Acute oral toxicity study of ethanol extract of *Ceiba pentandra* leaves as a glucose lowering agent in diabetic rats

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**ABSTRACT**

**Objective:** To evaluate the use of *Ceiba pentandra* (*C. pentandra*) as a glucose lowering agent and the attendant physiological changes in albino rats.

**Methods:** Acute oral toxicity study of the extract was carried out by the administration of 10, 100, 1000, 1600, 2900, and 5000 mg/kg body weight of *C. pentandra* to rats in their respective groups. Twenty healthy albino rats weighing between 140 and 150 g were randomly allotted to five groups of four rats each. 100 mg/kg body weight of alloxan monohydrate was i.p. administered to rats and rats with blood glucose ≥ 200 mg/dL were considered diabetic. 5 mg/kg body weight of standard drug, 200 and 400 mg/kg body weight of ethanol extract of *C. pentandra* were orally administered to diabetic rats in their respective groups once daily for 12 days while the control groups received 0.1 mL of normal saline for the same period. The blood glucose was checked after every 4 days and the experiment was terminated on the 17th day.

**Results:** The safe dose (LD50) of the extract was greater than 5000 mg/kg body weight. The extract treated groups exhibited a remarkable reduction in blood glucose [(87.72 ± 7.67) mg/dL for 200 mg/kg body weight dose and (86.33 ± 4.54) mg/dL for 400 mg/kg body weight dose] competitively with the normoglycemic group [(88.71 ± 4.56) mg/dL]. The body weight of the extract and standard drug treated groups appreciated significantly (*P* < 0.05) as compared with normoglycemic group. High density lipoprotein increased significantly in the extract treated groups with the 400 mg/kg body weight having the highest value of (18.61 ± 3.44) mg/dL and a concomitant reduction in the concentrations of low density lipoprotein, triacylglycerol, and cholesterol of same group. The 400 mg/kg body weight dose of the extract also demonstrated the highest reduction in the activities of aspartate transaminase [(59.66 ± 3.45) IU/L], alanine aminotransferase [(33.33 ± 4.34) IU/L] and alkaline phosphatase [(48.36 ± 3.41) IU/L] that elevated as a result of diabetes. The two extract treated groups demonstrated a marked reduction in Cl−, and elevation in HCO3− and Na+ levels. K+, urea, creatinine, total and conjugated bilirubin that were elevated in the diabetic untreated group were all ameliorated in the treatment groups.

**Conclusions:** Ethanol extract of *C. pentandra* has glucose lowering effect and can ameliorate the biochemical abnormalities associated with diabetes mellitus.

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All experimental procedures involving animals were conducted in accordance to Ethical clearance given and approved by Federal University of Technology, Minna/Nigerian Ethical Review Board (CUERB) in accordance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review.

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1. Introduction

Glucose is the most important controller of insulin secretion, and insulin secretion is exerted by a feedback effect of blood glucose on the β cell of the pancreas. When blood glucose concentration rises above fasting levels, insulin secretion increases and stimulates glucose uptake by the liver and the peripheral tissues thereby returning glucose to the normal levels. This provides an important negative feedback mechanism for controlling the blood glucose concentration. Diabetes mellitus is a chronic disease due primarily to a disorder in carbohydrate metabolism, as a result of deficiency or diminished effectiveness of insulin, resulting in hyperglycemia and glycosuria\[1\]. Diabetes affects more than 100 million people worldwide (6% of the population) and in the next 10 years is projected to affect five times more people than it does now\[2\]. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The disease may present with classical characteristic features such as blurring of vision, excessive thirst (polydipsia), excessive feeding (polyphagia), excessive urination (polyuria), and weight loss. In its most severe form, ketoacidosis may develop leading to stupor, coma and in absence of effective treatment, death ensues\[3\]. In conventional therapy, type 1 diabetes is treated with exogenous insulin, while type 2 is treated with oral hypoglycemic agent like biguanides and sulphonylureas\[4\]. Although these agents provide a good glycemic control, they can do little to prevent secondary complications. Besides, they are also associated with side-effects or diminution in response after prolonged use\[5\]. According to World Health Organization (WHO)\[6\], more than 80% of the world population depends on the traditional medicine for their primary health care and this practice is recommended especially in countries where access to conventional treatment of diabetes is inadequate. WHO however emphasizes the fact that safety should be an overriding criterion in the selection of herbal medicine for the use in health care programme. The medicinal potentials of Ceiba pentandra (C. pentandra) may be due to the presence of bioactive compounds (secondary metabolites) that produce definite physiological action in the human body. They are produced by specific parts of the plant and translocated to other parts for storage\[7\]. Alloxan is a cytotoxic glucose analogue widely used to induce diabetes in experimental animal models. It destroys the pancreatic β-cells with specific selectivity and produces selective necrosis of β-cells of islets of Langerhans when injected into animals\[8\].

