[a]	9.27±0.56[a]
MCH(pg)	20.71±0.39[a]	19.60±0.18[a]	19.80±0.14[a]	20.23±0.30[a]	20.36±0.21[a]
MCHC(g/dl)	36.26±0.40[a]	36.83±0.31[a]	36.06±0.25[a]	37.13±0.47[a]	36.81±0.36[a]

Values in each parameter with the same superscript are not significantly different (p<0.05). Mean±SEM, n = 3; KEY:RBC: red blood count, MCV: Mean cell volume, RDW-CV: red blood cell distribution width coefficient of variation, RDW-SD: red blood cell distribution width standard deviation, PCV: packed cell volume, PLT: platelet, MPV: mean platelet volume, PDW: platelet distribution width, WBC: White blood count, HGB: hemoglobin, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration

Table 2. Effect of dose variation of chloroform fraction of *Ceiba pentandra* leaf on hematological parameters of treated and untreated rats

Groups	Normoglycemic	Diabetic untreated	Standard drug (Glibenclimide)	Chloroform 200 mg/kg	Chloroform 400 mg/kg
RBC (10^{12}/L)	4.91±0.12[a]	1.60±0.18[b]	4.58±0.28[a]	4.63±0.18[a]	4.51±0.14[a]
MCV (f l)	56.93±1.01[a]	52.76±0.89[b]	54.90±0.76[a]	59.21±1.19[a]	58.71±1.03[a]
RDWCV (%)	17.23±0.39[a]	17.60±0.64[a]	17.90±0.35[a]	17.15±0.30[a]	17.26±0.23[a]
RDW-SD (f l)	39.10±1.23[a]	37.90±0.69[a]	38.26±1.11[a]	42.01±1.13[a]	43.56±0.85[a]
HCT (%)	29.70±1.56[a]	17.70±0.87[b]	25.26±1.79[a]	30.01±1.05[a]	30.31±0.88[a]
PLT(10^9/L)	168.03±7.58[a]	51.00±2.39[b]	109.33±2.37[a]	363.83±67.01[ab]	443.33±50.07[ab]
MPV (f l)	6.56±0.12[a]	6.50±0.18[a]	6.53±0.12[a]	6.93±0.14[a]	6.92±0.10[a]
PDW (f l)	10.12±0.18[a]	10.80±0.18[a]	10.03±0.23[a]	10.25±0.18[a]	10.20±0.13[a]
WBC(10^9/L)	5.31±0.97[a]	3.60±1.27[b]	4.93±0.82[a]	11.78±2.45[ab]	12.26±1.76[ab]
HGB (g/dl)	12.16±1.46[a]	6.66±0.20[b]	9.06±0.62[a]	10.68±0.34[a]	10.82±0.28[a]
MCH (pg)	20.65±0.35[a]	19.60±0.18[a]	19.80±0.14[a]	21.13±0.30[a]	21.02±0.25[a]
MCHC (g/dl)	36.13±0.31[a]	36.83±0.31[a]	36.06±0.25[a]	35.73±0.26[a]	35.86±0.23[a]

Values in each parameter with the same superscript are not significantly different (p<0.05). Mean ± SEM, n = 3

Diabetes induces dyslipidemia due to Insulin deficiency or insulin resistance because insulin has an inhibitory action on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-coA) reductase, a key role rate-limiting enzyme responsible for the metabolism of cholesterol rich low density lipid particles [33]. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. High density lipoprotien (HDL) is an anti-atherogenic lipoprotein. Figs. 5 and 6 showed a significant ($p>0.05$) decrease in low density lipoprotein (LDL), total cholesterol (CHOL) and triglyceride (TAG) levels in all diabetes treated groups when compared with diabetic untreated group. This might be due to the reduced hepatictriglyceride synthesis and/ or reduced lipolysis as a result of oral administration of the crude fractions.n-hexane fraction of 200 mg/kg b.w had more lowering effect ($p<0.05$) on CHOL and TAG whereas the diabetic rats treated with 400 mg/kg b.w showed a significant ($p<0.05$) increase compared to glibenclamide treated and normoglycemic groups. All the fractions increased HDL (Figs. 5 and 6), thus indicating a reversed atherogenic risk. But varying the doses of chloroform fraction revealed significant ($p<0.05$) increase compared to normoglycemic, standard drug and n-hexane treated groups. In contrast, the diabetic untreated group depicted a significant $p<0.05$ decrease in HDL levels.

