RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v22i1.5

Udeh *et al.*/*Sokoto Journal of Veterinary Sciences*, **22**(1): 34 - 40.

Evaluation of sub-acute toxicity profile of *Combretum dolichopetalum* (E&L) methanol leaf extract in Wistar rats

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Publication History:
Received: 18-07-2023
Revised: 31-10-2023
Accepted: 14-11-2023

Abstract

This study evaluated the safety of the ethnomedicinal plant, *Combretum. dolichopetalum* methanol extract (CDME) in Wistar rats using the sub-acute toxicity model. Twenty-four adult male Wistar rats were randomly divided into 4 groups of 6 rats each. Group A (control) received 5% dimethylsulfoxide (DMSO) at 5 ml/kg, while groups B -D received CDME at 50, 100 and 200 mg/kg, respectively. All treatments were administered orally and once daily for 28 consecutive days. The haematological profile, liver and kidney function tests, lipid profile as well as antioxidant status were evaluated. The 50 mg/kg extract significantly ($P<0.05$) reduced the red blood cell count, packed cell volume, haemoglobin, but had no effect on leucocytic profile of rats. There was no significant difference ($P>0.05$) in leucocyte profile between the control and groups given the extract. At 200 mg/kg, CDME significantly ($P<0.05$) increased total protein, alkaline phosphatase (ALP) and aspartate transaminase (AST) compared to the control group. Triglyceride, high density lipoprotein (HDL-C) and very low density lipoprotein (VLDL-C) were significantly ($P<0.05$) increased by the extract at both 100 and 200 mg/kg while low density lipoprotein (LDL-C) was significantly ($P<0.05$) decreased by the extract at those doses compared to the control. Urea level was significantly higher ($P<0.05$) in rats dosed at 100mg/kg while creatinine levels was not increased by the extract. The antioxidants Superoxide dismutase and glutathione reductase were significantly ($P<0.05$) higher in rats at all doses of the extract while serum catalase level was significantly lower ($P<0.05$). We conclude that *Combretum dolichopetalum* could cause a reduction in erythrocyte parameters and should be administered with caution in anaemic conditions as well as in liver diseases.

Keywords: *Combretum dolichopetalum*, Haematology, Safety, Serum biochemistry, Wistar rats

Introduction

Ethnopharmacology has gradually taken a prominent position in medicine globally, but most especially in the developing nations. This is because plants contain abundant secondary metabolites (phytochemicals) with potential pharmacological activity against various diseases (Ngatchic *et al*., 2020). Almost 80%

of the world's population depend on medicinal plants for respite from various illnesses as they are effective, affordable and readily available (WHO, 2005).

Combretum dolichopetalum Engl. & Diels (*Combretaceae*) is an herbal plant used widely in African traditional medicine for maintaining health and treating a variety of ailments. The plant is known as "achicha nza" (food of the sun bird) in Igbo and "okoso" in Edo languages respectively (Uzor *et al.,* 2014). The roots of this plant have been used in relieving menstrual pain, facilitate uterine contraction and milk-let down post-partum. The leaves have been reported to have wound healing, antiulcer, antidiarrhoeal activity (Ameyaw *et al*., 2012). The antiulcer, anti-hepatotoxic, trypanocidal, anti-inflammatory, antidiabetic and antispasmolytic activities of this plant have also been reported (Barku *et al*., 2014). Anti-diarrhoeal activity of the plant has been established (Onoja & Udeh, 2015). Despite the massive popularity of this plant amongst Africans and its efficacy against various ailments which has been scientifically ratified, its safety on prolonged usage has not been established because information on its toxicology is scarce. This study was therefore designed to study the sub-acute toxicity effects of *C. dolichopetalum* in Wistar rats.

Materials and Methods

Plant collection and identification

The fresh leaves of *C. dolichopetalum* (E&L) were sourced from Alakwo, Owerri in Imo State Nigeria (MOUAU/VPP/2014/013) and identified by a Taxonomist of Bioresource Development and Conservation Programme, Enugu State Nigeria.