C. pentandra Telugu: is a tropical tree of the order Malvales and the family Malvaceae (previously separated from the family Bombacaceae). It is native to Mexico, Central America and the Caribbean, North-South America, and tropical West Africa\[9\]. Kapok is the most used common name for the tree and may also be referred to the cotton-like fluff obtained from its seed pods. The tree is also known as the Javacotton, Java kapok, silk-cotton or ceiba. Its bark decoction has been used as a diuretic, aphrodisiac, and to treat headache, as well as type II diabetes. It is used as an additive in some versions of the hallucinogenic drink\[10\]. Diabetes and its associated complications have become a public health problem of considerable magnitude and is one of the top five global leading causes of death. In the year 2000, the excess global mortality attributable to diabetes and its late complications were estimated to be 2.9 million deaths, equivalent to 5.2% of all deaths. The unaffordability and side effects associated with the conventional antidiabetic drugs have resulted in increased search for alternative medicinal plants. The side effects of the new agent, and its ameliorative potentials on complications associated with diabetes will also be investigated.

2. Materials and methods

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, Randox diagnostic kits and normal saline.

2.2. Apparatus and equipment

These include: reflux extractor, milling machine, rotary evaporator, bucket centrifuge, water bath, Whatman filter paper, measuring cylinders, hand gloves, masking tape, beakers, syringes, spatula, glucometer, and weighing balance.

2.3. Sample collection

2.3.1. Plant collection

Adequate quantities of fresh leaves of C. pentandra were collected in July, 2015 from rural area of Gada Oli via Wawa, New-Bussa, Niger State, Nigeria and were identified and authenticated at the Herbarium of Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.

2.3.2. Preparation of plant material

The leaves were washed, blotted and air-dried at room temperature in the Departmental Laboratory for two weeks. They were homogenized into fine powder using milling machine.

2.3.3. Experimental animals

Healthy Swiss albino rats weighing 100–200 g were procured from Animal house of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were housed and accommodated in groups of four in polypropylene cages with stainless steel grill top and a bedding of clean paddy husk. Standard room temperature of 30–36 °C, 12 h light and dark cycles were also maintained. They were allowed access to commercial grower mesh to feed on ad libitum. Acclimatization of the animals to these conditions was for two weeks.

2.3.4. Extraction of plant sample

1500 g of dried powdered sample of C. pentandra was weighed and extracted with 300 mL ethanol using reflux method at temperature of 60 °C. The extract was thereof concentrated with rotary evaporator and then evaporated to dryness in a water bath at 100 °C. The crude extract yielded 130.11 g (9.29%) and was stored in a labeled sterile bottle kept at 4 °C in a refrigerator until ready for use.

2.4. Acute toxicity studies

The acute toxicity test of the ethanol extract was carried out by the method of Lorke\[11\]. Eighteen albino rats were fasted for
18 h 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 body weight. The animals were monitored for 24 h to check for convulsion, salivation, diarrhea, lethargy, sleep, coma, nervousness and/or mortality.

2.5. Grouping of animals for glucose lowering test

The experimental design consisted of 20 rats grouped as follows: Group 1 – Normoglycemic (positive control) received 0.1 mL of normal saline; Group 2 – Diabetic untreated (negative control); Group 3 – Diabetic treated with standard drug (5 mg/kg body weight of glibenclamide); Group 4 – Diabetic treated with 200 mg/kg body weight of crude ethanol extract of *C. pentandra* and Group 5 – Diabetic treated with 400 mg/kg body weight of crude ethanol extract of *C. pentandra*.

2.6. Hyperglycemic rats

The blood glucose of rats was elevated by the administration of 100 mg/kg body weight of alloxan monohydrate intraperitoneally after an overnight fast but with access to drinking water[19,21]. The animals were then housed in a controlled facility and allowed to drink 5% glucose solution to overcome the hypoglycemia[19,21]. The hyperglycemic state was confirmed by the measurement of fasting blood glucose concentration using glucometer with blood collected by tail vein puncture. Rats with blood glucose ≥ 200 mg/dL after 72 h were considered diabetic and used for the research.