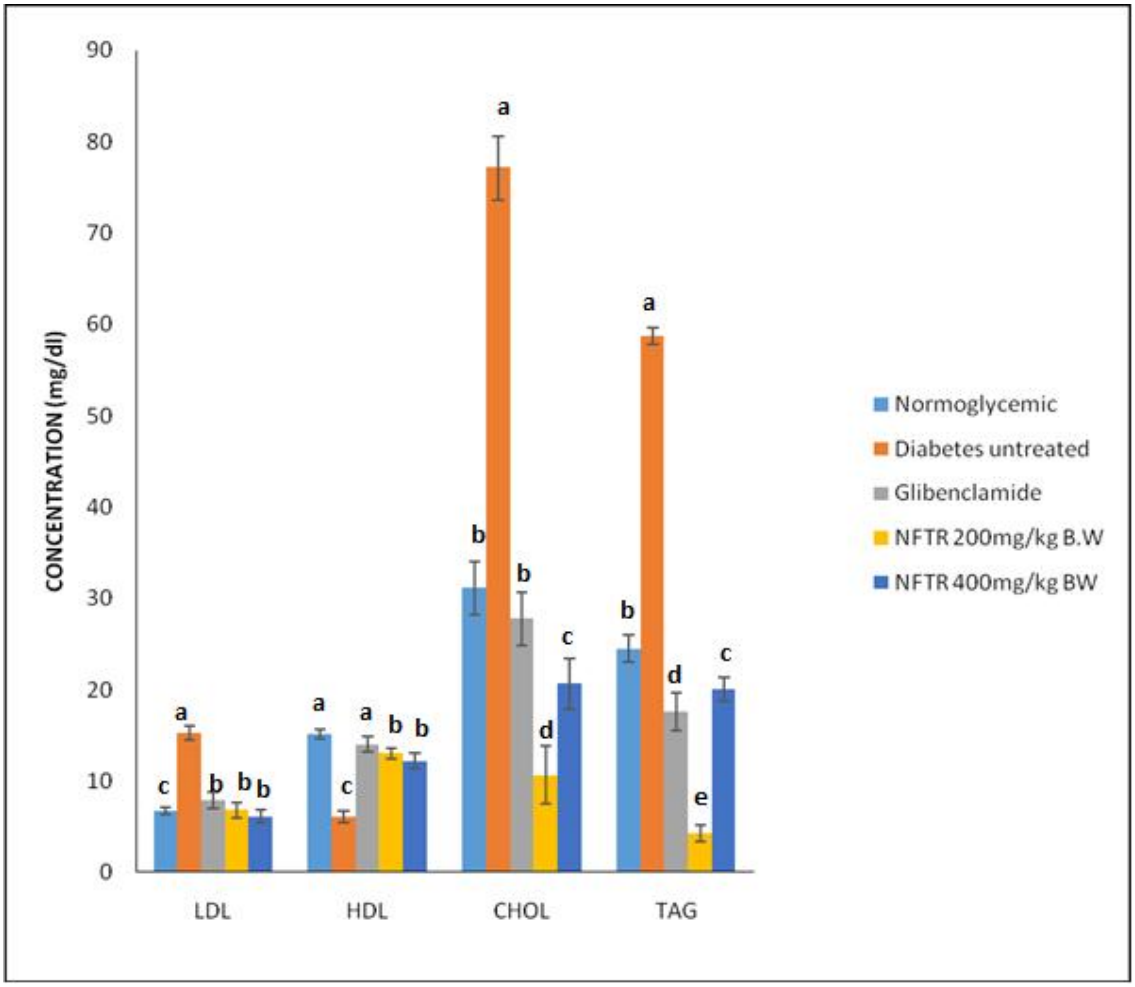

Fig. 5. Effect of n-hexane fraction on lipid profile of rats in all groups

Values in each parameter with the same alphabet are not significantly different ($p<0.05$). Mean±SEM, n = 3; LDL: low density lipoproteins; HDL: high density lipoproteins; CHOL: total cholesterol; TAG: Triglyceride; NFTR: n-hexane fraction treated rats

