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Research Article

Modulation of antioxidant activities, markers of hepatic and renal dysfunctions in alloxan-induced diabetic rats by *Combretum dolichopetalum*

Chinedum U. EMELIKE[1]*, Ojichukwuka E. CHIJIOKE-AGU[1] Favour-Ann K. NWOKE[1], Romanus OYIBE[1], Chinwendu G. ENWEREUZO[2], Eze F. AHUEKWE[3]

[1]*Alex Ekwueme Federal University, Department of Physiology, Faculty of Basic Medical Sciences, Ndufu-Alike, Nigeria; chinedum.emelike@funai.edu.ng (*corresponding author); melaninojii@gmail.com; favour.nwoke@funai.edu.ng; oyiberomanusagbo@gmail.com*

[2]*Middlesex University, Department of Cardiology, Faculty of Science and Technology, The Burroughs, NW4 4BT, Hendon, London, United Kingdom; ce476@live.mdx.ac.uk*

[3]*Covenant University, Department of Biological Sciences, College of Science and Technology, Ota, Nigeria; eze.ahuekwe@covenantuniversity.edu.ng*

Abstract

Diabetes mellitus is a metabolic dysfunction of insulin secretion exhibiting hyperglycemia with abnormalities in protein, fat and carbohydrate metabolism. The present study aimed to investigate the effect of *Combretum dolichopetalum* (CD) on hepatic parameters, renal indices and antioxidant markers of alloxan-induced diabetic rats. Twenty (20) male rats were used, and they were divided into four groups of five rats each. Group 1- Normal control received distilled water (non-diabetic control); Group 2- Diabetic control received distilled water; Group 3 and Group 4 are Diabetic rats treated with 200 mg/kg and 400 mg/kg of CD extract. There were significant decreases in the final body weights of Group 2 compared with Group 1 and an elevation of the body weights of Groups 3 and 4 compared with Group 2. There were significant increases in the blood glucose concentration of Group 2 compared with Group 1 and a reduction in blood glucose concentration of Groups 3 and 4 compared with Group 2. There were increases in the alanine amino transaminase (ALT), alkaline phosphatase (ALP) activities and serum total and conjugated bilirubin levels of Groups 2 and 3 compared with Group 1, but a reduction in the ALP, ALT activities and total bilirubin in Group 4. There was an elevation in the superoxide dismutase (SOD) the activity of Group 4. There were reductions in catalase activities and no change in the renal indices. The study showed ameliorating potentials on hyperglycemia, hepatoprotective activity and antioxidation effects.

Keywords: *Combretum dolichopetalum;* diabetes mellitus; metabolic disorder; oxidative stress

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Introduction

Diabetes mellitus is a looming health challenge that has taken the global health sector by storm. It is a functional flaw of the human body and loses its ability to convert sugar (glucose) into energy for consumption and use by the body (Ogbera and Ekpebegh, 2014). This condition is a metabolic dysfunction depicting chronic hyperglycemia with irregularities in the metabolism of protein, fat and carbohydrates arising from flaws around insulin secretion, action or both (ADA, 2009). Diabetes mellitus has proven to be responsible for long term damage, dysfunction and failure of vital organs. Hepatic and renal dysfunction appears almost simultaneously concerning individual independent organ failures or a collective of organ failures in fatally ill patients (Darmon *et al.,* 2014). These dysfunctions emerge simultaneously in disease cases involving the liver and kidney, primary hepatic dysfunction with secondary renal dysfunction and primary renal dysfunction with secondary hepatic dysfunction (Moore *et al.,* 1991). Simultaneous occurrence of these dysfunctions is ready to share similar pathogenetic mechanisms.

The liver is the body's detoxification organ and plays an essential role in maintaining metabolic homeostasis. Illnesses associated with the liver are of great essence because of this specific function (Kalra *et al.,* 2023). Free radicals produced from compounds are metabolised in the liver. These free radicals are further scavenged by antioxidants to maintain oxidative/antioxidative balance in the liver. However, when the oxidative/antioxidative balance scale is overturned, which leads to a condition termed "Oxidative Stress". Oxidative stress leads to damaging processes in the liver and ends up in liver diseases (Muriel and Arauz, 2012).

