

Research Article

Acute and Subacute Toxic Study of Aqueous Leaf Extract of *Combretum Molle*

Dodehe Yeo^{*}, Rita Bouagnon, Bernard Nazaire Djyh, Chonta Tuo and Jean David N'guessan

Laboratory of Pharmacodynamy, UFR Biosciences, University of Cocody-Abidjan, Ivory Coast.

Abstract

Purpose: The purpose of the present study was to evaluate the acute and subacute toxicity of the aqueous leaf extract of *Combretum molle*.

Methods: The acute toxicity of the extract was evaluated in rats. The animals were orally administered with doses ranging from 2000 to 8000 mg/kg and observed continuously for the first 4 h, then hourly for the next 24 h, and finally, 6-hourly for 72 h. Control animals received orally normal saline. The rats were observed carefully for mortality, pain as well as respiratory movements. For subacute toxicity, 6 groups of 6 rats (3 male and 3 female) each received intraperitoneally, normal saline (control), 400, 600, 800, 1000 and 1200 mg/kg of the extract, respectively, thrice daily for 15 days. At the end of the treatment period, the animals were sacrificed and their organs (liver, heart and kidney) removed for macroscopic examination.

Results: For the acute toxic test, no death and signs of poisoning were observed in the treated groups. In the subacute study, LD_{50} in the rats after intraperitoneal administration was 700 mg/kg (456 - 896, 95 % confidence interval). The clinical signs of poisoning (motor difficulties, decreased respiratory rate, and tremor preceding death) were observed, suggesting overt toxicity throughout the neuromuscular system. However, histological examination of vital organs showed normal architecture suggesting no morphological abnormalities in the heart, kidney and liver.

Conclusion: The results show that the aqueous leaf extract of *C. molle* is moderately toxic when given intraperitoneally.

Keywords: *Combretum molle*, Acute/subacute toxicity, Histopathology, Rat.

Received: 23 February 2011

Revised accepted: 28 February 2012

*Corresponding author: Email: dodeheyeo@yahoo.fr; Tel/Fax: 00225 22 44 44 73

INTRODUCTION

Combretum molle is a shrub or small, graceful, deciduous tree, 3 - 13 m high, with a crooked or lean trunk, occasionally swollen at the base, and up to 30 cm in diameter. It is found especially in savannah vegetation that cuts across from Senegal to West Cameroon, but generally exists in tropical Africa [1]. Traditional healers throughout Africa use this specie of Combretaceae for many medicinal purposes. These include treatment of fever, headaches, abdominal disorders, abdominal pains, gallstones, diarrhoea, dysentery, gastric ulcers, bilharziasis, hookworm, nosebleeds, sore throats, colds, chest coughs, pneumonia, conjunctivitis, dysmenorrhoea, infertility in women, venereal diseases including syphilis, and earache. It has also been used for the treatment of leprosy, scorpion and snake bites as well as toothache, heart diseases, backache, jaundice, stomach, gastric problems, constipation and general weakness. Other uses include treatment of swelling caused by mumps and cleansing of the urinary system. Species of Combretaceae have also been used as food supplement for babies [2-5].

Some studies have been performed on the biological activities of *C. molle*. Its leaf has anti-asthmatic and anti-tussive activities [6]. The plant induces considerable reduction in the variation of breathing amplitudes. Furthermore, the extract probably contains anticholinesterase substances as it exerted non-competitive inhibition of acetylcholinesterase with a Michaelis-Menten constant (KM) of 192 μ M and velocity at maximal concentration of substrate (Vmax) of 4444 μ M/min [7]. McGaw *et al* [8] found *C. molle* to have both anti-inflammatory and antischistosomal activities which may justify the traditional use of the plant for the treatment of malaria and pain.

Despite its long-time and varied uses, there are no data on the safety profile of the plant. This study was undertaken to determine the acute and subacute toxicity of the aqueous

leaf extract of *Combretum molle* given orally and intraperitoneally to Sprague Dawley (SD) rats.

