

Acute and sub-chronic toxicity studies of *Dichaetanthera africana* (Hook. F.) Jacq. Fel. (Melastomataceae) stem bark ethanol extract

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ABSTRACT

The safety of the ethanol stem bark extract of *Dichaetanthera africana* (DABE) was evaluated. A single oral dose of 1000 and 2000 mg/kg bw was administered to the rats in the acute toxicity study and the animals observed for 7 days. The crude extract was given by daily oral administration of 250–1000 mg/kg bw for 28 and 90 days respectively in the sub-chronic toxicity test. Body weight, rectal temperature, food and water consumptions were recorded weekly. Hematological and biochemical parameters and relative organ weights (liver, kidney, heart, and spleen) were determined at the end of the 28 and 90-day administration. Histopathological examination of the liver and kidney of rats was done. No adverse effects or mortality were observed throughout the period of the acute toxicity experiment ($LD_{50} > 2000$ mg/kg). DABE did not produce any mortality and there were no significant differences between groups in daily oral administration for 28 days. For 90 days, DABE caused behavior adverse effects at the dose of 1000 mg/kg bw. DABE resulted in significant changes in biochemical parameters at the dose of 500 and 1000 mg/kg bw. Liver histopathology revealed morphological alteration at these doses. The results suggested that DABE is relatively non-toxic in daily oral administration for 28 days. However, it becomes toxic for 90 consecutive days at the doses of 500 and 1000 mg/kg bw.

INTRODUCTION

Medicinal plants (crude extract, pure compound or derivative) are an unlimited source for the discovery of new medicines. Most of the natural products used in traditional medicine have solid scientific evidence regarding their biological

activities (Ochoa *et al.*, 2014; Musila *et al.*, 2017; Tang *et al.*, 2017). However, little information or evidence is available on the possible toxicity of medicinal plants to the consumers (Tang *et al.*, 2017). Regarding the discovery and development of drugs, the concerns of health authorities, the pharmaceutical industry and patients must be taken into consideration (Musila *et al.*, 2017). The prompt access to safe and efficient medicines, as well as animal welfare, are of primary interest to the general public, patients, and consumers. Plants used in traditional medicine could be expected to have low side effects due to their long-term use by local

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populations. Nonetheless, the surveys have indicated that many medicinal plants applied in traditional medicine showed adverse effects (Ochoa *et al.*, 2014). Therefore, the traditional use of any plant for medicinal purposes does not guarantee the safety of this plant. Thus, concerns remain about the potential toxic effects of the short-term and long-term use of the medicinal plants. The data from toxicity studies on medicinal plants or other derivatives must be obtained in order to establish their safety for humans essential for the development of pharmaceutical products. (Etame *et al.*, 2017). Therefore, the toxicological study of any medicinal plant extract intended to be used in animals or humans is crucial for the assessment of its potential toxic effects. *Dichaetanthera africana* is a tree of 9 to 15 m high, found in riverine woodland from Sierra Leone to Congo and Angola (Pauwels, 1993). The different parts of this plant are medicinally used in Cameroon, Gabon, Sierra Leone, Ivory Coast and Nigeria against coughs, chest pain and fatigue (Burkill, 1997; Fonge *et al.*, 2012). Previous studies on this plant revealed antioxidant, analgesic, antipyretic and anti-inflammatory properties and bioavailability profile of ethanol extract of the stem bark (Mokale *et al.*, 2017a; Mokale *et al.*, 2017b). Akoué *et al.* (2013) showed that the ethanol, hydroalcoholic and aqueous extracts of the branches of *Sakersia africana*, an isotype of *Dichaetanthera africana*, had high total phenol content and good antiradical activity. However, no toxicological study of this extract has been performed. The present study designed to evaluate the toxicity of the ethanol extract from the stem bark of *Dichaetanthera africana*.

MATERIALS AND METHODS

Collection of plant material

The stem bark of *D. africana* was collected from the Littoral Region of Cameroon (Babimbi II). The identification of the plant was done at the National Herbarium of Cameroon, where the sample was identified from that registered under the number 7157/SRFK.

Preparation of crude extract

The stem bark of *D. africana* (2 kg) was air-dried and powdered and then was macerated at room temperature in ethanol (5 L, 72 h) and then evaporated using a rotary evaporator (Büchi R200) to obtain a crude extract (76.3 g).

Experimental animals

Nulliparous and non-pregnant Wistar rats (140–200 g) were used and placed in wire mesh plastic cages under normal laboratory conditions. Animals were received a daily food and water *ad libitum*. This work was done according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). This guideline has been issued by the Ministry of Environment and Forests, Government of India. This study was approved by the Institutional Animal Ethics Committee (Registration No. 778/PO/a/03/CPCSEA; 03.09.).