2.7. Treatment with extract and standard drug

Ethanol extract and glibenclamide (standard drug) were dissolved in 10 mL normal saline (0.9% NaCl) before oral administration. Respective doses of extract and standard drug were then administered to rats once daily (10 am) for twelve days and the blood glucose was checked after every four days. On the seventeenth day, the rats were fasted for 12 h and euthanized. Blood samples were collected by carotid puncture into heparinized tubes, centrifuged at 1 000 r/min for 5 min 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.

2.8. Serum biochemical parameters

The biochemical parameters that were investigated include: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and conjugated bilirubin using Randox diagnostic kits.

2.9. Hematological parameters

The full blood count including 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.

2.10. 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.

2.11. 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-, HCO3-, Na+) were determined using Randox diagnostic kits.

2.12. Statistical analysis

SPSS software was used to analyze all the data. Results are expressed as mean ± SEM using ANOVA, followed by Turkey, Duncan and Dunnet's multiple comparison test and the values of *P* < 0.05 were considered to be statistically significant.

3. Results

No behavioral changes such as alertness, motor activity, breathlessness, restlessness, diarrhea, tremor, convulsion and coma were observed at the administered doses. The rats were physically active and no death was recorded even at the dose of up to 5000 mg/kg body weight (Table 1). Therefore, the LD50 is greater than 5000 mg/kg body weight.

Glucose concentrations decreased in all treated groups compared with the untreated group that continued to elevate until when all the animals were sacrificed. The 400 mg/kg bodyweight had a better hypoglycemic effect (Figure 1). The total body weight of rats in various groups appreciated as the treatment progressed except the diabetic untreated group that depreciated with the highest effect in standard drug treated group followed by 400 mg/kg body weight extract treated group (Figure 2).

All electrolytes were ameliorated by the administration extract. The extract at both doses was able to correct the potassium level in dose dependent manner as much as did the standard drug (Figure 3) as compared with diabetic untreated group.

Urea and creatinine that were significantly elevated (*P* < 0.05) in diabetic untreated group were significantly reduced when varied doses of extract and standard drug were orally administered to rats in the treatment groups (Figure 4). In fact,
the 400 mg/kg body weight and normoglycemic groups showed similar urea and creatinine concentration at the end of experiment.

The activities of AST, ALT and ALP that were significantly elevated \((P < 0.05)\) in the diabetic untreated group were significantly reduced when varied doses of extract and standard drug were orally administered to rats in the treatment groups (Figure 5). While the ALT and ALP activities tend towards normoglycemic group.

Total and conjugated bilirubin were elevated significantly \((P < 0.05)\) while albumin and total protein decreased significantly in the untreated group. These anomalies were ameliorated by the administration of the extract at 200 mg/kg body weight and 400 mg/kg body weight (Figure 6). Similar effect of improvement was also observed in standard treated group as compared to diabetic untreated group.

HDL decreased, while LDL, TAG, and cholesterol all increased in the untreated group as compared to with the extract and standard drug treated groups (Figure 7). Administration of extract at varied doses increased the HDL more than the standard drug. LDL, TAG and cholesterol all decreased significantly \((P < 0.05)\) when the animals were treated with the extract and the standard drug.

Some hematological parameters (except MCH) decreased significantly \((P < 0.05)\) in the diabetic untreated group, while the WBC increased in the same group (Figure 8). Administration of the extract at both doses (200 mg/kg body weight and 400 mg/kg body weight) and the standard drug normalizes the level of RBC and WBC. The positive effect was quite corresponding to the normoglycemic group.
4. Discussion

Blood glucose is a key marker for diagnosis and prognosis of diabetes mellitus. The evaluation of hypoglycemic activity of any agent using alloxan-induce hyperglycemia animal model has been described by several authors that have used plant extracts to monitor the blood glucose and the attendant abnormalities associated with diabetes. Alloxan selectively destroy the pancreatic β-cells of Langerhans that secrete insulin thereby leaving the less active cells that leave the animal in a diabetic state[14]. Treatment with ethanol extract of C. pentandra at 200 and 400 mg/kg body weight alleviated the hyperglycemic condition of the diabetic rats to near normal as indicated by the normoglycemic group. The 400 mg/kg body weight group had a swift hypoglycemic effect with a reduction of 60.95% the first day the blood glucose was measured after the establishment of the diabetic state (345 ± 4.56–182 ± 6.32). Similar hypoglycemic property from plant extract was earlier established by Frederick et al.[15]. The hypoglycemic property of the ethanol extract of C. pentandra, may be by the following mechanisms: that the extract has the ability to inhibit the endogenous glucose production, interferes with gastrointestinal glucose absorption, has insulin-like substances (insulin mimetic), inhibits insulinase activity and increases the secretion of insulin from the β-cells of pancreas[16]. It may also be possible that the extract was able to increase the β-cells in the pancreas by activating the regeneration of these cells or its release from bound insulin.