EXPERIMENTAL

Preparation of aqueous extract

The plant material (*C. molle* leaves) was collected in December 2009 from Korhogo, in northern Ivory Coast, and authenticated by Professor Assi L Aké of the National Floristic Garden (University of Cocody-Abidjan). A voucher specimen (no. 6129) was deposited at the National Floristic Garden (University of Cocody-Abidjan). The dried leaves were powdered in a mortar and about 100 g of the powder extracted with 2 L of distilled water for 48 h on a hot plate. The mixture was sequentially filtered through a cheese cloth, cotton wool and Whatman filter paper no.1, respectively. The filtrate obtained was concentrated under reduced pressure, using a rotary evaporator, to the desired consistency.

Animals

Adult Sprague Dawley (SD) rats used for the study (36 males and females each), weighing 180 - 200 g, were obtained from the animal house of the Physiology Department, University of Cocody-Abidjan, Ivory Coast.

The experimental procedures and protocols used in this study were approved by Ethical Committee of Health Sciences of the university [9,10]. The animals were housed in plastic cages (47×34×18 cm) in an air-conditioned environment with 6 rats in each cage. The temperature of the environment was 25 ± 2 °C with a 12 h light-dark cycle. Food and water were freely available to them.

Acute toxicity

The animals were divided into a control group and four treatment groups (2000, 4000, 6000 and 8000 mg/kg of extract), each group consisting of six animals. The repeat-dose

oral toxicity study was carried out according to OECD guideline 407 [11]. The control group (Group 1) received orally, normal saline (0.9% NaCl) while Groups 2 to 5 received 2000, 4000, 6000 and 8000 mg/kg body weight, respectively. Each treated group received the aqueous extract orally and clinical signs and symptoms were observed continuously for the first 4 h, then hourly for the next 24 h, and finally, 6-hourly for 72 h [12]. All the surviving animals were sacrificed after being anesthetized with sodium entobarbital.

Subcute toxicity test

The animals were divided into six groups of six animals each. The treatments were given by intraperitoneal injection. Group 1 served as control and received normal saline, while Groups 2 to 6 received 400, 600, 800, 1000 and 1200 mg/kg body weight, respectively, of the extract. Each group received the specified treatment dose thrice daily for 15 days. The animals were observed every 2 hours for toxic symptoms, signs of poisoning and mortality over a period of 30 days. The organs of the dead animals (liver, heart and kidney) were removed for macroscopic examination.

Determination of lethal dose (LD₅₀)

The mortality rate of the animals for each dose was computed [13,14]. The arithmetical method of Karber was used for the determination of LD₅₀ [15]. The interval mean of the dead animals in each group of animals was used as well as the difference between doses for the same interval. The product of interval mean and dose difference was obtained. The sum of the product was divided by the number of animals in a group and the resulting quotient was subtracted from the least lethal dose in order to obtain LD₅₀ value (Eq 1).

$$LD_{50} = LD - \frac{\sum ab}{N} \dots\dots\dots (1)$$

where LD is the apparent lethal dose of all the groups, N is the number of animals in

each group, a is the dose difference and b the mean mortality.

Histopathologic examination

For post-mortem, the rats were dissected and careful examination of the organs, liver, kidneys and heart were carried out. Tissue samples were fixed in 10 % formalin and dehydrated overnight using upgraded ethanol series and embedded in paraffin blocks. Ultrathin sections were de-waxed by xylene, hydrated through a degraded ethanol series, and stained with haematoxylin and eosin. A pathologist, blinded to the treatments, performed the histopathologic examination with an optical microscope [Nikon Eclipse E600, USA (x 400)]. Sections were assigned grades as reported by Billingham *et al* [16].

Statistical analysis

The LD₅₀ values were computed by probit analysis with a calculator. One-way analysis of variance (ANOVA) with Tukey post hoc test was applied to evaluate significant differences between groups with InStat Statistical Package (Graph Pad Software). Values of $p < 0.05$ were considered significant.

RESULTS

Acute toxicity

Oral administration of the aqueous extract of *C. molle* (2000 to 8000 mg/kg) did not produce significant changes in behavior, breathing, cutaneous effects (irritation, ulceration, caustic injuries and skin rashes), sensory nervous system responses, and gastrointestinal effects in the animals. No deaths occurred in any of the groups during the entire period of treatment. Also, no mortality was observed after oral ingestion of doses ranging from 4000 to 8000 mg/kg of extract weekly.