Acute oral toxicity

The acute toxicity test was performed using the OECD guidelines for acute toxicity (2001). Animals were divided into 3 groups of 6 animals each. The experimental rats were deprived of

food for 15 h and then they were weighed before the administration of the extract. The control group was orally administered water (10 mL/kg bw) while test groups received extract at the doses of 1000 and 2000 mg/kg bw. Animals were observed for toxic manifestations (reduction in locomotion, aggressiveness, reaction to stimuli, social interactions and aspect of feces) for the next 5 h and then rats were observed daily for signs of morbidity and mortality for 7 days. Rectal temperature and body weight of the animals during the first 5 hours and once every three days were noticed.

Sub-acute and sub-chronic oral toxicity

Twenty-four albino rats were used for each experiment. Animals were randomly divided into four groups of 6 rats each. The extract was given by the daily oral administration for 28 and 90 days to the test groups at the different doses (250, 500 and 1000 mg/kg). Control group received only distilled water (OECD, 1998; OECD, 2008). The animals were observed for signs of toxicity and mortality throughout the experimental period. The body weight, rectal temperature, water and food consumptions were recorded weekly. At the end of each experiment, the animals, fasted for 12 h, were sacrificed by decapitation under anesthesia (diethyl ether). Blood was collected by cardiac puncture into two EDTA tubes. The first tube was used for hematological parameters determination and in the second tube 2, plasma was separated by centrifugation for biochemical analysis. The organs (kidneys, liver, heart, and spleen) were weighted. Kidney and liver samples of each group were preserved in 10% formalin for histopathological examination.

Blood analysis

Hematological parameters such as white blood cell (WBC) count, red blood cell (RBC) count, platelet (PLT) count, hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined using an automatic analyzer (System H1, Bayer Diagnostics). The serum was analyzed for total protein, total cholesterol, HDL cholesterol, creatinine, urea, triglycerides, glucose, and alanine transaminase (ALT)/aspartate aminotransferase (AST) activities using specific commercial diagnostic kits (Fortress Diagnostics, London, UK).

Histopathological analysis

Kidney and liver samples were preserved in 10% formalin and processed by conventional techniques. Paraffin sections (5 µm thick) were stained with hematoxylin and eosin before microscopic examination.

Statistical analysis

Data are presented as mean \pm SD. One-way ANOVA with Newman-Keuls Multiple Comparison Test was performed to assess differences between groups (16.0 SPSS Windows software). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Acute oral toxicity

No adverse reactions or mortality were observed at 1000 and 2000 mg/kg oral administration of ethanol extract ($LD_{50} > 2000$ mg/kg). No significant differences in body weight

and rectal temperature (Figure 1) of the animals were observed

between control and test groups during the experiment.

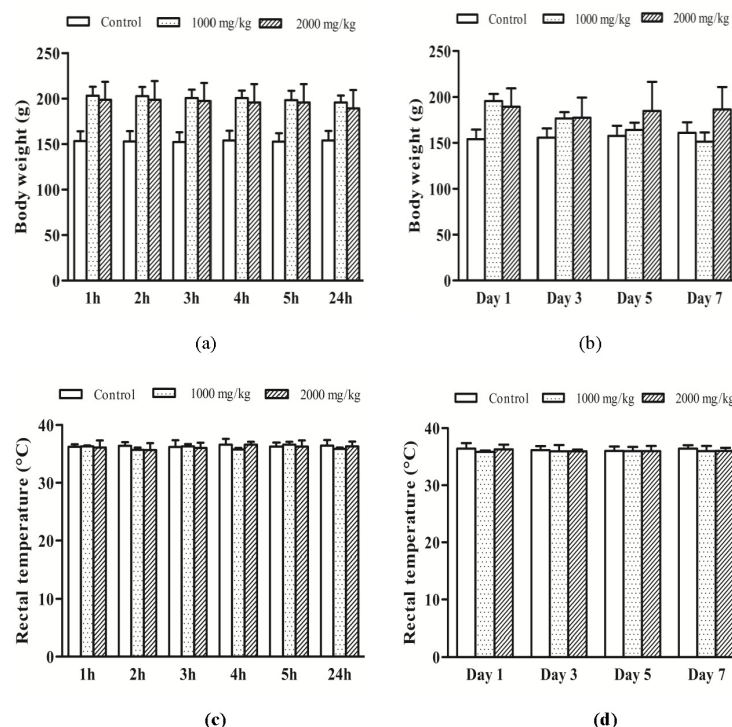


Fig. 1: Body weight (a and b) and rectal temperature (c and d) of experimental animals after oral administration of a single dose of the DABE. Each bar represents the data expressed as mean \pm SD for each group of rats, $n = 6$.