ALP (alkaline phosphatase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), creatinine and urea are good indicators used to assess liver and kidney damage respectively. ALT and AST are determined predominantly for hepatocellular damage. High level of AST indicates that the liver is damage due to toxicant effect during cardiac infection and muscle injury. ALT is however more specific to the liver for detecting hepatocellular damage [34]. Increase in the serum level of ALP is due to increased synthesis in presence of increasing biliary pressure [35]. Generally there was significant decrease ($p<0.05$) in ALP, ALT, AST level in all diabetic treated group (Figs. 8 and 9) which indicate that the extract has hepato-protective potentials [32]. Significant decrease ($p<0.05$) in ALT level observed in standard drug treated group, 200 mg/kg b.w of n-hexane fraction and 400 mg/kg b.w of both n-hexane and chloroform fractions compared to normoglycemic was due to gradual decrease of diabetic complications. Since body cells contain more AST than ALT [36] higher concentration of AST than ALT is expected. As for the diabetic untreated group, significant increase was observed in ALP, ALT, AST. In all the groups, Serum albumin and total protein showed no significant difference.

Considering the values in Figs. 9 and 10, there was significant elevation in sodium and bicarbonate levels, with a concomitant reduction in potassium and chloride levels in all the diabetic treated groups. This was due to serum electrolyte imbalance associated with diabetic condition, and resulting in hyponatremia, hyperkalemia, and hyperchloremia [37]. Because of the relative effect of hyperchloremia, the bicarbonate in the extracellular fluid remains at a reduced level while the anion gap is diminished [38]. But groups treated with standard drug experienced a significant ($p<0.05$) reduction in sodium level than the fraction treated groups when compared with normoglycemic group.

