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Acute and Subacute Toxicity Study of the Combination of Aqueous Extracts of the Trunk Bark of *Musanga cecropioides* R. Br. (Cecropiaceae) and the Fruits of *Combretum micranthum* G. Don (Combretaceae)

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Original Research Article

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Abstract: The main objective of the work was to evaluate the acute and subacute toxicities of the combinated of aqueous extracts of the bark trunk of *Musanga cecropioides* and the fruit of *Combretum micranthum*. The respective extraction yields of 12% and 3.6% were obtained for *Combretum micranthum* and *Musanga cecropioides*. Tests of alkaloids, sterols, terpenes, phenols, flavonoids, saponins, coumarins, reducing sugars, tannins were positive with at least one solvent while those of anthraquinones and anthocyanins were negative, with all solvents whatever the species considered. At the end of the fourteen days of study of the acute toxicity, no abnormality of the studied parameters was observed, except for the aggressiveness of the rats, during the first five days. Most parameters varied in different groups during the subacute toxicity study. The values of blood biochemical and hematological parameters of the rats fluctuated sometimes. The study pharmacological activities *in vitro* and *in vivo* would allow the development of medicinal products based on these medicinal plants.

Keywords: *Musanga cecropioides*, *Combretum micranthum*, bark, fruit, toxicities, rats.

INTRODUCTION

Traditional medicine is an alternative medicine of which practices that can or can not be explained, used to diagnose, prevent or eliminate a physical, mental or social illness based on practical experiences and transmitted from generation to generation orally or in writing [1].

Medicinal plants are any plant or part of a plant used for therapeutic purposes. These plants are widely used in developed countries where they are widely available and offer an alternative to pharmaceuticals. In developing countries, people depend on them for primary health care and subsistence [2]. Plants are very valuable resources for the vast majority of rural populations in Africa, where more than 80% of these populations use for health problems [3].

Population growth, inaccessibility to modern medicines and expensive their costs in these countries are contributing to increase demand for traditional medicines. In Cameroon, medicinal plants are particularly important for poor communities who find most of their resources for self-consumption, trade and culture [4]. However, the majority of plants used in traditional medicine can have toxic effects. Toxicity is defined as all undesirable effects in a living organism, caused by a relatively high single-dose drug or small, long-term doses [5]. The study of the toxicity of a substance is the set of pharmacological tests, which determine the degree of harmfulness of the latter in order to regulate its use. The action of a toxic substance is evaluated according to parameters such as its mode of administration (oral, intravenous, intraperitoneal), the dose administered, the observed mortality rate, the weight change, the histology of certain organs, modification of certain biochemical parameters of the blood called toxicity markers (total cholesterols, bilirubin, creatinine, urea) [6].

MATERIAL AND METHODS

MATERIAL

Two types of equipment were used, biological material and technical equipment. The biological material was composed of plant material

and animal material. The plant material consisted of the trunk bark of *Musanga cecropioides* and the fruit of *Combretum micranthum*, harvested in October 2017, respectively in a plantation of Nkongsamba, Moungo Department and in a young rainforest of Logbadjeck, Department of Sanaga Maritime. Both departments belong to the Coastal Region of Cameroon. The animal material consisted of albino white rats, both male and female of the Wistar strain.

The technical material consisted of extraction equipment, phytochemical screening material, toxicity study materiel, glass ware, consumables, reagents and chemical solvents.

METHODOLOGY

Extraction

The organs used were washed under a stream of continuous water, cut into small pieces and dried in an oven at 50 °C for 10 days, then pulverized with a grinder [7]. The aqueous crude extracts were obtained after maceration of 200 g of vegetable powder of each sample in 1 L of distilled water for 48 hours and then evaporated in an oven [8, 7]. The extraction yield (RE) was calculated with respect to the weight of dry vegetable matter according to the formula:

$$RE = \frac{\text{Mass of extract obtened}}{\text{Mass of powder}} \times 100$$

Phytochimical screening

Detection tests of large groups of phytochemicals were performed on five residues obtained from ethanol, methanol, ethyl acetate, hexane and distilled water as extraction solvents. Colorimetric and precipitation techniques described in the work of Tona *et al.*, [9] and Longanga *et al*., [10] were used.