Sub-chronic oral toxicity

Food-water consumptions, rectal temperature, and body weight evolution

EtOHDA administered daily for 28 days induced a significant increase in food consumption into the test groups (Figure 2a) throughout the treatment. Water consumption increased significantly during 3rd week (Figure 2b). During 90 days of treatment, food consumption decreased significantly in the 8th week, remained stable and not significant with respect to the control group (Figure 2c). No significant differences in water consumption were observed between the groups of rats (Figure 2d). The increase in food consumption resulted in a very marked gain in body weight of the animals which received the plant

extract for 28 days (Figure 3b). This effect is significantly greater as the dose increases. However, there was no significant increase in body weight for 90 days between the groups (Figure 3d). The rectal temperature of the animals did not significantly vary during the 28 and 90 days of treatment (Figures 3e and 3f). Changes such as sedation, defecation and salivation were observed in animals from the groups treated with 500 and 1000 mg/kg of DABE. These changes decreased with time.

Relative organ weights

No significant changes in weight of the organs (liver, kidney, heart, and spleen) were observed between the treated as compared to the control rats after oral administration of DABE over 28 and 90 days (Table 1).

Table 1: Relative organ weights (g/100g bw) recorded at the end of the study from experimental animals after 28 days and 90 days of oral administration of the DABE.

Treatment	Dose (mg/kg)	Organ weights (g)			
		Kidney	Liver	Heart	Spleen
28 days	0	0.83 \pm 0.16	3.23 \pm 0.63	0.48 \pm 0.10	0.45 \pm 0.14
	250	0.83 \pm 0.07	3.48 \pm 0.14	0.44 \pm 0.03	0.50 \pm 0.05
	500	0.90 \pm 0.09	3.74 \pm 0.38	0.48 \pm 0.03	0.53 \pm 0.06
	1000	0.90 \pm 0.08	3.73 \pm 0.26	0.51 \pm 0.05	0.57 \pm 0.11
90 days	0	0.97 \pm 0.04	3.73 \pm 0.13	0.47 \pm 0.02	0.51 \pm 0.03
	25	0.90 \pm 0.05	3.72 \pm 0.25	0.47 \pm 0.02	0.48 \pm 0.05
	500	0.92 \pm 0.08	3.64 \pm 0.17	0.49 \pm 0.03	0.52 \pm 0.04
	1000	0.91 \pm 0.06	3.87 \pm 0.37	0.45 \pm 0.05	0.54 \pm 0.03

Data are expressed as mean \pm SD, $n = 6$. No statistically significant difference was observed between test and control groups.

Table 2: Biochemical parameters in experimental animals after 28 days and 90 days of the DABE administration.

Parameters	28 days				90 days			
	Control	250 mg/kg	500 mg/kg	1000 mg/kg	Control	250 mg/kg	500 mg/kg	1000 mg/kg
CHOL (mg/dl)	43.16 ± 5.00	51.31 ± 8.64	54.67 ± 8.85	57.43 ± 10.08*	44.96 ± 7.00	51.91 ± 7.01	56.95 ± 8.29	58.87 ± 9.59*
HDL CHOL (mg/dl)	16.15 ± 4.12	16.48 ± 1.75	18.11 ± 3.40	16.90 ± 1.89	15.07 ± 1.42	21.38 ± 3.83	22.34 ± 6.30	23.75 ± 5.90*
LDL CHOL (mg/dl)	13.26 ± 2.73	19.22 ± 6.62	18.11 ± 6.03	22.94 ± 8.64	19.03 ± 2.11	20.13 ± 5.44	25.19 ± 4.38	20.85 ± 3.73
TGY (mg/dl)	68.75 ± 10.09	78.02 ± 11.54	79.22 ± 12.38	87.89 ± 7.01*	54.26 ± 5.63	52.01 ± 5.92	47.08 ± 6.42	71.30 ± 9.47**
ALT (U/L)	17.12 ± 1.73	20.66 ± 3.73	17.49 ± 1.62	20.90 ± 1.98	12.00 ± 2.36	13.22 ± 1.60	15.27 ± 2.03*	17.88 ± 1.44***
AST (U/L)	37.60 ± 3.32	30.74 ± 3.13	33.62 ± 6.98	32.24 ± 7.53	37.15 ± 4.91	39.12 ± 5.30	47.07 ± 4.71**	55.82 ± 3.50***
TP (g/l)	71.70 ± 8.39	72.43 ± 4.93	74.21 ± 2.56	74.04 ± 4.79	67.63 ± 5.86	66.44 ± 2.51	65.95 ± 5.80	64.42 ± 1.91
ALB (g/l)	19.11 ± 2.73	25.58 ± 5.74	25.05 ± 6.44	20.97 ± 4.36	26.31 ± 2.73	23.41 ± 3.90	21.51 ± 4.40	18.47 ± 2.05**
GLU (mg/dl)	120.29 ± 23.85	129.43 ± 17.61	136.15 ± 24.97	153.36 ± 38.07	145.69 ± 17.41	231.58 ± 25.06***	209.54 ± 16.62***	207.79 ± 19.65***
CRE (μmol/l)	69.77 ± 11.74	64.67 ± 5.27	66.94 ± 14.54	68.07 ± 13.78	78.28 ± 7.76	78.85 ± 18.81	76.58 ± 5.59	77.15 ± 6.69
UR (mg/dl)	35.00 ± 8.46	34.75 ± 7.32	33.97 ± 3.28	34.97 ± 3.09	58.88 ± 6.35	45.02 ± 5.16**	45.16 ± 4.75**	40.03 ± 4.77***