The use of medicinal plants in the treatment and prevention of specific illnesses/ailments have demonstrated a vital role in the global healthcare system (Dasgupta and Bratati, 2007; Okoro *et al*., 2021; Ekakitie *et al*., 2021; Aloke *et al*., 2021a; Aloke *et al*., 2021b; Aloke *et al*., 2021c; Emelike *et al*., 2021). Phenolics have the capacity to scavenge free radicals resulting from their redox properties, commonly found in leaves, flowering tissues and woody parts such as stems and barks of plants (Larson, 1988).

Combretum dolichopetalum is an indigenous African plant predominantly found in the Eastern parts of Nigeria. The plant serves a vital role in food nutrition and is also significant in ethnomedicine. In food nutrition, the leaves are boiled and used as soup (Ameyaw *et al*., 2012; Uzor *et al*., 2014; Barku *et al*., 2014; Emelike *et al*., 2020). The study of the medicinal properties of plants to bring relief to ailments has evolved. It delivers the arsenal required to fight off oxidative stress-related diseases such as hepatic and renal dysfunctions, cancer, cardiovascular diseases and other neurodegenerative disorders. It has become predominant due to the assemblage of free radicals in the body (Sahoo *et al*., 2012).

The present study aimed to determine the modulation of antioxidant activities, markers of hepatic and renal dysfunctions in alloxan-induced diabetic rats by *Combretum dolichopetalum.*

Materials and Methods

Plant materials

Fresh matured leaves of *C. dolichopetalum* were located and collected in 2019 from its natural habitat in Nsukka, Enugu. Mr C. J. Onyeukwu, a taxonomist of the Department of Plant Science and Biotechnology, University of Nigeria, authenticated the plant. A voucher specimen (UNH No.49a) was deposited at the herbarium.

Preparation of extract

The leaves extract was washed and air-dried at room temperature for 7 days. It was grounded to a coarse powder using an electric blender (model ms-233, China). About two kilograms (2 kg) were extracted for 48 h with methanol in a Soxhlet extraction following the method of Jensen (2007). Following the extraction, the

extract was collected and dried at a low temperature (40 °C) to obtain the pale dark green used for animal experiments.

Chemicals

Alloxan monohydrate used in this study was sourced from Sigma-Aldrich Chemical Company, United States of America. All other chemicals employed were of standard grade.

Animal experiments

Twenty (20) male rats weighing about 120-200 g were used for the study obtained from the Animal House, Department of Physiology, University of Nigeria Enugu Campus. They were acclimatized to their feed (Vital feed®, Nigeria) and water (which they had access to *ad libitum*) for two weeks before the commencement of the experiment. The study protocol was approved by the College of Medicine Research Ethics Committee of the University, with protocol number 026/02/2017. The study followed the established institutional guidelines and the NIH guidelines for experimental animals.

Induction of experimental diabetes mellitus

After two (2) weeks of acclimatization, a freshly prepared solution of alloxan monohydrate (0.5 g dissolved in 8.5 ml of distilled water) was administered intraperitoneally to fifteen (15) rats at a dosage of 150 mg/kg body weight at a fasting state. The remaining five (5) rats served as the non-diabetic control group. Blood samples were collected from the tail vein of the rats for blood glucose concentration analysis using a blood glucometer (Accu-Chek ®, India) before the commencement of the administration of the extract. The alloxan-treated rats with fasting blood glucose levels>200 mg/dl after seven days of induction and evidence of hyperglycemia considered to be diabetic were used for the study.

Experimental procedure

The normal rats and rats with stable diabetes mellitus were assigned into four groups of five rats per group.

The groups were as follows:

Group 1- Normal control received distilled water (non-diabetic control)

Group 2- Diabetic control also received distilled water.

Group 3- Diabetic rats treated with 200 mg/kg of CD extract.

Group 4- Diabetic rats treated with 400 mg/kg of CD extract.