Preparation of plant extract

Fresh leaves of *C. dolichopetalum* were dried for four weeks at room temperature and pulverized using electric blender. The ground leaves were then extracted in 80% methanol using soxhlet apparatus. The extract was dried using a hot air oven at the temperature of 35°C, enoded as CMDE and stored in a refrigerator (4^{o}C). The percentage yield of the sample was determined using the formula as follows:

$$\text{Percentage yield (\%)} = \frac{\text{weight of the extract}}{\text{Weight of the dried powder}} \times 100$$

Handling of experimental animals

Male Wistar rats weighing between 103-171 g were obtained from the University of Nigeria, Nsukka. The rats were housed at the animal house, Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Nigeria. The animals were kept in aluminium cages at room temperature under a 12 h dark/light cycle. They were fed with standard rat pellets (Vital® feeds, Nigeria) and allowed access to water *ad libitum*. The study was performed in accordance with the ethical guidelines stipulated by the ethical committee of Michael Okpara University of Agriculture, Umudike, Nigeria and was assigned the following ethical approval number; MOUAU/CVM/REC/202350. These guidelines were in accordance with the international accepted guidelines for laboratory animal use and care.

Experimental design

Twenty-four (24) adult male Wistar rats were randomly divided into 4 groups of 6 rats each. Group A (control) received 5% dimethylsulfoxide (DMSO) at 5 ml/kg, while groups B-D received CDME 50, 100 and 200 mg/kg, respectively. All treatments were administered orally and once daily for 28 consecutive days, after which 5 mL of blood samples were collected through the ocular puncture into each of plain and EDTA vacutainers. The blood samples in EDTA container were used for haematology, while the blood in the plain containers was allowed to clot and the serum harvested were used for antioxidant and biochemical analyses.

Determination of haematological indices

Haemoglobin concentration (Hb) and packed cell volume (PCV) were determined by cyanomethemoglobin and haematocrit methods respectively as described in Brar *et al.* (2000). Total white blood cell count (TWBC), differential leucocyte count and red blood cell count (RBC), were carried out on the blood collected in the EDTA bottles using improved Neubauer haemocytometer and Wintrobe's hematocrit as described by Dacie & Lewis (1991). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined according to the method described by Jain (1986).

Assay of biochemical parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities as well as serum total bilirubin, conjugated bilirubin, total protein, urea, creatinine, total cholesterol, triacylglycerol and high-density lipoprotein cholesterol (HDL-C) concentration were evaluated using a commercially available reagent kit (Randox Diagnostic Laboratories, United Kingdom). The assay was carried out according to the

manufacturer's instructions. Serum low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation (Friedewald *et al*., 1972). LDL-C = [TC – {HDL-C+ (TG/5)}] where VLDL-C = (TG/5) (Bhandari *et al*., 2013). Very low-density lipoprotein-cholesterol (VLDL-C) was calculated according to the method of Wilson *et al*. (1981) as VLDL = 0.2 x TG (where TG is total glycerides).

Determination of lipid peroxidation (LPO) in serum

The level of the thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured by the method described by Draper & Hadley (1990). Superoxide dismutase activity was assayed as described by Xin *et al*. (1991). Catalase activity was determined using the method of Aebi (1983).

Data analysis

Data obtained from the study were expressed as mean ± standard error of the mean (mean ± SEM). Statistical analysis was performed by one analysis of variance (one-way ANOVA) at 95 % confidence level using SPSS statistical software. Mean differences were separated using the Least Significant Difference (LSD).

Results

The effects of *Combretum dolichopetalum* methanol extract (CDME) treatment on the haematological profiles respectively presented in Tables 1 and 2. The extract at 50 mg/kg caused a significant (P< 0.05) reduction in red blood cell counts, haemoglobin, PCV, MCV and MCH; but significantly (P<0.05) increased MCHC levels of the treated groups when compared with the control group (Table 1). The extract did not produce any significant (P>0.05) effect on the white blood cells and their differentials (Table 2) when compared with the control.

The effect of CDME treatment on serum enzyme markers of liver function in Wistar rats is presented in Table 3. The extract (100 mg/kg and 200 mg/kg) significantly lowered (P<0.05) serum AST and ALT activities as well as the serum levels of total protein of the treated groups when group compared with the control group. Serum bilirubin was significantly (P<0.05) lower in treated rats at 50 mg/kg. Also, the extract at same doses significantly increased (P<0.05) serum levels of ALP in the treated groups when compared with the control group.