Evaluation of acute and subacute toxicity

Acute toxicity

The acute toxicity experiment was conducted according to guideline 423 of the OECD [11]. Male or female rats of Wistar strain were fasted all night prior to the experiment. Four (4) groups of six (6) randomized rats received increasing doses of aqueous extracts (50, 300, 2000 and 5000 mg/Kg). The control group received the solvent for diluting the extracts. The rats once treated were observed during the 2 hours following the administration of the extract, at the end of which they were fed. They were then observed after 4 hours, 8 hours, and 14 days during which symptoms of intoxication (change in coat, motility, tremor, grooming, breathing, sensitivity to noise and death) were noted. Dead rats in each lot were counted for LD_{50} determination. The extract was administered to the rats orally.

Subacute toxicity

Subacute toxicity was determined from OECD Guideline 407 [12]. Adult male and female albino rats of Wistar strain were divided into three (3) experimental groups of six (6) animals each, including three males and three females. The limit tests were carried out at the dose of 1000 mg/Kg on the second group, while on the third, the subacute toxicity tests were carried out with the dose of 2 x171 mg/Kg, corresponding to the dose of the traditional healer. Group 1, control received distilled water. Administrations continued for 28 days with 6 days of administration out of 7 per week. After 28 days, the organs removed (kidneys, liver, lungs, heart, pancreas) were rinsed with 0.9% saline, then observed *in situ* and weighed.

Blood samples

Blood samples were taken from fasted and anesthetized rats by diethyl ether. They were made at the level of the jugular. The blood sample was collected in three types of tubes (heparin tubes, EDTA tubes, and dry tubes). EDTA tubes and dry tubes were intended for hematological analyzes. The heparinized tubes were centrifuged at 4000 rs/min for 10 min and the resulting serum was stored at -20 °C for blood biochemistry analyzes.

Biochemical examinations

The following serum parameters: Glucose (Glu), Creatinine (Creat), Uric Acid (AU), Total Cholesterol (Chol T), Total Triglycerides (TG), Transaminase Glutamate Oxolo-Acetate (TGO), Transaminase Glutamate Pyruvate (TGP), were measured by enzymatic methods. These assays were performed at the Laboratory of Hematology and Pathology of the General Hospital of Douala.

Hematological examinations

The hematological parameters for the blood count formula (FNS) were: Red blood cells (GR), mean cell volume (VGM), hematocrit (Hte), platelet (PLT), mean platelet count (VPM), white blood cells (GB), Hemoglobin (HGB), Corpuscle Hemoglobin (TCH), Mean Hemoglobin Corpuscle Concentration (CCMH), Neutrophils (N). These parameters were made by the Medonic (Beckman coulteur-USA), at the Laboratory of Hematology and Pathology of the General Hospital of Douala.

RESULTS

Yield extraction

The aqueous extracts of the drugs from both plants were prepared from 200 g of powder. The mass extracts obtained after drying in an oven at 50 °C were 24 g and 7.2 g respectively for *Combretum micranthum* and *Musanga cecropioides* with extraction yields (RE) of 12% and 3.6%, respectively.

Phytochimical screening

The phytochemical screening of bark extract of *Musanga cecropioides* has made it possible to highlight the presence of various phytochemical groups. Alkaloid, sterol, terpenes and coumarin tests were found to be positive, but at low concentrations with the hexane extract. With the ethyl acetate extract, the alkaloid test was positive with average concentrations, while the phenol and tannin tests were positive at low concentrations. The distilled water extract revealed the presence of alkaloids and saponins at high concentrations, phenols and flavonoids at medium concentrations, followed by sterols and sugars at low concentrations. In the ethanol extract, the alkaloid test was found to be positive with average concentrations, while the phenol, tannin and reducing sugar tests were positive at low concentrations. In the methanol extract, flavonoid and saponin tests were found to be positive at high concentrations, while sterol and reducing sugars tests were positive at medium concentrations and those for terpenes, phenols, coumarins, flavonoids, and tannins were found to be positive with low concentrations. However, it should be noted that only alkaloids were positive with all extraction solvents, while anthraquinones and anthocyanins were negative (Table-1).

