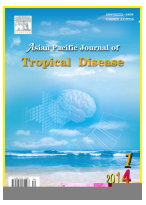
HOSTED BY

Contents listsavailableat ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Original article doi:

©2015 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

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 Engineeringand Biotechnology, Global Institute for Bioexploration Unit (GIBEX),Federal University of Technology, Minna, Nigeria*

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

ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 25 May, 2nd revised form 27 May 2015
Accepted 21 Jun 2015
Available online 30 Jul 2015

Keywords:
Ceiba pentandra
Diabetes
Hypoglycaemic activity
Haematological parameter
Normoglycemic group

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.
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 experiment was terminated on the 17th day, the rats were euthanized, and the blood samples were collected for hematological and biochemical parameters. 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. Other haematological parameters favorably compared with the normoglycemic group. Body weight of the treated groups remained stable as against the reference group II. Activities of the serum alanine aminotransferase and aspartate aminotransferase increased significantly ($P < 0.05$) in standard drug, 400 mg/kg body weight and untreated groups in reference to the normoglycemic group, while urea, creatinine, total and conjugated bilirubin all increased significantly ($P < 0.05$) in the standard drug and untreated groups. Serum chloride and potassium also exhibited increased levels in standard drug and untreated groups but exhibited a reverse trend in bicarbonate and sodium. Total cholesterol and triglyceride increased in untreated and treated groups as against the normoglycemic group, while the high density lipoprotein increased in all groups as 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

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

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).

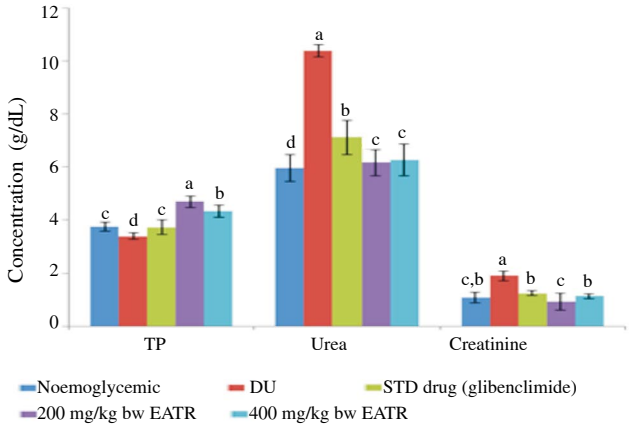


Figure 4. Comparative effects of ethyl acetate fraction of *C. pentandra* leaf on TP, urea, and creatinine.

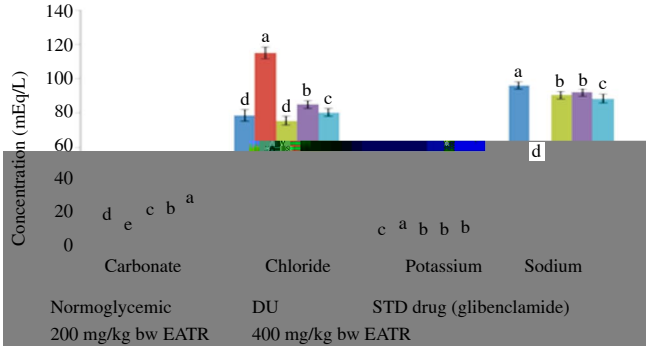


Figure 5. Comparative effect of ethyl acetate fraction of *C. pentandra* leaf on serum electrolytes in all groups.

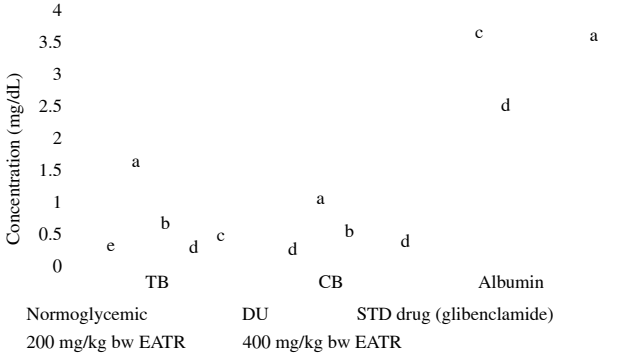


Figure 6. Effect of ethylacetate fraction of *C. pentandra* albumin, total bilirubin, and conjugated bilirubin.
TB: Total bilirubin; CB: Conjugated bilirubin.