The effect of CDME on lipid profile is presented in Table 4. There was no significant (P<0.05) change in the serum cholesterol levels in the treated groups when compared with the control group. The extract

Table 1: Effect of *Combretum dolichopetalum* methanol extract on erythrocytic profile

Parameter	Control	CDME 50mg/kg	CDME 100mg/kg	CDME 200mg/kg
Haemoglobin (g/dL)	20.45 ± 0.13	18.20 ± 0.42*	19.45 ± 0.31	19.40 ± 0.37*
PCV (%)	51.25 ± 0.63	43.75 ± 1.70*	47.50 ± 0.65*	47.25 ± 1.25*
RBC (x106/µL)	8.02 ± 0.07	6.88 ± 0.26*	7.51 ± 0.11	7.51 ± 0.19
MCV (fL)	63.94 ± 0.28	63.60 ± 0.20	63.25 ± 0.15*	62.89 ± 0.08*
MCH (pg)	25.52 ± 0.11	26.51 ± 0.49*	25.90 ± 0.18	25.84 ± 0.17
MCHC (g/dL)	39.91 ± 0.33	41.69 ± 0.80*	40.94 ± 0.20	41.08 ± 0.30

*p < 0.05 when compared with the control

Table 2: Leucocytic profile of Wistar rats given *Combretum dolichopetalum* methanol leaf extract

Parameter	Control	CDME 50mg/kg	CDME 100mg/kg	CDME 200mg/kg
TWBC (x10^3/µL)	10.06 ± 0.40	10.43 ± 1.35	12.21 ± 0.46	11.06 ± 0.71
Relative lymphocyte (%)	59.00 ± 0.91	56.25 ± 0.75	55.75 ± 0.63	56.25 ± 1.65
Relative neutrophil (%)	32.75 ± 0.75	36.50 ± 1.19	36.00 ± 1.08	37.00 ± 2.16
Relative monocyte (%)	5.75 ± 0.25	4.75 ± 0.48	5.75 ± 0.63	4.75 ± 0.25
Relative eosinophil (%)	2.50 ± 0.29	2.50 ± 0.65	2.25 ± 0.25	2.00 ± 0.71
Relative basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Absolute Lymphocyte (x10^3/µL)	5.93 ± 0.19	5.87 ± 0.78	6.80 ± 0.23	6.19 ± 0.23
Absolute neutrophil (x10^3/µL)	3.30 ± 0.20	3.79 ± 0.49	4.40 ± 0.25	4.13 ± 0.48
Absolute monocyte (x10^3/µL)	0.58 ± 0.04	0.50 ± 0.09	0.70 ± 0.07	0.53 ± 0.05
Absolute eosinophil (x10^3/µL)	0.25 ± 0.03	0.26 ± 0.08	0.28 ± 0.04	0.21 ± 0.07
Absolute basophil (x10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

No significant difference P > 0.05 when compared with the control, TWBC = total white blood cell

Table 3: Effect of *C. dolichopetalum* methanol leaf extract on liver function markers

Parameter	Control	CDME 50 mg/kg	CDME 100mg/kg	CDME 200mg/kg
Total Protein (g/dL)	7.25 ± 0.07	7.18 ± 0.09	7.16 ± 0.02	6.74 ± 0.04*
Albumin (g/dL)	4.28 ± 0.05	4.09 ± 0.03	4.14 ± 0.06	4.11 ± 0.16
Globulin (g/dL)	2.96 ± 0.06	3.09 ± 0.08	3.02 ± 0.08	2.64 ± 0.19
ALP (IU/L)	47.67 ± 0.96	48.69 ± 0.35	49.85 ± 0.54*	51.91 ± 0.31*
AST (IU/L)	61.43 ± 3.00	44.83 ± 2.50)	51.43 ± 2.27*	47.38 ± 1.13*
ALT (IU/L)	16.33 ± 1.01	10.72 ± 0.52*	15.40 ± 0.18	14.35 ± 0.79
Total Bilirubin (mg/dL)	0.16 ± 0.00	0.12 ± 0.01*	0.17 ± 0.00	0.15 ± 0.01
Direct Bilirubin (mg/dL)	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.01
Conjugated bilirubin (mg/dL)	0.13 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.12 ± 0.01

*p < 0.05 when compared with the control, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = Aspartate aminotransferase

Table 4: Effect of *C. dolichopetalum* methanol leaf extract on lipid profile of rats

