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Acute oral toxicity activity of aqueous extract of *Combretum glutinosum* Perr. ex De leaves in wistar rats

Alowanou Goué G.[1], Olounladé Abiodoun P.[1,2*], Azando Erick Virgile B.[1,3], Dossou Tobi R.[1], Dedehou Vidjinnangni Fifamè Grâce N.[1], Daga Florian D.[1], Adenilé Adam D., Hounzangbé-Adoté Sylvie M.[1]

[1]Laboratoire d'Ethnopharmacologie et de Santé Animale, Faculté des Sciences Agronomiques, Université d'Abomey Calavi, 01 BP 526 Cotonou, Bénin

[2]Laboratoire Pluridisciplinaire, Ecole Nationale Supérieure des Sciences et Techniques Agronomiques, Université d'Agriculture de Kétou, BP : 95 Kétou, Bénin

[3]Ecole Nationale Supérieure des Sciences et Techniques Agronomiques de Djougou, Université de Parakou, Bénin

*Corresponding Author E-mail: abiodoun.olounlade@coraf.org

ABSTRACT

Combretum glutinosum Perr. ex is tropical and subtropical medicinal plant widely used in traditional African to treat a range diseases such as parasitic diseases. The anthelmintic activity has been demonstrated in previous studies. Toxicity acute evaluation of Combretum glutinosum aqueous extract leaves was performed in this study, according to OECD 401 Guidelines for testing of chemicals. A single dose of 2000 mg/kg bw were given in one single administration by gavage female rats. No animal died and no behavioral signs of acute toxicity were observed. No statistically significant differences (p>0,05), compared to control animals in the weight of animal, some organs and the values of some hematological and biochemical parameters were observed. It was concluded that the aqueous extract of C. glutinosum is safe for oral use at single dose of 2000 mg/kg bw. On condition of chronic evaluation of the toxicity of the aqueous extract of C. glutinosum leaves, could therefore be continuously used by small breeders to control small ruminant helminthes.

***Key words**: Acute toxicity, Combretum glutinosum, Rats; Aqueous extract, Veterinary medicine.*

INTRODUCTION

The use of plant parts in the treatment of human disease is as old as the disease itself and herbal medicine was the major form of medicine in Benin. Phytomedicine has been an integral part of traditional health care system in most parts of the world for thousands of years[1]. Despite development of pharmaceutical industry, many reports estimate that approximately 80% of population in developing countries still relies on traditional medicine for their primary health care[2]. *Combretum glutinosum* is a very large genus comprising of about 250 species found in both temperate and tropical regions of the world[3] and widely used in traditional African especially in West Africa to treat a range of diseases. The roots stem, bark and leaves of *C. glutinosum* are used in the treatment of scrotal elephantiasis, dysentery, ringworms, syphilis, typhoid fever; eyesore and ear ache[4]. It was also reported that the stem bark and leaves of *C. glutinosum* are used as antipyretic and in the treatment of stomach ache, gonorrhea and typhoid[5]. Alowanou *et al.*,[6] have done documentation on the plant through a review about pharmacological and phytochemical activities. According to them, the plant has a many properties such as antibacterial, antifungal, antidiabetic, antispasmodic, anti-inflammatory, antimalarial effects, which justify the plant' medicinal use. As well, phytochemical studies carried out on plant have showed the presence of many classes of secondary metabolites, including quinones, catechic and gallic tannins, alkaloids, sterols, polyterpenes,

polyphenols, reducing compounds, flavonoids, saponins, phenols and tannins[7-8]. According to ethno - veterinary surveys in Benin as in other African countries such as Benin, Camerooun, Ivory Coast and Burkina Faso, the plant is used as anthelmintic in traditional human and veterinary medicine[9-10-11]. In the scientific validation of this usage, Alowanou *et al.,*[12] have demonstrated *in vitro* the anthelmintic properties of leave powder extracts of plant on three life - cycle stages (eggs, larvae and adult worms) of the parasitic nematode, *Haemonchus contortus.* The plant has been effective on the three stages of parasite. On condition to plant, assessment *in vivo* we can conclude that this plant could be used as alternative to synthetic compounds and a great asset for the control of gastro-intestinal nematodes, particularly *H. contortus.* However, it is necessary to have detailed scientific analyses and adequate information on the toxicity of the plant to strengthen these results. The way to determine the safe or unsafe use of these plants is the assessment of how it affects hematological and biochemical parameters. Changes from normal physiological levels of these parameters after administration of a chemical agent to the experimental animals is an indication of adverse effects of such agent on living organisms[13]. In the quest to evaluate and validate the plants use as anthelmintic, the acute toxic evaluation was done.