Table-1: Phytochemical screening of bark extract of *Musanga cecropioides*

Secondary metabolites	Hexane extract	Ethyl acetate extract	Distilled water extract	Ethanol extract	Methanol extract
Alcaloids	+	++	+++	++	+++
Sterols	+	-	+	-	++
Terpenes	+	-	-	-	+
Phenols	-	+	++	+	+
Coumarins	+	-	-	-	+
Flavonoids	-	-	++	-	+
Tannins	-	+	-	+	+
Saponins	-	-	+++	-	+++
Reducing sugars	-	-	+	+	++
Anthraquinones	-	-	-	-	-
anthocyanins	-	-	-	-	-

(+++): Positive test (strong concentration); (++): Positive test (average concentration); (+): Positive test (low concentration); (-): Negative test

The phytochemical screening of *Combretum micranthum* fruit extract was performed. The flavonoid test was positive with the hexane extract, but at moderate concentrations. With the ethyl acetate extract, all tests were negative. The distilled water extract showed the presence of phenols and flavonoids with high concentrations; saponins at average concentrations; alkaloids, sterols and coumarins at low concentrations. With the ethanol extract, flavonoid and saponin tests were found to be positive, but at medium concentrations, while those of alkaloid, phenol, tannin, reducing sugar tests, were found to be positive at low concentrations. With the methanol extract, flavonoid and saponin tests were positive at high concentrations, while phenol and reducing sugar tests were positive at medium concentrations and those for sterol, terpene and tannin tests were positive, with low concentrations. However, it should be noted that no secondary metabolite test was positive with all solvents. Anthraquinone and anthocyanin tests were negative with all solvents and no tests were positive for ethyl acetate (Table-2).

Table-2: Phytochemical screening of fruit extract of *Combretum micranthum*

Secondary metabolites	Hexane extract	Acetate ethyl extract	Distilled water extract	Ethanol extract	Methanol extract
Alcaloids	-	-	+	+	-
Sterols	-	-	+	-	+
Terpenes	-	-	-	-	+
Phenols	-	-	+++	+	++
Coumarins	-	-	+	-	-
Flavonoids	++	-	+++	++	+++
Tannins	-	-	-	+	+
Saponins	-	-	++	++	+++
Reducing sugars	-	-	-	+	++
Anthraquinones	-	-	-	-	-
anthocyanins	-	-	-	-	-

(+++): Positive test (strong concentration); (++): Positive test (average concentration); (+): Positive test (low concentration); (-): Negative test

Acute toxicity of the combinated extract

The acute toxicity study focused on the observation of physiological changes in albino rats of Wistar strain. The combinated extract of *M. cecropioides* and *C. micranthum* in the proportions 50% -50% was administered to the rats, followed by observation for 8 h and 14 days, after administration. At the end of 14 days of observation, there was no abnormality of the studied parameters except for the aggressiveness of the rats during the first five days. No death was recorded. The LD_{50} was therefore greater than 5000 mg/Kg (Table-3).

Subacute toxicity of the combined extract

The combinated extract of *M. cecropioides* and *C. micranthum* in the proportions 50% -50% was administered to the rats, followed by observation for 4 h and 4 weeks after administration. In group 1, no anomality in the parameters was observed at the end of the 4 weeks of observation, apart from the aggressiveness of the rats. In group 2, the aggressiveness of the rats during the first week of observation, a decrease in the reaction to sound during the third week (rat 1, rat 4), then excessive motility (rat 4), tremor (rats 4, 5) and somnolence (rats 4, 5, 6), during the fourth week. In group 3, no abnormality in the parameters was observed during the first and second weeks apart from the aggressiveness of the rats during the first week. During the third and fourth week, salivary secretions, nasal excretions, and sneezes (rats 1, 2, 3) were noted (Table-4).