CHOL: Total cholesterol, HDL CHOL: HDL cholesterol, LDL CHOL: LDL cholesterol, TGY: Triglycerides, ALT: Alanine transaminase, AST: Aspartate transaminase, TP: Total Protein, ALB: Albumin, GLU: Glucose, CRE: Creatinine, UR: Urea. Data are expressed as mean ± SD, $n = 6$. *, **, *** indicate a significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively, compared to the appropriate control group (Newman-Keuls).

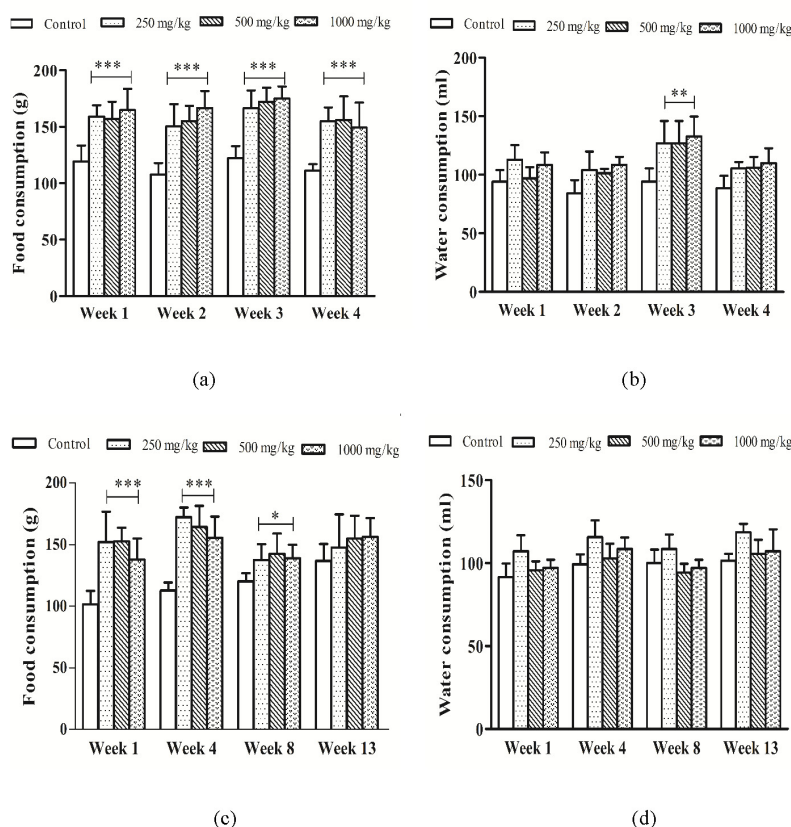


Fig. 2: Effect of daily intake of the DABE on the food and water consumptions in rats for 28 (a and b) and 90 days (c and d). Each bar represents the data expressed as mean ± SD for each group of rats, $n = 6$. For a given week, *, **, *** indicate a significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively (Newman-Keuls).

Hematological and biochemical parameters

The effects of administration of DABE after 28 and 90 days on plasma biochemical parameters in experimental rats are presented in Table 2. Except for total cholesterol and triglycerides, there were no significant differences in all the other

biochemical parameters evaluated between the groups after 28 days of administration. Total cholesterol and triglycerides levels significantly increased ($p < 0.05$) only at the dose of 1000 mg/kg bw. After 90 days of administration, we observed a significant increase in ALT, AST (500 and 1000 mg/kg bw, $p < 0.001$),

albumin (1000 mg/kg bw, $p < 0.01$), total cholesterol, HDL cholesterol, triglycerides ($p < 0.05$) and glucose levels (all the doses, $p < 0.001$) compared to the control. We also observed a significant decrease in urea level ($p < 0.05$) of the animals which received different doses of plant extract. No significant difference was detected on hematological parameters between test and control groups (Table 3).

Liver and kidney histopathology

Histopathological analysis of liver and kidney did not show morphological alteration for 28 days (Figure 4). However, daily administration of DABE had significant effects on the hepatic tissues of the treated rats for 90 days (Figure 5). The histological study of liver showed vascular congestion and leukocyte infiltration at the doses of 500 and 1000 mg/kg bw.