Parameter	Control	CDME 50 mg/kg	CDME100 mg/kg	CDME 200 mg/kg
Cholesterol (mg/dL)	46.34 ± 1.52	43.41 ± 0.28	46.83 ± 4.16	40.73 ± 1.08
Triglyceride (mg/dL)	37.83 ± 1.74	43.35 ± 3.63	48.86 ± 2.90*	46.01 ± 0.38*
HDL-C (mg/dL)	8.80 ± 0.46	6.94 ± 1.17	9.72 ± 1.39	13.43 ± 0.89*
VLDL-C (mg/dL)	7.57 ± 0.35	8.67 ± 0.73	9.77 ± 0.58*	9.20 ± 0.08*
LDL-C (mg/dL)	29.98 ± 1.48	27.80 ± 1.18	27.34 ± 5.10	18.10 ± 1.95*

*p < 0.05 when compared with the control, HDL-C = high density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol

(100 and 200 mg/kg) caused significant (P < 0.05) higher serum levels of triglycerides, LDL-C, HDL-C and VLDL-C in the treated rats when compared with the control groups.

Table 5: Effect of *C. dolichopetalum* methanol leaf extract on kidney function markers

Parameter	Control	CDME 50mg/kg	CDME 100mg/kg	CDME 200mg/kg
Urea (mg/dL)	25.62 ± 0.30	21.88 ± 0.28*	31.80 ± 0.98*	25.91 ± 0.41
Creatinine (mg/dL)	1.27 ± 0.10	1.12 ± 0.06	1.13 ± 0.02	0.95 ± 0.07*
Urea/Creatinine ratio	20.60 ± 1.51	19.64 ± 0.92	28.19 ± 1.25*	27.93 ± 2.60*

*p < 0.05 when compared with the control

The effect of CDME treatment on serum levels of creatinine and urea is presented in Table 5. There was a significant (P<0.05) change in the serum urea level in the treated (50 and 100 mg/kg) groups when compared with the control group. The extract (50 mg/kg) did not produce any significant (P>0.05) change in the serum creatinine level in the treated groups. The extract (100 and 200 mg/kg) caused a significant (P<0.05) increase in the serum creatinine levels when compared with the control group. The extract (100 and 200 mg/kg) produced a significant (P<0.05) increase in the urea-creatinine ratio in serum of the treated groups when compared to the control groups.

The effect of CDME on *in vivo* antioxidant activity is represented in Table 6. The extract did not produce any significant (P>0.05) difference in the MDA levels of the treated groups compared to the untreated groups. The extract produced a significant (P<0.05) change in the activities of the SOD of the treated group when compared with the control group. The extract produced a significant (P<0.05) reduction in the activities of catalase in the treatment group when compared with the control group.

Discussion

The blood indices (red blood cells, white blood cells and their differentials) serve as an indicator of the physiological and pathologial status of the body and significant changes imply that the administered chemical is either protective or toxic to the haematopoietic tissue (Blann, 2014).

At all concentrations, the extract produced a significant (P<0.05) decrease in the Hb, PCV, and RBC but no significant (P>0.05) change in the white blood cells, when compared with the control. This suggests that this plant may be erythropoietic so care should be taken in patients with anaemia (Abubakar *et al*., 2019). This is in contrast with the findings of Emelike *et al*. (2021) who reported changes in both

Table 6: *In vivo* antioxidant effects of *C. dolichopetalum* methanol leaf extract on Wistar rats

Parameter	Control	Extract 50 mg/kg	Extract 100 mg/kg	Extract 200 mg/kg
MDA (nanomole/g protein)	16.13 ± 0.60	15.64 ± 0.73	19.92 ± 2.11	18.77 ± 1.29
SOD (IU/g protein)	1.44 ± 0.12	2.29 ± 0.06*	2.26 ± 0.02*	2.58 ± 0.03*
CAT (U/g protein)	17.72 ± 0.58	11.79 ± 0.73*	12.66 ± 0.54*	11.86 ± 0.47*
GSH (µg/L)	89.28 ± 4.56	85.56 ± 2.63	188.80 ± 16.60*	147.19 ± 11.80*

*p < 0.05 when compared with the control, SOD = superoxide dismutase, MDA = malondialdehyde, GSH = glutathione, CAT = catalase

white blood cells and red blood cells, but in agreement with Obakiro *et al.*(2021). White blood cells (WBCs) are major immune cells of the body. They provide immunity and defend the body against invasion by pathogens or toxins. Therefore, the non-significant difference in WBC count and its differentials between the treatment and control groups suggested that the administered doses did not interfere with the differentiation of haematopoietic stem cells into leucocytes.