Table-3: Acute toxicity of combinated extracts

Parameters Observed	Periods of study																	
	1h	2h	4h	8h	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
Motility	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Coat	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Convulsion	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Salivation	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Agressiveness	Vsb	Vsb	Vsb	Vsb	Vsb	Vsb	Vsb	Vsb	Vsb	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Vigilance	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Weird	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Drowsiness	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Sensitivity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Stool	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

N = Normal ; Vsb = Visible ; Abs = Absent ; 0 = Zero

Table-4: Subacute toxicity of combinated extracts

Groups and doses administered (mg/Kg)		Observed parameters									
		Eye modification	Motility	tremor	Clonic mouvements	Secretions and excretions	Agressiveness	Drowsiness	Reaction to sound	Sneezing	Death
Group 1 : 0 mg/Kg	Week 1	Abs	N	Abs	Abs	Abs	Visible	Abs	N	Abs	0
	Week 2	Abs	N	Abs	Abs	Abs	Abs	Abs	N	Abs	0
	Week 3	Abs	N	Abs	Abs	Abs	Abs	Abs	N	Abs	0
	Week 4	Abs	N	Abs	Abs	Abs	Abs	Abs	N	Abs	0
Group 2 : 1000 mg/Kg	Week 1	Abs	N	Abs	Abs	Abs	Visible	Abs	N	Abs	0
	Week 2	Abs	N	Abs	Abs	Abs	Abs	Abs	N	Abs	0
	Week 3	Abs	N	Abs	Abs	Abs	Abs	Abs	Ebs	Abs	0
	Week 4	Abs	Agited	Visibles	Abs	Abs	Abs	Visible	N	Abs	0
Group 3 : 171 mg/kg	Week 1	Abs	N	Abs	Abs	Abs	Visible	Abs	N	Abs	0
	Week 2	Abs	N	Abs	Abs	Abs	Abs	Abs	N	Abs	0
	Week 3	Abs	N	Abs	Abs	Visible	Abs	Abs	N	Visible	0
	Week 4	Abs	N	Abs	Abs	Visible	Abs	Abs	N	Visible	0

N = normal ; Abs = absent ; 0 = zéro ; Ebs = en baisse

Variations in average masses of rat groups

The average masses were increasing during the observation period (209.33 ± 31.6 on day 0 to 241.66 ± 28.41 on day 28) and (208.16 ± 48.57 on day 0 to 229.33 ± 43.73 on day 28) for groups 1 and 2 respectively. A comparison of these values with the ANOVA test revealed very significant differences. The average masses of group 3 were decreasing during the period of experiment (247 ± 42.14 at day 0 to 238.66 ±50.65 at day 28) with a difference of 8.34 g. A comparison of these values with the ANOVA test revealed very significant differences.

Variations in mass averages of rat groups in function of sex

The masses of rat groups fluctuated during the experiment. Growth of females was greater than that of males in group 1. Differences in averages masses of males and females were 41.33 g and 16.67 g on day 0 and day 28, respectively. The finding was the same in

group 2. The differences in average masses of males and females were respectively 81 g and 51.33 g on day 0 and day 28. In group 3, the growth of males was greater than that of females. Differences in masses of males and females were 73.34 g and 83.33 g on day 0 and day 28, respectively. The differences between these values were significant (Table-5).

Table-5: Variations in average masses of rat groups in function of sex

N° Group		D0	D2	D4	D6	D8	D10	D12	D14	D16	D18	D20	D22	D24	D26	D28
Group 1 ***	F	188.67	199.33	201.00	206.00	204.00	213.00	211.67	215.33	217.33	221.00	217.00	223.00	222.67	229.67	233.33
	M	230.00	243.00	239.00	243.33	244.00	252.67	250.00	251.00	254.33	253.00	245.67	243.33	243.33	247.67	250.00
Group 2***	F	167.67	175.33	175.67	180.67	179.67	185.00	186.67	189.00	193.00	194.33	193.67	198.00	195.33	201.00	203.67
	M	248.67	252.33	257.33	261.33	255.33	259.00	253.67	238.33	251.33	253.67	249.67	251.67	252.67	253.00	255.00
Group 3***	F	210.33	206.67	208.67	204.33	209.67	207.67	211.00	204.00	202.00	206.33	202.67	203.33	199.00	198.67	197.00
	M	283.67	274.33	274.33	270.33	276.00	269.33	272.67	273.00	268.67	285.00	282.00	282.00	286.33	279.00	280.33