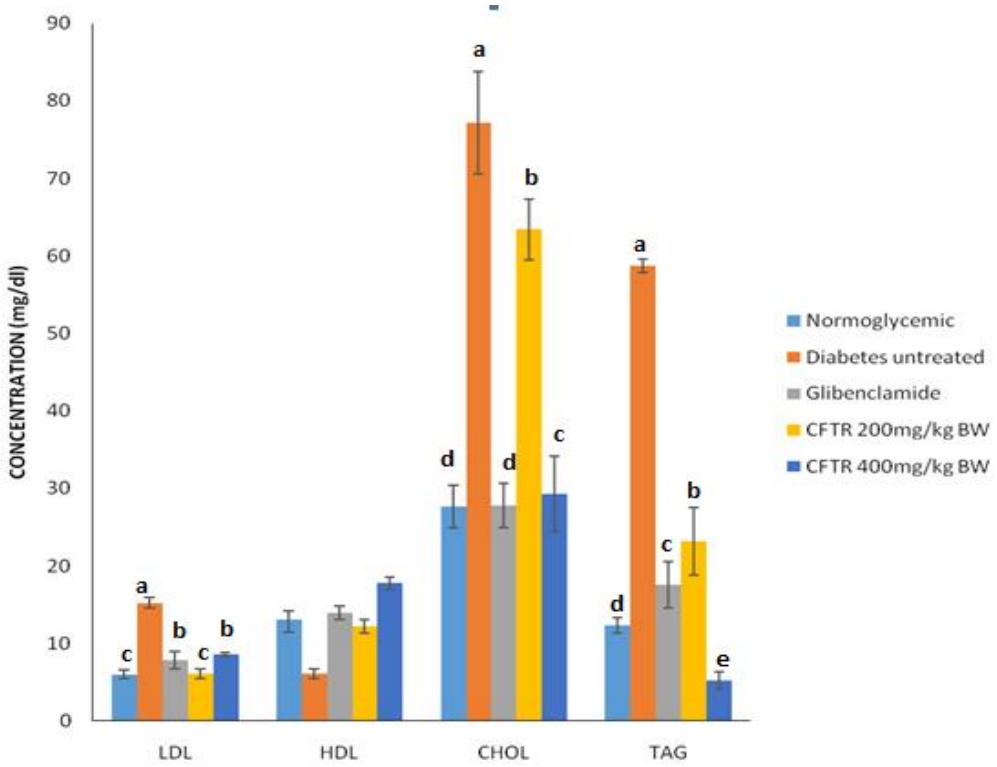

Fig. 6. Effect of chloroform fraction on lipid profile of rats in all groups

Values in each parameter with the same alphabet are not significantly different ($p<0.05$). Mean ± SEM, n = 3; LDL: low density lipoproteins; HDL: high density lipoproteins; CHOL: total cholesterol; TAG: triglyceride; CFTR: chloroform fraction treated rats

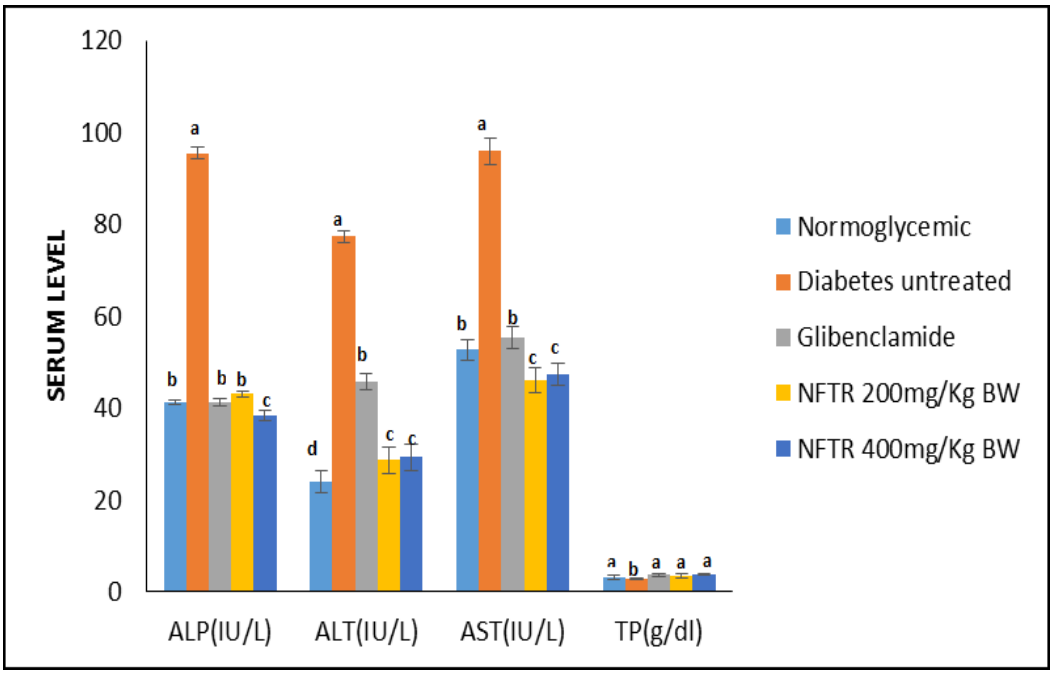

Fig. 7. Effect of n-hexane on fractions on some liver enzymes in all groups

Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TP: total protein, NFTR: n-hexane fractions