MATERIALS AND METHODS

Laboratory animals

Female Wistar nulliparous and non-pregnant rats weighing between 150 to 200g and aged 8 to 12 weeks used in this study were obtained from Laboratory Cytogenetic of Faculty of Health Sciences, University of Abomey-Calavi. The rats were housed in environmentally protected transparent polypropylene cages with stainless steel wire tops for a period of two weeks before commencement of the tests. The rats had free access to food and water *ad libitum.* Experimental diets were placed in special containers to minimize spillage. Environmental conditions included 23 – 25 °C, relative humidity of 45 – 55 %, and a 12-h light/dark cycle.

Collection and Preparation of plant

Plants screened were leaves *C. glutinosum*. The samples of plant were collected from Kandi, North of Benin. The specimens were identified at national herbarium of University of Abomey-Calavi of Benin under the numbers: AA6528/HNB. Then, plant leaves were dried indoors at room temperature during two weeks and the dried leaves was crushed in powder. The powdered material was stocked in a plastic container.

Preparation of plant extract

25 g of powdered leaves from *C. glutinosum* measured in a bottle with scales of precision were extracted in 250 ml of distilled water for two hours at 50°C. This was then filtered and the filtrate was concentrated under low pressure at 40°C to obtain dry powder extract. The extract was reconstituted in distilled water to give the required dose of 1ml/100g body weight (bw).

Evaluation method of acute oral toxicity of plants

Experimental Design

Acute oral toxicity study was performed according to OECD 401 Guidelines for testing of chemicals[14] following use as assays limit the single dose of 2000 mg/kg bw. Rats were randomly divided on (02) two groups of three animals per box. Control group received distilled water while the experimental group was administered with *C. glutinosum* aqueous leaves extract. Their weight was taken at the beginning of the experiment and then every 7 days. The animals were kept fasting for overnight providing only water, after which the plant extract were administered orally at the dose of 1ml/100g bw by gavage to rats of group test. On others hands, rats of control group have received 1ml of distilled water. After administration of the extract, the animals were observed every 30 min for 08 h on the first day and once daily for 14 days. During this period, the liveliness, sensibility to painful stimulation, visual acuity and texture of the faeces were assessed.

Collection of blood sample

Two weeks after plant extract administration; rats were pulling on sleep with chloroform and then sacrificed in order to take rat's blood and organs. Blood samples were taken on a level with orbital retro

of rats eye then collected into two clean dry heparin and no heparin capillary tubes of 75 x 1,5 mm for hematological and biochemical parameters determination respectively.

Evaluation of hematological parameters

The analysis of blood samples was performed using an analyzer, SYSMEX KS 21N. The hematological parameters including the number of Red Blood Cells (RBC), White Blood Cells (WBC), Level Corpuscular Haemoglobin concentration (LCHC), Hemoglobin concentration (Hb) the Platelet count (Plt), Mean Corpuscular Volume (MCV), Content and Mean Corpuscular Concentration in Hemoglobin (MCHC) count were determined.

Assessment of biochemical parameters

The serum biochemistry parameters including Glycaemia, cholesterol, creatinine, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were evaluated spectrophotometrically using an automated MIDRAY BS 200

Statistical analysis

Results were expressed as the mean value ± standard error of mean (SEM). Differences between control and experimental group were determined by one-way analysis of variance (ANOVA) using R software.

RESULTS

General observation

At the end of the both weeks of the experiment (Day 14), no harmful effect such as convulsion, agitation, diarrhea, trembling, respiratory problem, weight loss was observed. In additional any death was noted during all experimentation period after plant extract administration.

Body weights

Table 1 and 2 illustrate the effects of aqueous leaves extract of *C. glutinosum* on the body weight and relative organ weights of rats respectively. The results showed a significant difference (p<0,001) in body weight changes between group treated and control animals with time (p<0,05) (table 1)). Animals body weight on D7 and D14 appear to be the same at first when rats were treat with aqueous leaves extract of plant and with the rats of control group (table 2).