*The differences were significant for p <0.05; * = insignificant differences; ** = moderately significant differences; *** = very significant differences; Jn: Jour n*

Evolution of the average masses of the internal organs of the rat groups

Weighing of the masses of the internal organs showed that the average mass of the heart of group 3 (0.80 g) was the highest followed by that of the control group (0.76 g) and that of group 2 (0.70 g). The average mass of group 3 was not significantly different from that of the control group, but significantly different from the group 2. The average mass of the livers of the control group (5.62 g) was the highest, followed by group 2 (5.18 g) and group 3 (4.87 g). These three values were very significantly different from each other. The average mass of group 3 (1.24 g) was the highest, followed by group 2 (1.17 g) and control group (1.02 g). These three values were very significantly different from each other. Speaking of the lungs, the average mass of group 2 (2.25 g) was the highest, followed by control group (2.03 g) and group 3 (1,.75 g). These three values were very significantly different from each other. The average mass of the pancreas of group 3 (0.7 g) was the highest, followed by control group (0.64 g) and group 2 (0.5 g). These values were found to be significantly different from each other (Figure-1).

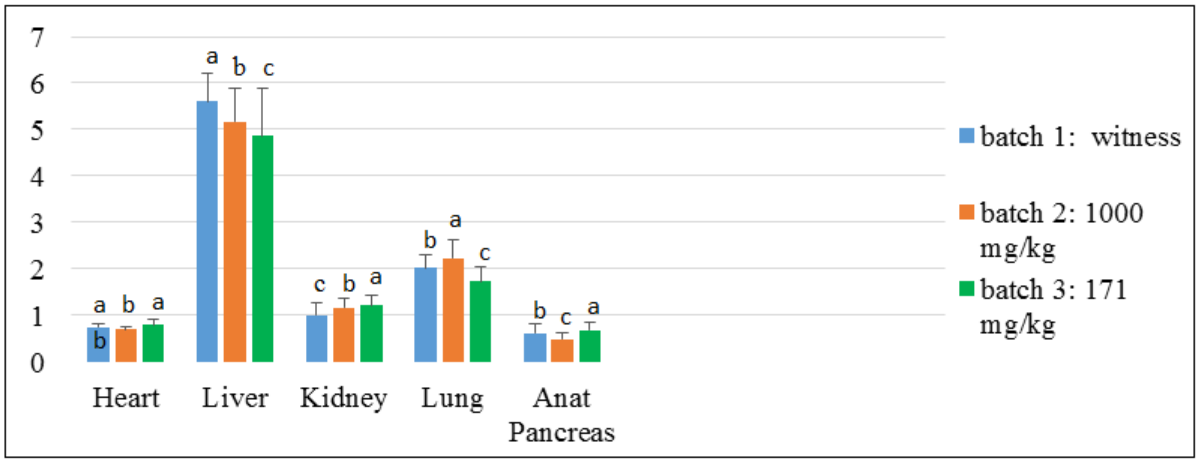

Fig-1: Average masses of the internal organs of rat groups

Biochemical parameters of rat groups

The analysis of the blood biochemical parameters of rat groups after subacute toxicity study showed that the average value of the creatinine level of the control group was lower than that of groups 2 and 3 with significant differences. The average uric acid, cholesterol, triglyceride and blood glucose levels of the control group rats were higher than those of groups 2 and 3 with significant differences. The average HDL cholesterol value of the control group rats was higher than that of groups 2 and 3, with a significant difference in group 3 and not significant in group 2. The GOT and GPT values of the control group were higher than those of groups 2 and 3 with, very significant differences (Table-6).