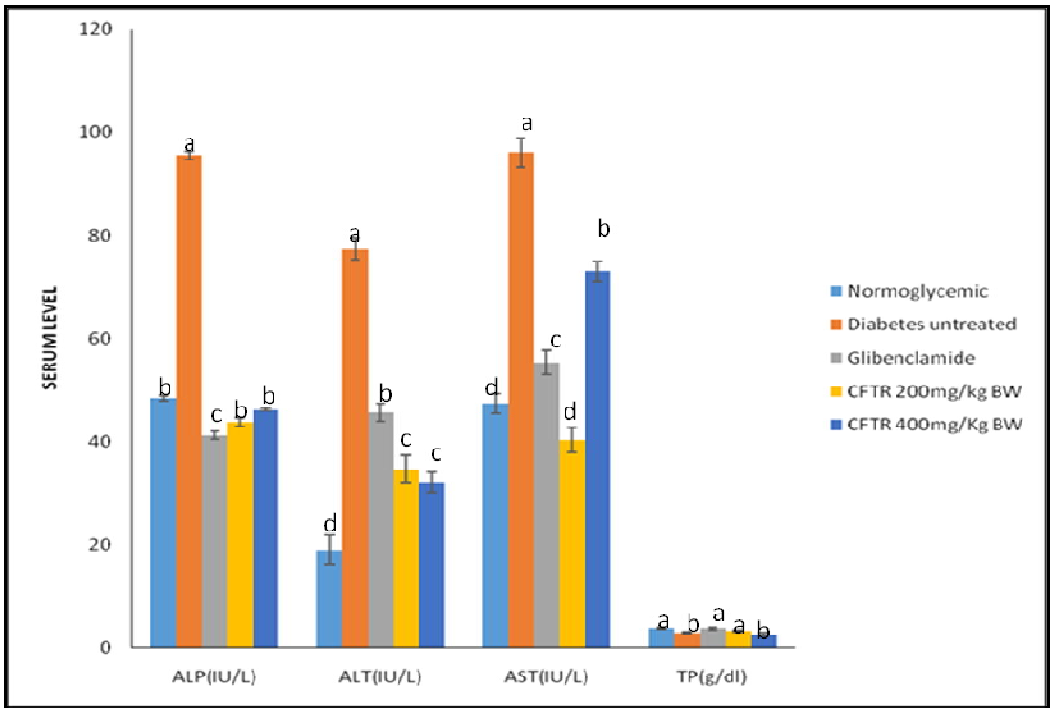

Fig. 8. Effect of chloroform fractions on some liver enzymes of rats in all groups

Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TP: total protein; CFTR: chloroform fractions