Bodyweight (before and after administration) was measured using a digital electronic weigh Scout Pro SP 401 (China). At the end of twenty-eight (28) days of the administration, the rats fasted overnight, and blood was collected from their tail for blood glucose analysis using a blood glucose meter. The rats were anaesthetized with 2% sodium pentobarbital (75 mg/kg) intraperitoneally. Venous blood was obtained via the orbital, poured into plain tubes and allowed to clot. Sera were obtained from the clotted sample after centrifuging at 3000 rpm for 10 minutes for the analysis of antioxidant activity and liver and renal function parameters.

Body weights

The changes in the weights of the rats were recorded using a digital electronic weighing scale model number Scout Pro SP 401 (China).

Liver function tests

The liver indices analysed include total bilirubin and direct bilirubin. Others include alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) using A25 Biosystem (Barcelona, Spain).

Kidney function tests

Electrolytes (sodium, potassium, chloride and bicarbonate) were estimated with Easylyte® analyzer Medica Corporation, Bedford, USA while urea and creatinine were analysed with A25 Biosystem, Barcelona, Spain.

Antioxidant activity

The level of superoxide dismutase (SOD) was measured following the procedure of Assady *et al*. (2011). The activity of catalase (CAT) in serum was determined according to the method described by Hadwan (2018). The serum concentration of reduced glutathione (GSH) was measured by the method described by Alisik (2019).

Statistical analysis

Results were analysed using GraphPad Prism (GraphPad® Software, San Diego, CA, USA). One-way ANOVA following the Turkey post hoc test was used for data comparison. The values were regarded as significant at $P<0.05$. Results were expressed as mean ± standard error of the mean

Results

Effect of C. dolichopetalum on body weight of rats

Table 1 shows the body weight and percentage change in the body weights of the rats. There were no significant differences (P>0.05) in the initial body weights of all the rats in the respective groups and no significant differences (P>0.05) in the final body weights of Groups 3 and 4 compared with Group 1.

In contrast, there were significant decreases (P<0.05) in the final body weights of Group 2 (24.7±1.00 loss of weight) compared with the Group 1 but significant elevation (P<0.05) of the final body weights of Groups 3 and 4 compared with the Groups 2.

Table 1. Effect of *C. dolichopetalum* on body weight of rats

Parameters	Group 1	Group 2	Group 3	Group 4
Initial body weight (g)	178.2 ± 2.00	179.1±1.90	180.2±2.50	180.3±1.00
Final body weight (g)	205.1±2.30	157.0±1.30*	197.3±1.40#	206.2±3.40#
Weight difference (g)	26.3±2.10 (increase)	24.7±1.00 (decrease)	14.7±1.00 (increase)	24.0±2.89 (increase)

Values are mean ± SEM, n=5, *= $P<0.05$ versus normal control group #= $P<0.05$ versus diabetic control

Effect of C. dolichopetalum on blood glucose concentration of rats

Table 2 shows the blood glucose concentration of the rats that were studied. There were significant differences (P<0.05) in the blood glucose concentration of Groups 2, 3 and 4 compared with Group 1 in the initial blood glucose. Also, there were significant increases (P<0.05) in the final blood glucose concentration of Group 2 compared with Group1, but a significant reduction (P<0.05) of their hyperglycemia in Groups 3 and 4 compared with Group 2.

Table 2. Effect of *C. dolichopetalum* on blood glucose concentration of rats

Parameters	Group 1	Group 2	Group 3	Group 4
Initial glucose level (mg/dL)	76.2 ± 5.03	299.1±1.40*	308.2±4.50*	314.0±4.80*
Final glucose level (mg/dL)	100.1±4.30	306.0±3.30*	174.3±1.50*#	113.2±3.10*#
Glucose difference (mg/dL)	24.3±2.10 (increase)	26.7±3.30 (increase)	133.4±5.90 (decrease)	201.0±6.30 (decrease)

Values are mean ± SEM, n=5, *= *P*< 0.05 versus normal control group #= *P*< 0.05 versus diabetic control

Effect of C. dolichopetalum on the liver function test of rats

The serum AST activities of Group 3 were significantly (P<0.05) increased when compared with Group 1. There were significant increases (P<0.05) in the ALP, ALT activities and Serum Total and Conjugated bilirubin levels of Groups 2 and 3 compared with Group 1. Also, there were significant increases (P<0.05) in the ALP and ALT activities of Group 3 when compared with Group 2 but a significant reduction (P<0.05) of the ALP, ALT activities and Total bilirubin in Group 4 when compared with Group 2 (Table 3).