Sub-acute toxicity

In the animals that received the extract intraperitoneally, abdominal muscle contractions and ataxia were observed, and these persisted for a few hours. At the 6th h, the animals became drowsy and less responsive to stimuli. Some of the animals died and mortality was as well as the severity of the toxic effects

were dose-related (Table 1). However, at the 24th h, most of the survivors had recovered from these symptoms. LD₅₀ of *C. molle* in rats was determined to be 700 mg/kg (range: 456 - 895 mg/kg, 95% confidence interval) after intraperitoneal injection. The results are given in Tables 2 and 3.

Table 1: Mortality rate (%) of rats in acute toxicity test

Treatment group	Period (h)					
	0-6	6-8	8-10	10-12	12-22	22-24
Control	0	0	0	0	0	0
400 mg/kg	0	0	0	0	0	0
600 mg/kg	0	0	0	16.7±2.0	33.3±1.0	33.3±4.0
800 mg/kg	0	16.7±2.0**	16.7±2.0**	33.3±1.0**	33.3±1.0**	83.3±9.0**
1000 mg/kg	0	33.3±3.0 ^a	33.3±3.0 ^a	50.0±9.0 ^a	66.7±4.0 ^a	83.3±5.0 ^a
1200 mg/kg	16.7±3.0	83.3±1.0 ^{a*}	50.0±5.0 ^{a*}	66.7±4.0 ^{a*}	83.3±9.0 ^{a*}	100

** $p < 0.01$ compared with 600 mg/kg and 800 mg/kg groups; ^a $p < 0.001$ compared with 1000 mg/kg group and 600 mg/kg groups; ^{a*} $p < 0.001$ compared with 1200 mg/kg and 1000 mg/kg groups

Table 2: Mortality rate in subacute toxicity test, and corrected values after intraperitoneal administration of the extract

Treatment group	Dose (mg/ml)	Log dose	Mortality rate (%)	Corrected (%)
Control	Vehicle		0	0
400 mg/kg	400	2.60	0	4.17
600 mg/kg	600	2.78	33.33	33.33
800 mg/kg	800	2.90	83.33	83.33
1000 mg/kg	1000	3	83.33	83.33
1200 mg/kg	1200	3.08	100	95.83

Corrected % formula: For 0% dead, $100(0.25/n)$ [15].

Table 3: Determination of LD₅₀

Group	Difference of consecutive doses (a)	Number of dead animals	Mean mortality between two consecutive doses (b)	Probits (a x b)
Control	0	0	0	0
400 mg/kg	400	0	0	0
600 mg/kg	200	2	1	200
800 mg/kg	200	5	3,5	700
1000 mg/kg	200	5	5	1000
1200 mg/kg	200	6	5,5	1100

Note: Sum of the product = 2100; LD₅₀ = $LD - \Sigma ab/N$; N = 6; $\Sigma ab=3000$; Least lethal dose = 600; LD₅₀ = $1200 - 3000/6 = 1200 - 500 = 700$ mg/kg.

Histopathological results

Histopathological data on the liver, heart and kidney of the rats are shown in Figs 1 – 3, respectively. No changes were observed in

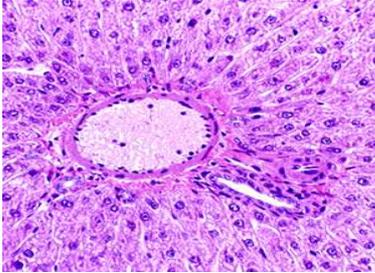


Fig 1a: Histopathology of normal rat liver (control) with normal parenchymal cells. The section showing structure of liver with sheets of hepatocytes arranged around central vein. Hepatocytes, sinusoids appeared normal, stained with haematoxyline-eosin (400 x magnification).

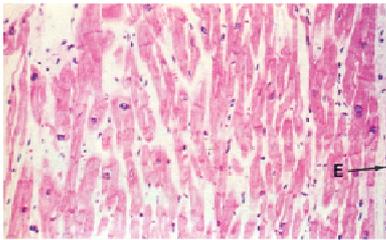


Fig 2a: Representative photomicrograph of cardiac tissue (control). Section showing normal myofibrillar organization and cytoplasmic vacuolation. stained with haematoxyline-eosin (400 x magnification).