Table 1: Analysis of variance of rats' body weight in function treatment by *C. glutinosum* aqueous leaves extract

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	1	16806	16806	76,764	6,56E-08***
Date	2	2372	1186	5,417	0,0144*
Residuals	18	3941	219		
Signif. Codes	'***' ;0,001	'**' ;0,01	'*' ;0,05	',' ;0,1'	

Table 2: Effect of *C. glutinosum* aqueous leaves extract on body weights of rats at D1, D7 and D14

	Weight (g)		
Treatment groups	D1	D7	D14
Control	118,33±7,14	145,75±9,28	147,98±8,04a
Test	168,13±3,46	187,33±4,08b	188,48±3,21b

Values are Mean ± SD, n=2, *Values are significantly different from the control group at p<0.05.

Relative organ weights

Table 3 and 4 illustrate the effects of aqueous leaves extract of *C. glutinosum* on the relative organ weights of rats respectively. The relative organ weights of rats treat with aqueous leaves extract of plant liver, kidney, lung, spleen, and heart were compared favorably with those of the control and no significant

change (p>0,05) (table 3) was observed at the single dose of the extract used. However, there are a significant variation (p<0,001) on organs weight between organs of test group and those of control group. Liver weight of test group is the highest and inversely the lung of control group is the highest (table 4).

Table 3: Analysis of variance of rats' relative organs weight in function treatment by *C. glutinosum* aqueous leaves extract

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	1	0,42	0,418	3,444	0,0783
organs	4	61,35	15,338	126,447	6,7E-14***
Residuals	20	2,43	0,121		
Signif. Codes	'***' ;0,001	'**' ;0,01	'*' ;0,05	',' ;0,1'	

Table 4: Effect of *C. glutinosum* aqueous leaves extract on relative organs weight of rats

	Weight (g)	
Organs	Control group	Test group
Heart	0,4111±0,0401a	0,4439±0,0274a
Liver	3,595±0,425a	4,47±0,185b
Lung	1,086±0,134b	0,7058±0,0673a
Spleen	0,4109±0,0796a	0,5199±0,0707a
Kidney	0,6757±0,0246a	0,7428±0,0154a

Values are Mean ± SD, n=2, *Values are significantly different from the control group at p<0.05.

Biochemical parameters

The effects of *C. glutinosum* aqueous leaves extract on the biochemical parameters of rats were shown in the tables 5 and 6. The aqueous leaves extracts of *C. glutinosum* at single dose of 2000 mg/kg bw showed no statistical significant different (p>0.05) in the levels of all biochemical parameters of experimental group when compared with the control (table 5). However, There are a variation on biochemical parameters values compared to control group (p<0,001) (table 5). The plasma SGOT and the SGPT levels in the serum were significantly different in the test group compared to control group (table 6).

Table 5: Analysis of variance of biochemical parameters in Wistar rats in function treatment by *C. glutinosum* aqueous leaves extract

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	1	124	124	0,315	0,581
Biochemical-values	4	259363	64841	164,268	5,41E-15***
Residuals	20	7895	395		
Signif. Codes	'***' ;0,001	'**' ;0,01	'*' ;0,05	',' ;0,1'	

Table 6: Effect of aqueous leaves extracts of *C. glutinosum* on some serum biochemical parameters in wistar rats for 14 days

	Treatment group	
Parameter	Control	Test
Cholesterol	0,7833±0,0677	0,8467±0,0722
creatinine	9,667±0,667	7,67±1,2
Glycaemia	1,707±0,544	0,63±0,0709
SGOT	222±7,37	209,3±31,6*
SGPT	123,67±2,6	97±9,29*

Values are Mean ± SD, n=2, *Values are significantly different from the control group at p<0.05

Hematological parameters

The results of the effect of single dose of 2000 mg/kg bw of aqueous extract of *C. glutinosum* on the hematological parameters are shown in tables 7 and 8. Effect of aqueous extract of *C. glutinosum* on the hematological parameters in the treated group was not significantly different (p>0,05) from the control group (table 7). Nevertheless, there are a variation on hematological values (p<0,001) compared to control group. The platelet count was significantly different in the test group compared to control group inversely the mean corpuscular volume in the control group was higher than the test group (table 8).