Table-6: Average biochemical parameters of rat groups

N° of group	Creat	Uric acid	CH	TG	HDL	Trans		Glycemia
						GOT	GPT	
Group 1	6.43 ± 0.73*	57.87 ± 20.85*	0.95 ± 0.16*	0.94 ± 0.44*	0.4 ± 0.07*	348.17± 200.17**	119,18±133.29*	0.97± 0.08*
Group 2	8.54 ± 2.34**	50.61 ± 16.02**	0.86± 0.18**	0.69 ± 0.18***	0.39 ± 0.11*	390.88± 129.15*	65,7± 16.44**	0.61± 0.07**
Group 3	9.51 ± 0.82***	45.91 ± 10.46***	0.86± 0.15**	0.89 ± 0.20**	0.23± 0.04**	27.67 ± 11.66***	23,17 ± 12,2***	0.64± 0.04**

Values are presented as mean ± standard deviation. Creatine: Creatinine; CH: Cholesterol; TG: Triglycerides; HDL: HDL cholesterol; Trans: Transaminases; GOT: Transaminase Glutamate Oxolo-acetate; GPT: Transaminase Glutamate Pyruvate. The differences were significant for p <0.05.

Biochemical parameters of rat groups in function of sex

Creatinine was higher in males than females in groups 1 and 3 with a significant difference in group 1 and not significant in group 3, whereas in group 2, it is higher in females than males, with a significant difference. Cholesterol was higher in females than males in group 1 with a significant difference, while it was higher in males than females in groups 2 and 3 with, significant differences. Triglycerides were higher in females than males in groups 1 and 3, with significant differences, whereas they were higher in males than females in group 2, with a significant difference. HDL was higher in females in lot 1 whereas in groups 2 and 3 it was higher in males, but with insignificant differences. Glycemia was higher in females than in males in groups 1 and 3, with a significant difference in group 1 and not significant in group 3. In group 2, it was higher in males than in females. However, the highest blood glucose level was found in the females of group 1 (Table-7).

Table-7: Average biochemical parameters of rat groups

N° of group		Creat	Uric acid	CH	TG	HDL	Trans		Glycemia
							GOT	GPT	
Group 1	F	6.33 ± 1.06*	54.90 ±23.90*	0.95± 0.01*	0.89 ± .38*	0.41 ± .10*	439.47±272.03**	187.30± 74.02*	1.01 ± 0.05*
	M	9.90 ± 1.89**	40.83 ±12.68**	0.91 ±0.16*	0.74 ±0.23**	0.30 ± 0.05*	33.67 ± 16.16*	51.07± 14.52**	0.93 ±0.09**
Group 2	F	12.30 ± 5.59*	49.49 ± 7.82*	0.74 ± 0.14*	0.89 ± 0.11*	0.21 ± 0.04*	16.00 ± 6.08*	76.50 ± 12.90*	0.68 ± 0.10*
	M	10.37 ± 2.37**	45.24 ± 17.78**	0.77 ± 0.25*	0.94 ± 0.17**	0.25 ± 0.10*	23.33 ± 9.71**	54.90± 12.62**	0.71 ± 0.10*
Group 3	F	9.08 ± 0.71*	47.32 ± 13.69*	0.78 ± 0.15*	0.91 ± 0.12*	0.23 ± 0.02*	23.33 ± 3.21*	30.00 ± 9.54*	0.67 ± 0.01*
	M	9.94 ± 0.80*	44.50 ± 8.93**	0.93 ± 0.11**	0.86 ± 0.28**	0.24 ± 0.06*	33.00 ± 15.62**	16.33 ± 12.10**	0.61 ± 0.03*

Values are presented as mean ± standard deviation. Creatine: creatinine; CH: Cholesterol; TG: Triglycerides; HDL: HDL cholesterol; Trans: Transaminases; GOT: Transaminase Glutamate Oxolo-acetate; GPT = Transaminase Glutamate Pyruvate. The differences were significant for p <0.05.