Table 3. Effect of *C. dolichopetalum* on the liver function test of rats

Parameters	Group 1	Group 2	Group 3	Group 4
AST (IU/L)	32.90±1.69	40.90±2.33	49.47±2.78*	26.63±1.98
ALP (IU/L)	110±3.40	127±2.40*	204±6.50*#	96.9±2.40#
ALT (IU/L)	26.2±2.39	38.9±3.32*	92.3±4.22*#	21.7±1.07#
Total bilirubin (µmol/L)	9.50±0.93	19.3±0.77*	23.7±2.90*	10.6±2.35#
Conjugated bilirubin (µmol/L)	2.20±0.15	3.10±0.12*	3.37±0.15*	2.50±0.17

Values are mean ± SEM, n=5, *= *P*< 0.05 versus normal control group #= *P*< 0.05 versus diabetic control

Effect of C. dolichopetalum on renal indices of rats

There was no significant (P>0.05) change in the renal indices of Groups 2, 3 and 4 when compared with Group 1 (Table 4).

Table 4. Effect of *C. dolichopetalum* on renal indices of rats

Parameters	Group 1	Group 2	Group 3	Group 4
Sodium (mmol/L)	140±2.03	143±0.58	142±1.00	144±0.88
Potassium (mmol/L)	12.7±3.61	10.6±1.82	11.2±1.44	7.27±1.84
Chloride (mmol/L)	101±2.08	103±0.58	102±1.15	104±0.58
Bicarbonate (mmol/L)	24.3±1.20	25.0±1.15	22.3±0.88	24.7±0.33
Urea (mmol/L)	6.43±0.79	9.63±2.10	9.0±1.74	8.47±1.98
Creatinine (mmol/L)	94.0±8.66	89.3±7.8	86.3±3.9	86.0±3.00

Values are mean ± SEM, n=5, *= *P*< 0.05 versus normal control group #= *P*< 0.05 versus diabetic control

Effect of C. dolichopetalum on antioxidant of rats

The oxidative stress markers of the rats are shown in Table 5. There were no significant differences (P>0.05) in the SOD activities of Groups 2 and 3 compared with Group 1. On the other hand, there was a significant elevation (P<0.05) in the SOD activity of Group 4 compared with Groups 1 and 2. There were significant reductions (P<0.05) in the catalase activities of Groups 2 and 3 compared with Group 1.

Table 5. Effect of *C. dolichopetalum* on antioxidant of rats

Parameters	Group 1	Group 2	Group 3	Group 4
SOD (U/M)	9.46±0.41	8.92±0.97	11.2±0.55	12.6±0.39*#
GPx (U/M)	0.80±0.14	1.28±0.14	0.97±0.11	0.96±0.12
CAT (KU/L)	65.9±2.6	46.7±2.31*	39.7±0.26*	57.9±6.20

Values are mean ± SEM, n=5, *= P< 0.05 versus normal control group #= P< 0.05 versus diabetic control

Discussion

In the present study, there was an increase in the body weight of Group 1 (Normal control), which suggests the increased synthesis of tissue proteins (Eleazu *et al*., 2019). The reduction in the body weight in the diabetic control group (Group 2) could be a result of alloxan destroying the pancreatic cells leading to insulin deficiency, which causes raised production of ketone bodies. Thus, elevated ketone bodies with increased lipolysis result in a loss in body weight (Cotter *et al*., 2013). However, the gain in body weight observed in diabetic rats treated with *C. dolichopetalum* could be attributed to better modulation of hyperglycemia in the diabetic rats and reduction in fasting blood glucose which could improve body weight in alloxan-induced diabetic rats (Yin *et al*., 2018). Additionally, the ability of the *C. dolichopetalum* extracts to enhance body weight may be due to its ability to lower elevated blood glucose via increased glucose metabolism, and this may be attributed to the protective effect of the extract at a higher dose. This is in agreement with the report of Eleazu *et al.* (2014).