The body weight of the two groups treated with ethanol extract of C. pentandra increased as the treatment progressed in days and as long as there was a de-elevation in blood glucose (Table 1) with the 400 mg/kg body weight having a better ameliorating effect. Weight loss is a clinical feature of diabetic mellitus[17], probably due to excessive break down of tissue protein and lipid and thus all animals in the diabetic groups showed significant weight loss fourth day after the establishment of diabetes and a concomitant reversible of weight loss days after the treatment commenced. The transient gain in weight may be attributable to improved metabolic activities and the maintenance of glucose homeostasis.

The kidneys regulate the excretion of urea and reabsorption of electrolytes into the blood. Filtration occurs at the glomeruli while reabsorption takes place in the renal tubules[18]. When there is compromise of normal glomerular function, substances normally cleared by the kidneys such as urea and creatinine accumulate in the biological fluid. The significant increase in serum urea, creatinine, chloride (hyperchloremia) and potassium observed in diabetic untreated group suggests that the normal excretion of these biomolecules and electrolytes by the kidney was adversely affected. Furthermore, the decrease in the levels of sodium and bicarbonate in diabetic untreated group suggests that some aspects of tubular functioning as it relates to these electrolytes have been compromised. The bicarbonate ion acts as a buffer to maintain the normal acidity in blood and other fluids in the body. Disruptions in the normal bicarbonate level may be due to diseases kidney or metabolic conditions. Treatment of rats in the diabetic group with ethanol leaf extract of C. pentandra and glibenclamide resulted in significant improvement in kidney function as indicated by the marked decrease in serum sodium, potassium, urea, and creatinine (that were elevated in diabetic group), and an increase in bicarbonate and sodium (that were reduced in diabetic group). Similar nephroprotective activities have been reported for other medicinal plants such as Murraya koenigii[19]. Ammonia released during deamination is removed from the blood by conversion into urea. Urea is high when the glomerular filtration is high as seen in the diabetic untreated group. Creatinine is filtered in the glomerular filtrate and passes on through the tubular system and is excreted in the urine. In kidney dysfunction, creatinine is reabsorbed rather than excreted in urine. This may be the case with the diabetic untreated group where creatinine level was high.

In assessing the hepatic status of animals (hepatoprotective or hepatotoxicity) when medicinal plant extract is administered, determination of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and total protein play a major role. The alteration in the activities of these biomarkers as indicated in the diabetic untreated group (Figure 3) signifies damages to the liver including the biliary system. The elevated levels of the enzyme (ALT, AST and ALP) could be as a result of inflammation of liver cell, the enzymes leaking into the blood stream leading to a rise in their levels (Figure 3). Alkaline phosphates (ALP) is particularly a biomarker enzyme for assessing the integrity of plasma membrane, and such increase in its activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate ester of the tissue[20]. Both doses of the C. pentandra (particularly the 400 mg/kg body weight) compared favorably with the glibenclamide in alleviating the high level observed in the diabetic untreated group.

Diabetes mellitus is associated with altered protein metabolism because much of structural proteins are used in gluconeogenesis. This is because insulin is an important physiological factor that plays a key role in the maintenance of
protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation[20]. The decrease in total protein and albumin of the diabetic untreated group could be as a result of continuing utilization of protein and lipid as sources of energy and a compromise of the synthetic ability of the liver. Administration of ethanol leaf extract of C. pentandra however, tends to shift the levels of albumin and total protein to normal. This could be linked to the hypoglycemic potentials of the extract that potentiates the activities of insulin and thus makes carbohydrate available as source of energy. Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. It is excreted by the liver into the bile and the intestine. Hemoglobin when catabolized by the reticuloendothelial system is released into the bloodstream where it binds strongly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide which are water soluble metabolites. Elevations of bilirubin in diabetic induced rats have been reported with significant improvement after treatment with Moringa oleifera oil[21]. Elevations in both direct and total bilirubin observed in diabetic untreated group may be due to excessive hemolysis, destruction of red blood cells or obstruction of the biliary tract. The treatment groups ameliorated the elevated levels probably due to strong tissue repair ability of the extract.