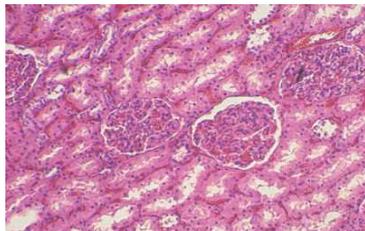


Figure 3a: Histopathology kidney after administration of 0.9 % saline solution Section showing normal glomerulus and distal convoluted tubules (haematoxylin and eosin staining, magnification x 400)

the heart and kidney of the rats following extract administration. However, Fig 1b shows cell infiltration of the portal vein, indicating inflammatory effects in the liver.

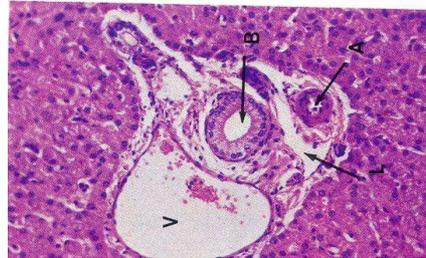


Fig 1b: Histopathology of rat liver treated with aqueous extract (1000 mg/kg of *Combretum molle* extract). V = portal tract; A = Hepatic duct; B= Artery. The section showing structure of liver with an infiltration of cells through the portal vein, stained with haematoxyline-eosin (400 x magnification).

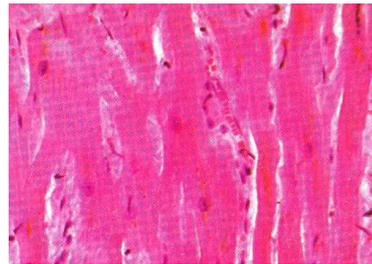


Fig 2b: Histopathology of rat heart in *C.molle* treated group (1200mg/kg body weight). Cytoplasmic vacuolation and myofibrillar organization were normal, stained with haematoxyline-eosin (400 x magnification).

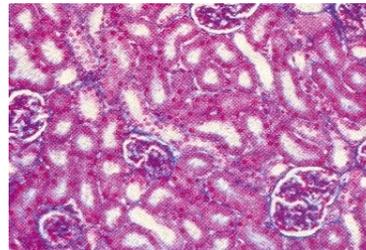


Figure 3b: Histopathology of kidney after administration of 1200 mg/kg of *C.molle*. There was no damage to glomerulus and distal convoluted tubules (haematoxylin and eosin staining, magnification x 400).

leading to increasing demand. Experimental screening method is, therefore, important in order to ascertain the safety of these herbal remedies [17]. A previous study showed the antiantihistaminic effect of *C. molle* aqueous extract at 7.14 mg/kg body weight in rabbit [18]. In the acute toxicity study, there was no mortality observed even at a maximum oral dose of 8000 mg/kg of the aqueous extract. Also, no changes in the behavior and in the sensory nervous system responses were observed. No death or signs of poisoning were observed after oral administration of the extract; suggesting oral doses of *C. molle* are not toxic. However, histological analysis showed infiltration of inflammatory cells at the level of the portal vein in the liver. Inflammation was probably due to passive immune response to the extract

The maximum tolerated dose (MTD) obtained intraperitoneally was 400 mg/kg body weight. The clinical signs of toxicity observed after intraperitoneal administration, such as motor difficulties, decreased respiratory rate, and tremor preceding death, suggest overt toxicity throughout the neuromuscular system. The LD₅₀ (intraperitoneal injection) of *C. molle* extract in rats was 700 mg/kg; indicating that the plant is moderately toxic [19].

Macroscopic analysis of the target organs of the treated animals (liver, heart, and kidney) did not show significant changes in color and texture when compared with control [14].

CONCLUSION

Our findings did not show any damage to the kidney and heart of rats following oral administration of high doses of *C. molle* extract. Furthermore, no mortality was observed following oral administration. However, the extract seems to be moderately toxic after intraperitoneal administration. It also induced infiltration of inflammatory cells into the portal vein of the liver; a phenomenon that could compromise the medicinal use of this plant in folk medicine. However further studies are necessary, such as hematological assay and more in-depth

morphological experiments, to confirm this evidence.