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).

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).

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).

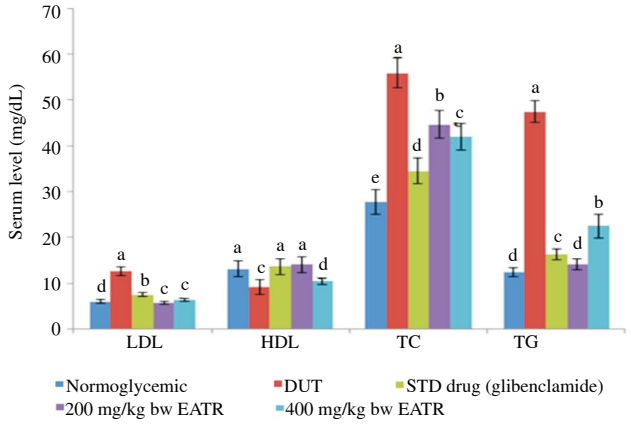


Figure 7. Comparative effect of ethyl acetate fraction of *C. pentandra* leaf on lipid profile.

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 (hepatitis and cirrhosis) and hemolytic disease of the newborn[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.

Acknowledgments

Acknowledgement is given to all the authors and the biochemistry laboratory and technical staff, Federal University of Technology, Minna, Nigeria. They really assist us to complete the research in good time. We all worked effectively to give life to this research work.

References

[1] Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect* 2001; **109**: 69-75.

[2] Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000; **106**(2): 171-6.

[3] Diabetes mellitus. Report of a WHO Study Group. *World Health Organ Tech Rep Ser* 1985; **727**: 1-113.

[4] Muhammad HL, Makun HA, Kabiru AY, Mann A, Busari MB, Abdullah AS, et al. *In vitro* antibacterial activity and *in vivo* acute toxicological studies of *Nelsonia campestris* aqueous leaf exrtact. *Int J Biochem Res Rev* 2015; **7**(1): 27-35.

[5] Ueda H, Kaneda N, Kawanishi K, Alves SM, Moriyasu M. A new isoflavone glycoside from *Ceiba pentandra* (L.) Gaertner. *Chem Pharm Bull (Tokyo)* 2002; **50**(3): 403-4.

[6] Elumalai A, Mathangi N, Didala A, Kasarla R, Venkatesh Y. A review on *Ceiba pentandra* and its medicinal features. *Asian J Pharm Technol* 2012; **2**(3): 83-6.

[7] Friday TE, Omale J, Olupinyo O, Adah G. Investigations on the nutritional and medicinal potentials of *Ceiba pentandra* leaf. *Int J Plant Physiol Biochem* 2011; **3**(6): 95-101.

[8] Sarkiyayi M, Ibrahim S, Abubakar MS. Toxicological studies of *Ceiba pentandra* Linn. *Afr J Biochem Res* 2009; **3**(7): 279-81.

[9] Aloke C, Nwachukwu N, Idenyi JN, Ugwuja EI, Nwachi EU, Edeogu CO, et al. Hypoglycaemic and hypolipidaemic effects of feed formulated with *Ceiba pentandra* leaves in alloxan induced diabetic rats. *Australian J Basic Appl Sci* 2010; **4**(9): 4473-7.

[10] Bairwa NK, Sethiya NK, Mishra SH. Protective effect of stem bark of *Ceiba pentandra* Linn against paracetamol-induced hepatotoxicity in rats. *Pharmacognosy Res* 2010; **2**: 26-30.

[11] Jamil M, ul Haq I, Mirza B, Qayyum M. Isolation of antibacterial compounds from *Quercus dilatata* L. through bioassay guided fractionation. *Ann Clin Microbiol Antimicrob* 2012; **11**: 11.

[12] Peschke E, Ebelt H, Bromme HJ, Peschke D. 'Classical' and 'new' diabetogens--comparison of their effect on isolated rat pancreatic islets *in vitro*. *Cell Mol Life Sci* 2007; **57**(1): 158-64.