Table 7: Analysis of variance of hematological parameters in Wistar rats in function of treatment by *C. glutinosum* aqueous leaves extract

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	1	241	241	0,632	0,433
Hematological-values	7	3244701	463529	1216,793	<2e-16 ***
Residuals	32	12190	381		
Signif. Codes	'***' ;0,001	'**' ;0,01	'*' ;0,05	',' ;0,1'	

Table 8: Effect of aqueous leaves extracts of *C. glutinosum* on some hematological parameters in Wistar rats for 14 days

	Treatment group	
Parameter	Control	Test
MCHC	28,467±0,584	29,3±0,656
WBC	9,967±0,797	10,43±2,63
RBC	6,86±0,0346	6,98±0,447
Hb	12,7±0,306	12,367±0,549
Ht	44,8±1,93	42,17±2,14
Plt	790,7±43,6	976±97,1*
MCCH	18,533±0,376	17,6±0,379
MCV	65,23±2,47*	60,47±1,02

Values are Mean ± SD, n= 2, *Values are significantly different from the control group at p<0.05

Hb: Haemoglobin concentration; WBC: White blood cell count. LCHC: Level Corpuscular Haemoglobin concentration, Plt: Platelets, RBC: Red Blood Cell, MCV: Mean Corpuscular Volume, MCCH: mean corpuscular concentration in hemoglobin, Ht: Hematocrit

DISCUSSION

Small breeders of developing countries such as Benin[11,] Cameroon[10,] and Burkina Faso[9] uses the leaves of *C. glutinosum* against gastrointestinal parasites of sheep and goat in veterinary medicine. To validate this traditional use, Olounladé *et al.,*[12] have tested in vitro the leaves of the plant on three life - cycle stages of *Haemonchus contortus.* The present study is complementary to these previously study and will contribute to validate the plant use in veterinary medicine.

Thus, following the administration of single dose of 2000 mg/kg body weight of *C. glutinosum* aqueous leaves extract to rats, No mortality was observed by oral way testing up to values of group test nor was any significant clinical sign of toxicity in the test led at limit dose of 2000 mg/kg body weight not noted. Significant differences in body weight changes between group treated and control animals with time was observed. Therefore, the mean gain in weight was high in-group, which received *C. glutinosum* aqueous leaves extract, which may be due to the beneficial effect of the extract. After the consumption of C. glutinosum aqueous leaves extract, animals showed a slight stimulation of appetite for food and water.

It is known that in addition to their therapeutic properties, medicinal plants can affect positively the nutritional status of an animal[15].

Then, no statistically significant differences, compared to control animals, the values of some biochemical and hematological parameters were observed in this study. No variation in total count were also observed in a study by the administration of *C. glutinosum* aqueous extract leaves that produced any changes in hematological index. Blood cells are produced in the bone marrow. Known hemotoxicants such as paracetamol cause reduction in RBCs leading to anemia and some bioactive phytochemicals affect HCT levels[16].

The aminotransferases (SGPT and SGOT) are 'markers' of liver damage and can thus be used to assess liver cytolysis with SGPT being a more sensitive biomarker of hepatotoxicity than SGOT[17]. SGPT and SGOT are located in the cytoplasm and mitochondria of liver cells in high concentrations but low in blood. However, SGPT is more liver-specific. It is known that increased activities of these enzymes in serum are due to increased membrane permeability and leakage into the blood circulation when there is damage to liver cells[18]. The plasma SGOT and SGPT levels in the serum were not significantly different by the intake of *C. glutinosum* extract compared to control and this indicates that the activity of the liver was preserved. In the assessment of liver damage by drugs or any other hepatotoxin, the determination of enzyme levels such as SGPT and SGOT is largely used[19]. In additional, there are no statistically significances in the levels of cholesterol, Glycaemia and creatinine at the single dose level compared to control. Creatinine is marker of kidney function[20]. It is an indication that the extracts were not nephrotoxic.

CONCLUSION

The results of acute toxicity study showed the aqueous bark extract of *C. glutinosum* did not cause any signs of toxicity or produce mortality in rats using single dose 2000mg/kg bw. These results suggest that the plant could be used par small breeders to ruminant helminthes control on condition to sub-acute toxicity and organ histology test.

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