Table 3: Hematological parameters in experimental animals after 28 days and 90 days of the DABE administration.

Treatment	Dose (mg/kg)	WBC ($10^3/\text{mm}^3$)	RBC ($10^6/\text{mm}^3$)	HGB (g/dl)	HCT (%)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)	PLT ($10^3/\text{mm}^3$)
28 days	0	5.83 ± 0.99	7.02 ± 0.73	12.15 ± 0.78	32.61 ± 5.45	46.45 ± 7.33	17.30 ± 1.92	37.25 ± 5.18	310.50 ± 10.32
	250	5.40 ± 0.45	6.69 ± 0.27	13.03 ± 0.89	39.80 ± 4.40	59.49 ± 7.44	19.47 ± 1.44	32.73 ± 4.86	315.50 ± 12.04
	500	5.23 ± 0.37	6.33 ± 0.62	12.58 ± 0.67	37.75 ± 3.51	59.63 ± 10.34	19.87 ± 2.91	33.32 ± 3.10	320.16 ± 8.19
	1000	5.86 ± 0.46	6.27 ± 0.08	12.70 ± 0.89	37.58 ± 3.94	56.93 ± 7.48	20.25 ± 1.77	33.79 ± 1.87	318.33 ± 11.50
	0	18.25 ± 2.48	7.04 ± 0.74	13.28 ± 0.99	40.21 ± 2.91	56.83 ± 1.06	19.03 ± 0.71	33.46 ± 1.66	423.50 ± 56.04
90 days	250	21.58 ± 2.52	7.38 ± 0.44	13.71 ± 0.98	43.05 ± 2.38	58.16 ± 0.68	18.63 ± 1.78	31.93 ± 2.78	438.66 ± 49.31
	500	19.90 ± 3.38	7.94 ± 0.42	12.01 ± 0.94	36.23 ± 2.55	61.16 ± 5.70	20.61 ± 3.38	33.51 ± 3.51	443.16 ± 21.73
	1000	17.53 ± 2.01	7.24 ± 0.62	13.83 ± 1.12	40.98 ± 3.66	56.50 ± 2.34	19.11 ± 0.76	33.83 ± 1.80	357.66 ± 19.59

WBC: White Blood Cell Count, RBC: Red Blood Cell Count, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Cell Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PLT: Platelet Count. Data are expressed as mean \pm SD, $n = 6$. No statistically significant difference was observed between test and control groups.

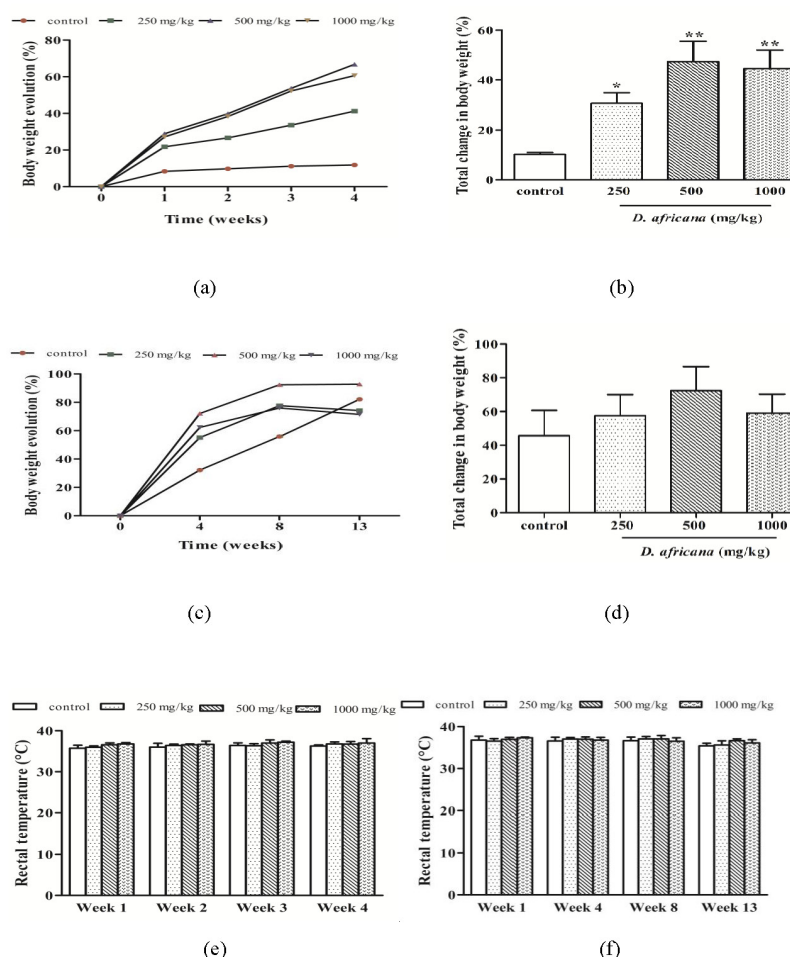


Fig. 3: Body weight evolution and rectal temperature of rats for 28 (a, b and e) and 90 days (c, d, and f). Each bar represents the data expressed as mean \pm SD for each group of rats, $n = 6$. For a given dose, *, ** indicate a significant difference at $p < 0.05$ and $p < 0.01$ respectively (Newman-Keuls).