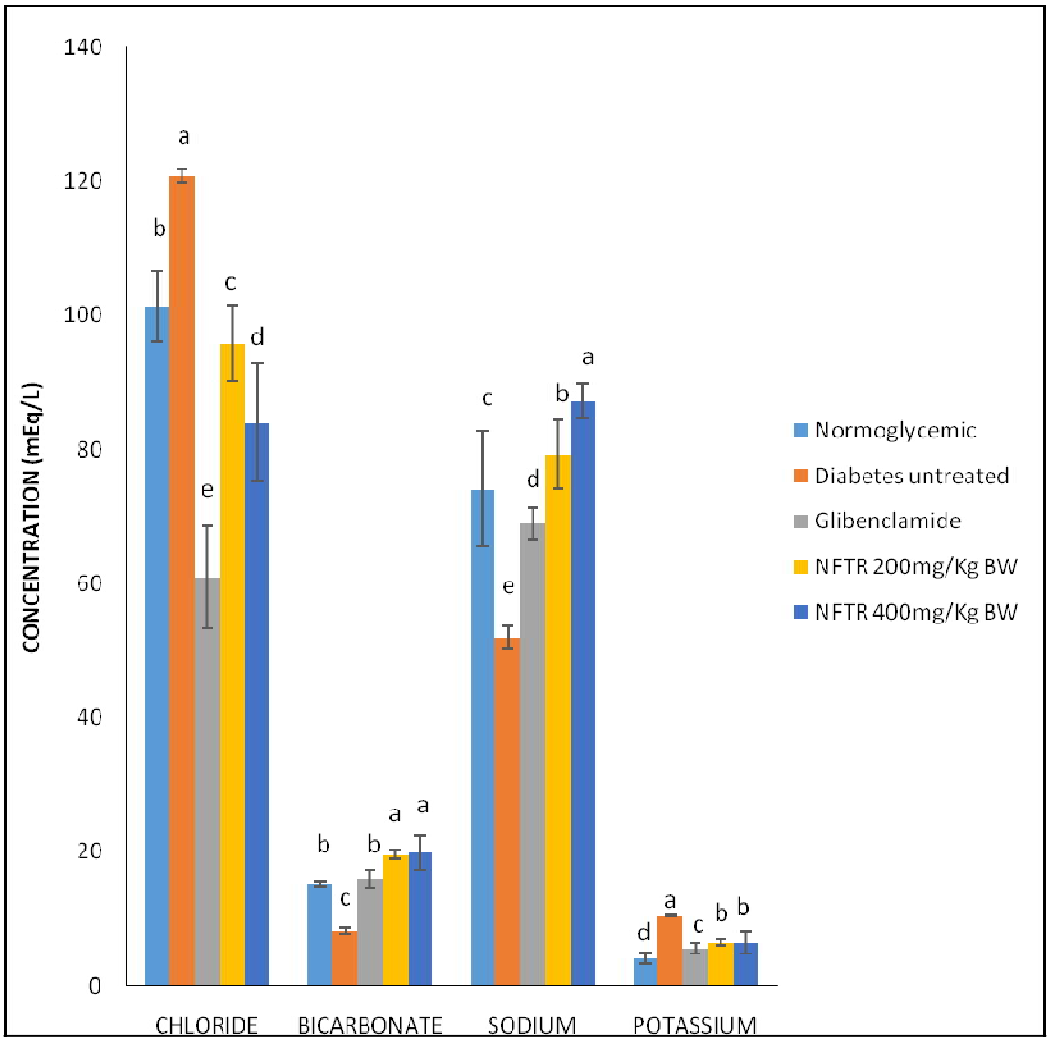

Fig. 9. Effect of n-hexane fraction on serum electrolytes of rats in all groups

NFTR: n-hexane fractions; Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3

Diabetic treated groups showed significant p<0.05 decrease in urea, total bilirubin (T.B), creatinine (CREAT) and conjugated bilirubin (C.B) when compared to untreated groups (Figs. 11 and 12). This findings support the report by [39] which suggested that the increase in plasma bilirubin (hyperbilirubenimia) may result from the decrease of liver uptake, conjugation or increase bilirubin production from hemolysis of red blood cells. All treated groups showed significant (p<0.05) increase in CREAT, T.B and C.B levels when compared with the normoglycaemic group. This elevation in plasma bilirubin observed indicates liver damage as confirmed by the changes in the activities of plasma liver enzymes.But increase in the serum level of ALP is due to increased synthesis in presence of increasing biliary pressure [35] and no changes was observed in groups treated with glibenclamide or varied doses of both fractions. Though diabetic rats treated with both fractions lowered CREAT, T.B and C.B more than the standard drug. This findings showed that the fractions is safe and has no hepatotoxic effect and is in line with the research carried out by [40, 41]. Further research should be carried out using other solvents for partitioning in order to determine bioactive compounds present singly or in combinations. The most active fractions could be isolated and their activities assayed to identify the active antidiabetic mediator(s).