The alloxan monohydrate rat model of diabetes is one of the most commonly used because it mimics many of the complications of human diabetes (Szkudelski, 2001). There is increasing evidence that alloxan causes diabetes by rapid depletion of β-cells by DNA alkylation and accumulation of cytotoxic free radicals resulting from initial islets inflammation and infiltration of activated macrophages and lymphocytes in inflammatory focus. This will reduce plasma insulin concentration, resulting in a sustained hyperglycemia state (Szkudelski, 2001). The reduction of the blood glucose concentrations of diabetic rats following the administration of *C. dolichopetalum* to the extent observed in this study indicates the ameliorating potentials of *C. dolichopetalum* on hyperglycemia. The hypoglycemic effect of the extracts may be attributed to the enhanced secretion of insulin from the beta cells of the pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity (Maniyar and Bhixavatimath, 2012; Emelike *et al*., 2020). Many researchers have reported that flavonoids are potent bioactive antioxidants and anti-diabetic agents and that the alkaloid content of plants could regulate insulin secretion. Also, saponins have been shown to exhibit blood-glucose-lowering potentials (Patel *et al*., 2012; Emelike *et al*., 2021).

The liver is necrotic in diabetic rats, which will lead to increased activities of total bilirubin, conjugated bilirubin, ALP, ALT and AST (Aloke *et al*., 2021b). They leak from the liver cytosol into the bloodstream. It is also an indicator of the hepatotoxicity of alloxan (Saeed *et al*., 2008). It is in agreement with this present study. However, the oral administration of *C. dolichopetalum* at 400 mg/kg suggests a non-deleterious effect on the total bilirubin and serum liver enzymes. The liver is an essential organ that helps in drug metabolism and other toxicants. The destruction of the liver cell results in the impairment of the liver cell membrane permeability, which results in the leakage of tissue contents into the bloodstream (Saeed *et al*., 2008). Hyperglycemia increases the generation of free radicals by glucose auto-oxidation, and the increment of free radicals may lead to liver damage (Tangvarasittichai, 2015).

The non-significant effect *C. dolichopetalum* had on the kidney indices of the normal rats, diabetic rats and diabetic treated rats suggests the non-deleterious effect of *C. dolichopetalum* on the kidney.

Oxidative stress is involved in the pathogenesis of many forms of genetic and acquired hypertension (Griendling *et al*., 2013). Poorly managed diabetes frequently results in nephropathy and cardiovascular complications (Bramlage *et al*., 2019). Moreover, the alleviation of oxidative stress with antioxidant therapy has been shown to ameliorate hypertension in several animal models (Griendling *et al*., 2013). Studies have shown that uncontrolled hyperglycemia in rats was associated with the activity of antioxidant enzymes (Eleazu *et al.,* 2014). However, administration of *C. dolichopetalum* at doses of 200 and 400 mg/kg increased SOD compared to Group 1 (Normal control). 400 mg/kg of *C. dolichopetalum* significantly increased SOD compared to Diabetic control (Group 2). It suggests that an increase in SOD activity is probably due to the regeneration of the damaged liver cells by the *C. dolichopetalum* extract and the viability of diabetic rats to secret

insulin. The effects of the *Combretum dolichopetalum* extract were dose-dependent. Furthermore, this potent antioxidant activity could be attributed to the phenolic content of the *Combretum dolichopetalum*.

Conclusions

The study showed ameliorating potentials on hyperglycemia, hepatoprotective activity and antioxidation effects.

Authors' Contributions

Conceptualization: CUE, RO and CGE; Data curation: CUE, RO and CGE; Formal analysis: CUE, RO and CGE; Investigation: CUE, RO and CGE; Methodology: CUE, OEC and FKN; Project administration: EFA; Resources: CUE, RO and CGE; Software: EFA; Supervision: CUE; Validation: CUE; Visualization: CUE, RO, CGE, OEC and FKN; Writing - original draft: CUE and OEC; Writing - review and editing: CUE, FKN and EFA. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The study protocol was approved by the College of Medicine Research Ethics Committee of the University, with protocol number 026/02/2017.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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