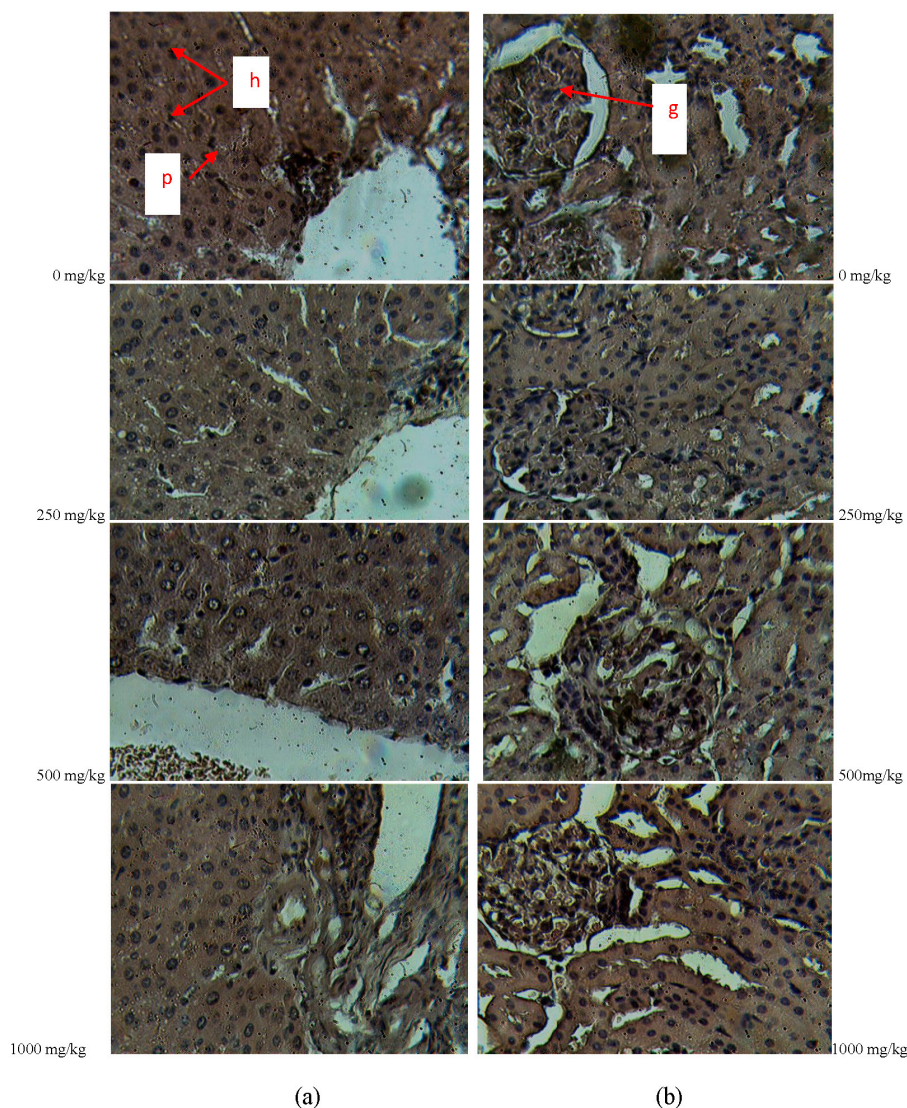


Fig. 4: Histology of the liver (a) and kidney (b) of the control rats and those exposed to various doses of DABE for 28 days. Histological analysis of the liver and kidney of the control rats show normal structure: Hepatocyte (h), parenchyma (p) and glomerula (g) respectively. H & E 400 \times .