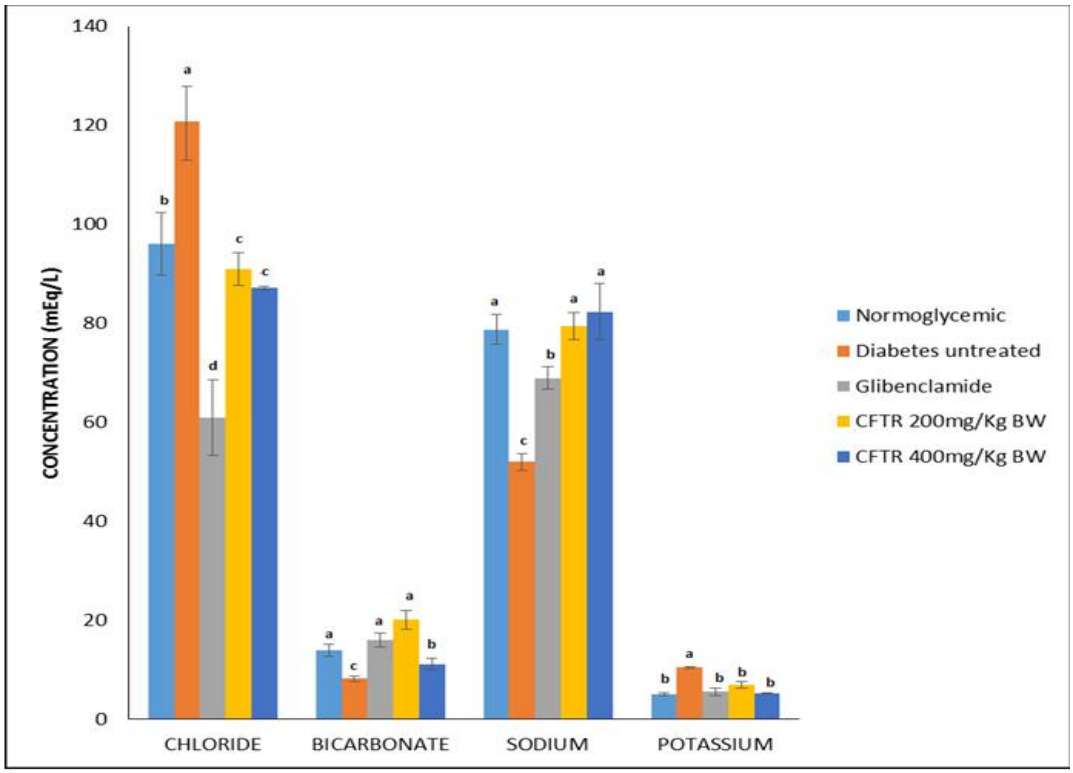

Fig. 10. Effect of chloroform fractions on serum electrolytes of rats in all groups

CFTR: chloroform fractions; Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3

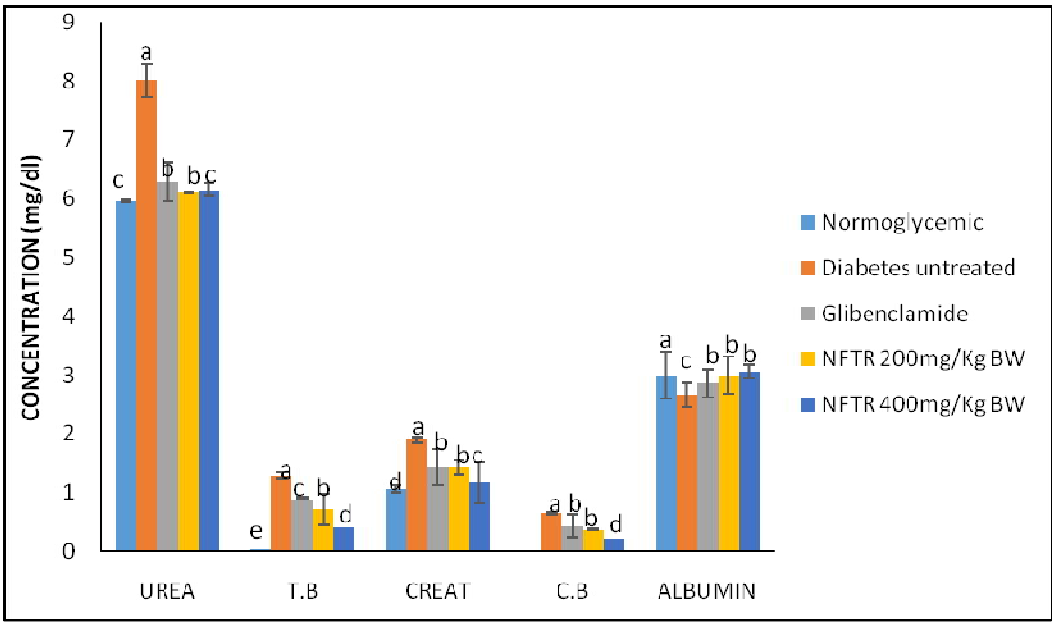

Fig. 11. Effect of n-hexane fractions on serum levels of some kidney function parameters of rats in all groups

Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3; T.B: total bilirubin; C.B: conjugated bilirubin; CREAT: creatinine; NFTR: n-Hexane fraction

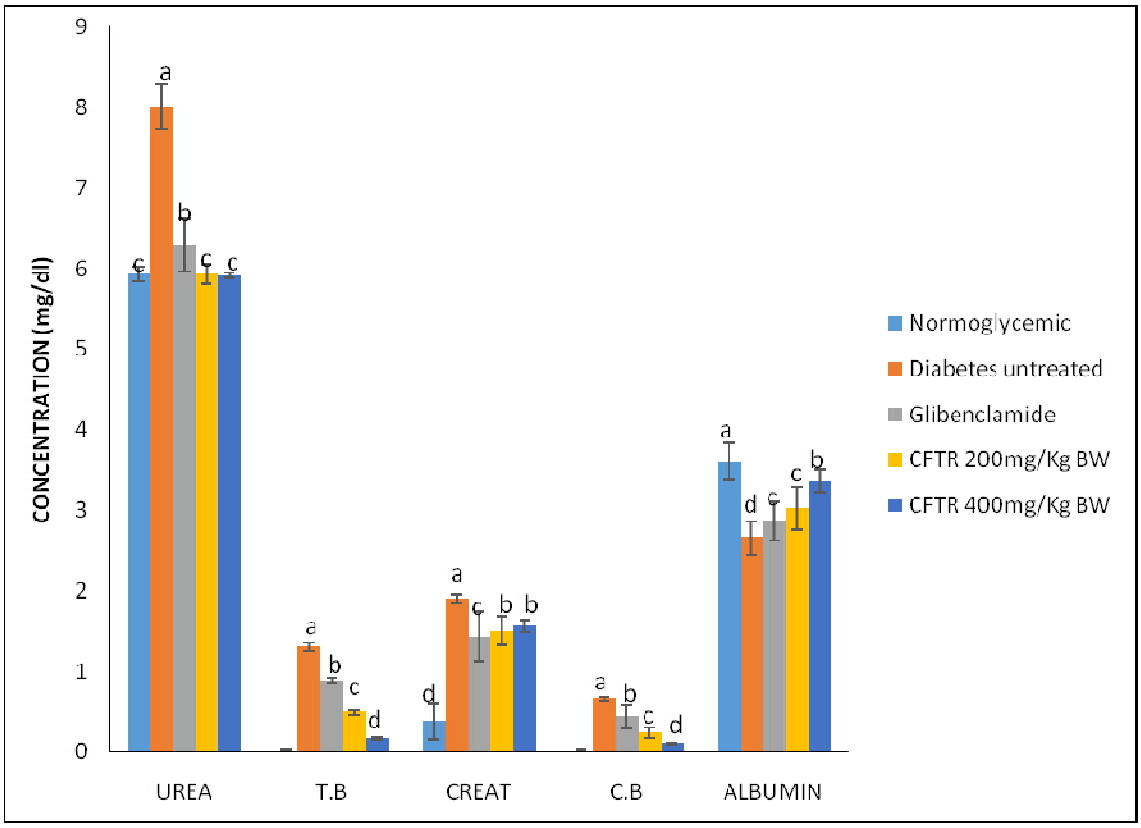

Fig. 12. Effect of chloroform fractions on serum levels of some kidney function parameters of rats in all groups

Values in each parameter with the same alphabet are not significantly different (p<0.05). Mean ± SEM, n = 3; T.B: Total bilirubin; C.B: Conjugated bilirubin; CREAT: Creatinine; CFTR: chloroform fraction treated rats

5. CONCLUSION

Varied doses of n-hexane and chloroform fractions of ethanol extract of *Ceiba pentandra* demonstrated potent hypoglycaemic properties, and improved the altered biochemical and hematological parameters associated with pathophysiology of diabetes mellitus. However, chloroform fraction demonstrated increased in platelets and white blood cells at a dose dependent fashion (from 200-400 mg/kg b.w). Base on this, higher doses greater than 400 mg might lead to thrombosis. Furthermore, hypolipidemic and hepatoprotective activities of both fractions have been established to be dose dependent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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