ACKNOWLEDGMENT

The authors are thankful to AUF (Agence Universitaire de la Francophonie) for providing scholarship for one of the authors and the Department of Medicine (University of Cocody-Abidjan, Ivory Coast) for assistance with the provision of material.

REFERENCES

1. Burkill HM, *The Useful Plants of West Tropical Africa, Families A-D*. Kew: Royal Botanic Gardens; United Kingdom; 2nd edition, 1985; p 960.
2. Masoko PA, Picard JB, Eloff JN. The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae). *S Afr J Bot*, 2007; 73: 173-183.
3. Letcher, RM, Nhamo, LRM. Chemical constituents of the Combretaceae. Part IV. Phenanthrene derivatives form the hardwood of the *Combretum heteroense*. *Journal of the Chemical Society Perkin Transactions*, 1973; 24: 127-129.
4. Iwu MM. *Handbook of African Medicinal Plants*. CRS Press, Florida, 1993; p 435.
5. Yéo D, N'guessan, JD, Sea T, Coulibaly A, Djaman AJ, Tako NA, Yavo JC. et Guédé-Guina F. Evaluation de l'activité antiasthmatique et antitussive de *Combretum molle*, plante médicamenteuse de la pharmacopée ivoirienne. *Phytothérapie*, 2008 ; pp 348-351.
6. McGaw LJ, Rabe T, Sparg SG, Jager AK, Eloff JN, van Staden J: An investigation on the biological activity of *Combretum* species. *J Ethnopharmacol* 2001; 75: 45-50.
7. Yéo D, Koffi E, Bidié AP, Tako NA, Bahi C, Méité S, Djaman AJ and Guédé-Guina F. In Vitro Anticholinesterase and Inhibitory Effects of the Aqueous Extract of *Combretum molle* (Combretaceae) Leaf on Rabbit Breathing. *Trop J Pharm Res*, 2010; 9: 469-473.
8. Bever BO. *Medicinal Plants in Tropical West Africa*. Cambridge University. Press, Great Britain, 1986; p 375.
9. OECD. Repeated dose oral toxicity test method. In: OECD Guidelines for testing of chemicals, N°407. Organization for Economic Cooperation and Development, Paris, France; 2008.
10. OECD. Guidelines for the Testing of Chemicals / Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. Organization for Economic Cooperation and Development, Paris, France; 2002.

11. Bhrger C, Fischer DR, Cordenunzzi DA, Batschauer de Borba AP, Filho VC, Soares dos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasilinsis*) (Asteraceae) in mice. *J. Pharm. Sci.* (www.cspsCanada.org), 2005; 8: 370-373.
12. Shah Ayub MA, Garg SK, Garg KM. Subacute toxicity studies on Pendimethalin in rats, *Indian J. Pharmacol*, 1997; 29: 322-324.
13. Ghosh MN. In *Satistical Analysis, Fundamentals of Experimental Pharmacology*, 2nd ed, Calcutta: Scientific Book Agency; 1984; p 189.
14. Miller LC and Tainter ML. Estimation of ED50 and its error by means of log-probit graph paper. *Proc Soc Exp Bio Med*; 1944; 57: 261-264..
15. Turner R. Quantal responses. Calculation of ED₅₀. In *Screening. Methods Pharmacol*, Academic Press, New York, 1965; pp 61-63.
16. Billingham ME, Mason JW, Bristow MR, Daniels JR. Anthracycline cardiomyopathy monitored by morphologic changes. *Cancer Treat Rep*, 1978; 62: 865-872.
17. Mythilypriya R, Shanthi P, Sachdanandam P (2007). Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats. *J. Health Sci*, 2007; 53(4): 351-358.
18. Yéo D, Koffi E, Bidié AP, Tako NA, Bahi C, Méité S, Djaman AJ and Guéde-Guina F
In Vitro Anticholinesterase and Inhibitory Effects of the Aqueous Extract of *Combretum molle* (Combre-taceae) Leaf on Rabbit Breathing. *Trop J Pharm Res*, 2010; 9 (5): 469-473.
19. Cotonat J. La toxicologie point des connaissances actuelles. *Presses universitaires de France*, 1996; pp 5-25.