DISCUSSION

Often mistakenly considered as safe because they are natural, phytotherapeutic products have bioactive compounds that are likely to cause adverse effects. In addition, the pharmacovigilance services in this area are insufficient. Thus, the determination of the frequency of the secondary effects related to their use becomes difficult. Thereby, all the natural products used in therapeutics must beforehand be submitted to efficacy and safety tests (Tang *et al.*, 2017). In the acute oral toxicity study, no adverse reactions or mortality were observed at 1000 and 2000 mg/kg oral administration of extract, thus the LD₅₀ of DABE can be estimated to be above 2000 mg/kg. This strongly suggests that DABE is relatively non-toxic since substances with an LD₅₀ value of 1000 mg/kg by the oral route are regarded as being safe or of low toxicity (OECD, 2001; Obici *et al.*, 2008). In addition, body weight and rectal temperature of the animals did not change. Several studies showed that species of the Melastomataceae family are non-toxic even at 5000 mg/kg (Zakaria *et al.*, 2006;

Sunilson *et al.*, 2009; Alnajjar *et al.*, 2012). Final and daily clinical observations in repeated dose studies are of the major importance (Feres *et al.*, 2006). Food consumption increased significantly in the groups treated with the extract for 28 days. This suggests that the extract will stimulate appetite. However, it decreased significantly from the eighth week for 90 days of treatment. This decrease indicates that the extract could be toxic beyond the 8th week of treatment. At high doses or long periods of administration, some chemical constituents of the crude plant extract can be metabolized into toxic end-products, which may interfere with the efficiency of gastric function (Choksi, 2007). There were no significant changes in water consumption of animals. An increase in the body weight of the test groups was observed relative to the control group for 28 days of treatment. A significant increase in food consumption could be responsible for the observed gain in body weight. However, adipose tissue is a dynamic organ that plays an important role in energy balance and mass changes depending on the metabolic needs of the