The liver is a very important organ in the body due to its expedient role in the detoxification of drugs and its optimal functionality could be assessed by the concentrations of various biomarker molecules or enzymes in the serum; as changes could indicate a disease state (Eleazu *et al*., 2014). The liver is the major source of seum AST, ALT and ALP enzymes, and their level in the serum increases during liver pathology. Serum AST levels are not just indicator of pathology of the liver, but also indicator of muscle and heart dysfunction (Nayak, 2007). Alkaline phosphatase is beneficial in the diagnosis of bile duct pathologies (Nayak, 2007). Increased bilirubin production is ascribed to conditions such as primary biliary cirrhosis, hepatic cholestasis or jaundice (El-Kabbaoui *et al*., 2017). In this present study, total protein levels in control and treated rats in lower doses were not significantly (P > 0.05) different. This suggests that CDME has no deleterious effect on the liver as abnormal protein levels could be indicative of liver injury. There was increase in the levels of ALP in treated rats compared to the control but the levels of other liver enzymes were lower compared to the control. Therefore, there is a likelihood of bile duct anomaly by chronic use of this plant especially at high doses, but not on the hepatocytes themselves as other hepatocyte specific enzymes were not increased. The extract did not elicit significant changes in the levels of total and direct bilirubin in the rats, this also corroborates the non-hepatotoxic action of the extract. This is however in contrast with the findings of Emelike *et al*., 2020. Rats given CDME had no changes in cholesterol levels, while LDL-C was not increased. These effects can be attributed to the presence of bioactive phytochemicals such as flavonoids in the extract which have been reported to have anti-hyperlipidemic activity (Ngatchic *et al*., 2020).

Urea and creatinine are indices of renal function. Urea is formed in the liver as an end product of protein metabolism and thereafter eliminated by the kidneys via the urea cycle (Nayak, 2007). In the event of renal impairment, the rate of elimination of urea by the kidneys will be affected leading to high concentration of urea in the blood. However, these extract did not increase creatinine levels. Although serum creatinine levels were not adversely affected by the extract, care should be taken in cases of kidney disease. Also, there is a likely risk of hyperuremia developing in patients who chronically use herbal remedies that contain this medicinal plant.

Superoxide dismutase (SOD) and catalase (CAT) constitute the first line of antioxidant defense system in the body, as they aid detoxification (Ighodaro & Akinloye, 2017). In this study, it was observed that the extract treatment caused a significant increase in SOD and decrease in CAT activities when compared with the control. This is in contrast with the study of Uzor *et al*. (2015) who reported high antioxidant activities in the root of *C. dolichopetalum* and identified ellagic acid as the major antioxidant principle of this plant. Free radicals are responsible for the lipid peroxidation that occurs in the cell of an organism. Malondialdehyde (MDA) is one of the final products of lipid peroxidation in cells. Therefore, excessive production of MDA is caused by an increase in free radicals. The reduction in MDA levels by the extract recorded in this study could be due to its rich flavonoids content as studies have shown that they have the capacity to trap free radicals and inhibit their effect on the peroxidation of membrane lipids (Ngatchic *et al*., 2020).

In conclusion, the present study carried out to evaluate the sub-acute profile of methanol leaf extract of *Combretum dolichopetalum* in Wistar rats.showed relatively good antioxidant and anti-hyperlipidemic properties as well as proved to be partially toxic to the liver and kidneys. These findings

justify its use in folkloric medicine for treatment of various ailment, but chronic usage should be avoided, since it may be associated with pathology in the liver and kidney, especially at high doses.

Further histopathological studies should be carried out to accurately grasp the extent of the effect of this plant on the liver and kidney. Studies of methanolic extracts of other parts of this plant, such as the stem and roots, as well as sub-chronic toxicity tests should also be conducted so as to have a holistic knowledge on the toxic effect of *C. dolichopetalum.*

Acknowledgement

We wish to thank Rev Fr. Dr. Kingsley Ndubueze for his assistance in sourcing the plant materials.

Funding

No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

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