Insulin can affect the adipocytes by inhibiting lipolysis and promoting the storage of triacylglycerol in adipocytes. Thus, lack of it or inadequate utilization as in diabetes enhanced the hydrolysis of triacylglycerol into diacylglycerol, unesterified fatty acids and free glycerol[22]. Dyslipidemia observed in diabetic state brings about inhibitory action on 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key role rate-limiting enzyme responsible for the metabolism of cholesterol rich low density lipid particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. High density lipoprotein (HDL) is an antiatherogenic lipoprotein that transports cholesterol from peripheral tissue into the liver and thereby acts as a protective factor against coronary heart disease[23]. The result showed a significant (P < 0.05) decrease in low density lipoprotein (LDL), total cholesterol and triacylglycerol (TAG) levels in all diabetic treated groups when compared with diabetic untreated group. This might be due to the reduced hepatosynthesis of triacylglycerol and/or reduced lipolysis as a result of extract administration. The increased HDL (Figure 3) indicates a reversed atherogenic risk. Because insulin is known to suppress very low density lipoprotein secretion, lack of this suppression by deficient insulin secretion in diabetes may lead to hypertriglyceridermia, which possibly can mediate the mechanism by which diabetes causes hyperlipidemia. There is a concomitant increase in the hydrolysis of triacylglycerol from very low density lipoprotein (VLDL) to low density lipoprotein as its concentration increases in circulation. The reversed lipids (HDL, LDL, TAG, and cholesterol) in Figure 6 may be that the hepatic LDL receptor which is increased by insulin (as a result of extract administration) was able to clear most of the blood cholesterol (VLDL and LDL carry most of the cholesterol), thereby increasing HDL (Figure 6) and reversing the atherogenic risk. In other words, reduction in the rate of cholesterol removal from the circulation appeared to be responsible for hypercholesterolemia observed in diabetic state. It is therefore assumed that the ethanol extract of C. pentandra have inhibiting effect against hyperglycridemia and hypercholesterolemia that are clinical features and complications of diabetes.

According to Comazzi et al.[24], hematological (RBC), mean cell volume, packed cell volume (PCV), platelet (PLT), hemoglobin count (HGB), mean corpuscular hemoglobin (MCH) and white blood count (WBC) complications consist mainly of abnormalities in the functions, morphology and metabolism of erythrocytes, leukocytes and platelets. The primary reason for assessing the red blood cell (RBC) is to check anemia and to evaluate normal hematopoiesis[25]. Packed cell volume (PCV) represents 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[26]. There was significant decrease (P < 0.05) in all hematological parameters (except the white blood cells that showed an increase) of diabetic untreated group and these were ameliorated by the administration of extract at varied doses. Low hematological indices in diabetic untreated group may be a result of anemia or the onset of glycosylation process because the reactive oxygen species generated in diabetic state has been implicated in red cell damage[27]. Both doses of C. pentandra ethanol extract show no damaging effect on all hematological parameters when compared to normoglycemic.

Platelets are fragment of cells that participate in blood clotting, and initiate repair of blood vessels, and are also considered as acute phase reactant to infection or inflammation. 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. The significant (P < 0.05) reduction in PLT and increase in WBC indicated in diabetic untreated group when compared to normoglycemic is in line with the studies carried out by Edet et al.[28]. They postulated that alloxan diabetogenesis may cause perturbation in the bone marrow stem cells. Diabetes induces dyslipidemia due to insulin deficiency or insulin resistance because insulin has an inhibitory action on 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key role rate-limiting enzyme responsible for the metabolism of cholesterol rich low density lipid particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. High density lipoprotein (HDL) is an antiatherogenic lipoprotein. It transports cholesterol from peripheral tissue into the liver and thereby acts as a protective factor against coronary heart disease[29]. The ethanol leaf extract of C. pentandra had hypoglycemic effect when orally administered to diabetic rats at varied doses. It was also able to ameliorate the biochemical anomalies that resulted from diabetes mellitus.

Conflict of interest statement

The authors report no conflict of interest.

References