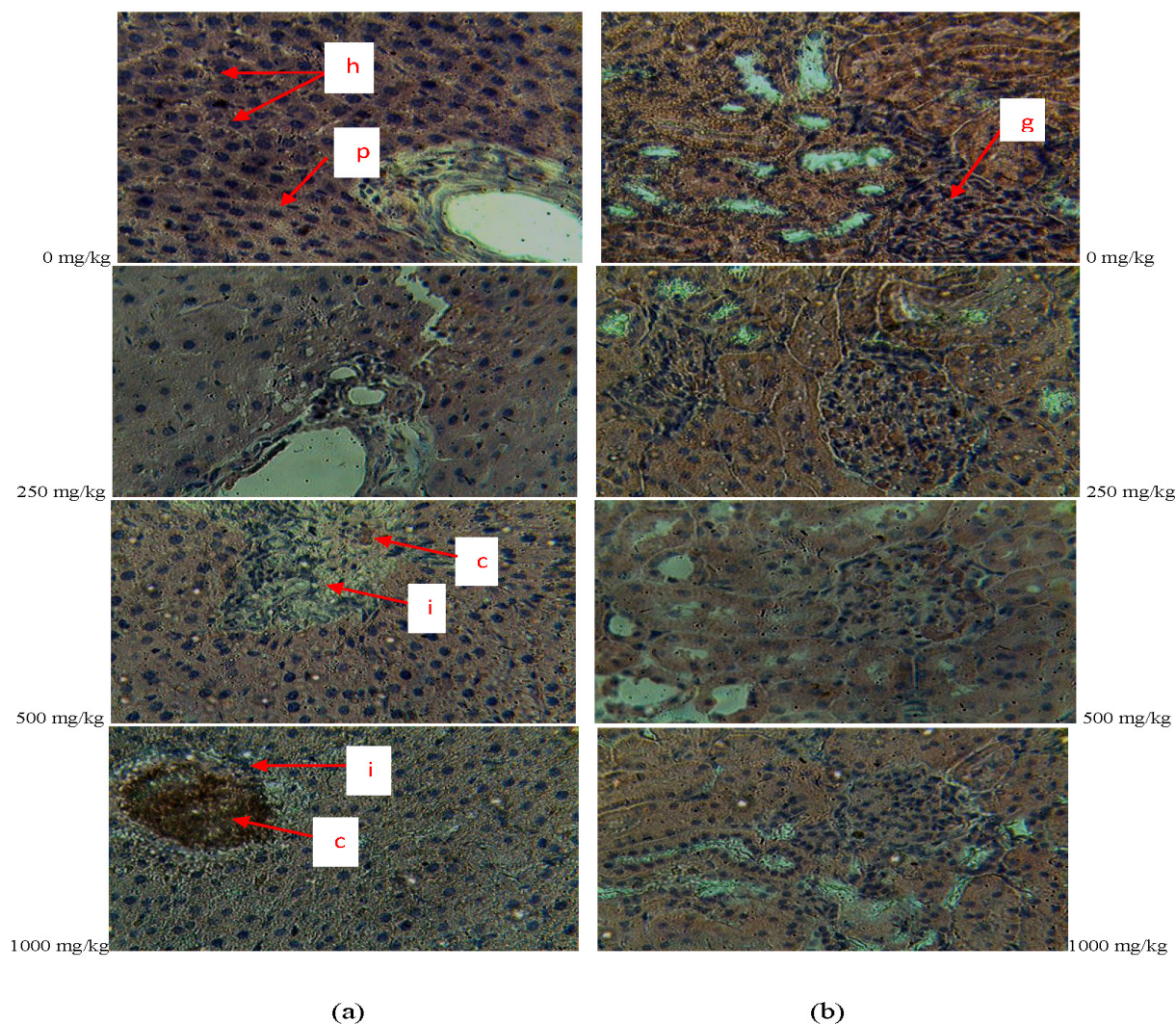


Fig. 5: Histology of the liver (a) and kidney (b) of the control rats and those exposed to various doses of DABE for 90 days. Vascular congestions (c) and inflammation sites (i) are present in the liver at the doses of 500 and 1000 mg/kg. Histological analysis of the liver and kidney of the control rats show normal structure: hepatocyte (h), parenchyma (p) and glomerula (g) respectively. H & E 400 \times .

body (Lafontan and Langin, 2009). It has been reported that the imbalance between intake and energy expenditure results from the abnormal excess growth of white adipose tissue, which can lead to overweight (obesity) (Jo *et al.*, 2009; Lim *et al.*, 2013). Further studies are needed to confirm the ability of *D. africana* to induce obesity. There was a non-significant increase in the body weight of the animals in the test groups relative to the control group during 90 days of treatment. However, a decrease in the body weight of the animals in the test groups was observed from the eighth week onwards. This would be due to the significant decrease in food consumption observed from the 8th week, which would reflect a loss of appetite resulting from the interference of the extract with the metabolism of carbohydrates, proteins or lipids (Klaassen and Casarett, 2001). The increase in the body weight of the animals had no effect on the weight of their organ. We observed normal values of all parameters analysed in experimental rats when compared to the control, indicating that DABE had no observed adverse effect on the hematopoietic system. This serves as an important index

of the physiological and pathological status (Guyton and Hall, 2006). Renal changes are likely to occur in preclinical toxicity studies because of the high doses administered and the fact that the kidneys eliminate many drugs and their metabolites. Analysis of liver markers revealed that prolonged use (90 days) of DABE had adverse effects on liver function. We observed a significant increase in serum levels of ALT and ASAT at doses of 500 and 1000 mg/kg bw. This suggests that, at these doses, the extract would affect hepatic function at the high doses during prolonged intake. This was reflected in the histopathological examination of liver, which found vascular congestion and leukocyte infiltration in groups of animals receiving these doses of extract. Significant reduction in serum level of albumin at a dose of 1000 mg/kg would result in hepatic or renal dysfunction (Upur *et al.*, 2009). Prolonged administration of the extract for 90 days also caused a significant decrease in serum urea level of the markers of renal function. On the other hand, the creatinine level did not change, which would result in liver failure. Therefore, DABE had no

adverse effect on renal function. Some factors associated with arteriosclerosis and cardiovascular diseases are the levels of lipids and lipoproteins in the blood (Wang *et al.*, 2010). The significant increase in total cholesterol and triglyceride levels after treatment with extract at the dose of 1000 mg/kg b.w presents a high risk of hypercholesterolemia and hypertriglyceridemia. This is related to a significant increase in the observed glucose level. This may be due to altered insulin activity or insufficient secretion caused by the ethanol extract, causing hyperglycemia and consequently activating hormone-sensitive lipases in the adipose to release lipids (Gauvreau *et al.*, 2011). This is also reflected in the significant increase in body weight of the animals (Changhyun and Uhee, 2012). *D. africana* presented a hyperglycemic effect confirm by the vascular and inflammatory changes observed in treated groups that could be due to the laying of fat droplets in those tissues.

CONCLUSION

This study provides information on the toxicological profile of DABE. The results obtained suggest that DABE is relatively non-toxic in daily oral administration for a period of 28 days. However, it becomes toxic for 90 days at the doses of 500 and 1000 mg/kg bw. These uphold indigenous knowledge on its safe folkloric use in humans and provide justification for specifically designed studies to investigate other beneficial pharmacological effects and clinical studies in humans.

ABBREVIATIONS

DABE: ethanol stem bark extract of *Dichaetanthera africana*; bw: Body weight; LD₅₀: Lethal dose; EDTA: Ethylene diamine tetraacetic acid; WBC: White blood cell counts; RBC: red blood cell counts; PLT: platelet counts; HCT: hematocrit; HGB: hemoglobin; MCV: mean corpuscular volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; ALT: alanine transaminase; AST: aspartate aminotransferase.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHORS' CONTRIBUTIONS

RANN, ASM, and RSP designated the study and supervised the work. CEG co-supervised the work. ALMK, LRYT, and ADT work in the laboratory to carry out the experiments. ATT prepared the plant extract. GAA, RMEE, DNNN, PVTF, and RSM helped in preparing the manuscript. All authors read and approved the final manuscript.

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