[13] Frederick E, Kayode A, Sylvester U. Evaluation of the methanolic extract of mistletoe (*Tapinanthus bangwensis*) leaves from orange trees for phytochemical properties and its physiologic effects on steptozotocin induced diabetes mellitus in laboratory animals. *World Appl Sci J* 2010; **9**(9): 975-9.

[14] Musabayane CT, Bwititi PT, Oyewole JA. Effects of oral administration of some herbal extracts on food consumption and blood glucose levels in normal and streptozotocin-treated diabetic rats. *Methods Find Exp Clin Pharmacol* 2006; **28**(4): 223-8.

[15] Santos MC, Louzada RA, Souza EC, Fortunato RS, Vasconcelos AL, Souza KL, et al. Diabetes mellitus increases reactive oxygen species production in the thyroid of male rats. *Endocrinology* 2013; **154**(3): 1361-72.

[16] Etuk EU, Bello SO, Isezuo SA, Mohammed BJ. Ethnobotanical survey of medicinal plants used for the treatment of diabetes mellitus in the north-western region of Nigeria. *J Ethnopharmacol* 2009; **121**: 135-9.

[17] Saif-ur-Rehman, Jafri SA, Ahmed I, Shakoor A, Iqbal HM, Ahmad BM, et al. Investigation of hypoglycemic effect of *Ceiba pentandra* root bark extract in normal and alloxan induced diabetic albino rats. *Int J Agro Vet Med Sci* 2010; **4**(3): 88-95.

[18] Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. *Toxicology* 1993; **81**(3): 173-9.

[19] Saini V. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. *World J Diabetes* 2010; **1**(3): 68-75.

[20] Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated haemoglobin, diadetes, and cardiovascular risk in non-diabetic adults. *N Engl J Med* 2010; **362**: 800-11.

[21] Yadav JP, Saini S, Kalia AN, Dangi AS. Hypoglycemic and hypolipidemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan-induced diabetic rats. *Indian J Pharmacol* 2008; **40**: 23-7.

[22] Adesokan AA, Oyewole OI, Turay BM. Kidney and liver function parameters in alloxan-induced diabetic rats treated with *Aloe barbadensis* juice extract. *Sierra Leone J Biomed Res* 2009; **1**(1): 33-7.

[23] Sheriff M, Tukur MA, Bilkisu MM, Sera S, Falmata AS. The effect of oral administration of honey and glucophage alone or their combination on the serum biochemical parameters of induced diabetic rats. *Res Pharm Biotech* 2011; **3**(9): 118-22.

[24] Goran HM. Serum bilirubin. Clinical biochemistry. 2009; Retrieved from http:/www.win2pdf.com., retrieved on16th February, 2013.

[25] Krautwald M, Münch G. Advanced glycation end productsas biomarkersand gerontotoxins: a basis to explore methylglyoxal-lowering agents for Alzheimer's disease? *Exp Gerontol* 2010; **45**(10): 744-51.

[26] Comazzi S, Spagnolo V, Bonfanti U. Erythrocyte changes in canine diabetes mellitus: *in vitro* effects of hyperglycaemia and ketoacidosis. *Comp Clin Path* 2004; **12**: 199-205.

[27] Rao G, Kamath U, Raghothama C, Pradeep KS, Rao P. Maternal and fetal indicators of oxidative stress in various obstetric complications. *Indian J Clin Biochem* 2003; **18**: 80-6.

[28] Thomas M, Tsalamandris C, Macisaac R, Jerums G. Anaemia in diabetes: an emerging complication of microvascular disease. *Curr Diabetes Rev* 2005; **1**: 107-26.

[29] Edet EE, Akpanabiatu MI, Uboh FE, Edet TE, Eno AE, Itam EH, et al. *Gongronema latifolium* crude leaf extract reverses alterations in hematological indices and weight loss in diabetic rats. *J Pharmacol Toxicol* 2011; **6**: 174-81.

[30] Johnson M, Olufunmilayo LA, Adegboyega CC, Adetayo OM. Evaluation of antidiabetic and the effect of methanolic leaf extract of *Jatropha curcas* on some biochemical parameters inalloxan induced diabetic malealbino rats. *Eur J Med Plants* 2014; **4**(12): 1501-12.