Hematological parameters of the rat groups

After studying subacute toxicity, the hematological parameters of the rat groups were determined. The values for these parameters except for Neutrophils and lymphocytes were all higher in the control group than in group 2, and lower in the control group than in group 3 (Table-8).

Table-8: Hematological parameters of rat groups

N° of group	GB	GR	Hb	Hte	VGM	TCMH	CCMH	PLT	N	L
Group 1	5.85±2.76*#	5.87± 0.89*#	13.07±1,08*#	35.40±5.590*#	60.38±3.19*#	22.47±2.34*#	37.35±4.20*	354.67±123.92*	6.00 ± 4.43*	79.5±11.26*
Batch 2	5.2±2.59*	5.79± 1.56*	11.50±2.57*	34.40±10.50*	58.85±2.23*	20.08±2.37*	34.37±5.24#	270.33±92.07**	7.33±6,30*	77.5±13.00*
Batch 3	7.17±1.32#	6.38± 0.73#	15.52±1.72#	39.80±5.09#	63.2±1.50#	25.18±1.81#	36.12±2.95*#	514.67±63.76***	7.33±3.50*	79.17±9.02*

Values are presented as mean ± standard deviation. GB: White blood cells; GR: Red blood cells; Hb: hemoglobin; The: Hematocrites; VGM: Average Globular Volume; TCMH: Mean Corpuscle Content in Hemoglobin; MCHC: Mean Corpuscle Concentration in Hemoglobin; PLT: Platelets; N: Neutrophils; L: Lymphocytes. The differences were significant for p <0.05.

Hematological parameters of rat groups in function of sex

The level of white blood cells was higher in males in group 1, while in 2 and 3, it was more in females. Red blood cell and hemoglobin levels were higher in males, in all three groupss. The hematocyte count was higher in males in groups 1 and 2, while in group 3, it was higher in females. Blood cell volume and corpuscular hemoglobin content were higher in females in group 1, but higher in males in 2 and 3. The average corpuscular hemoglobin concentration was higher in females in groups 1 and 3, whereas in group 2, it was higher in males than in females, with a significant difference. Blood platelet counts were higher in males than females in groups 1 and 2, with significant differences, but higher in females than males in group 3 with a significant difference (Table-9).

Table-9: Hematological parameters of rat groups in function of sex

N° of group l		GB	GR	Hb	Hte	VGM	TCMH	CCMH	Placelets	N	L
Group 1	F	5.07±2.40	5.45±1.43*	12.50±0.80	34.10±9.83	62.47±20	23.27±4.60	37.47±8.84	315.00±209.10	4.33±3.53	84.33±7.07
	M	6.63±2.46*	6.29±0.66	13.63±1.20*	36.70±4.85*	58.30±1.57*	21.67±0.81*	37.23±2.16	394.33±72.40*	7.67±5.86*	74.67±14.10*
Group 2	F	5.67±2.59	5.36±0.13*	10.27±0.63	31.43±0.70	58.50±0.26	19.00±0.79	32.60±1.44*	258.67±14.74	11.33±6.81	68.67±11.15
	M	4.73±3.07*	6.23±2.34	12.73±3.41*	37.37±15.70*	59.20±3.46	21.17±3.15*	36.13±7.57	282.00±143.82*	3.33±2.31*	86.33±8,02*
Group 3	F	7.83±1.71	6.29±0.33	14.83±0.47	40.37±3.13	62.87±10	24.40±1.93	36.43±1.95	534.33±64.29	7.00±5.29	79.67±11.68
	M	6.50±0.36*	6.47±1.10	16.2±2.40*	39.23±7.35	63.53±20	25.97±1.61*	35.80±4.20	495.00±69.78*	7.67±1.53	78.67±8.14

Values are presented as mean ± standard deviation. GB: White blood cells; GR: Red blood cells; Hb: Hemoglobin; The: Hematocrites; VGM: Average Globular Volume; TCMH: Mean Corpuscle Content in Hemoglobin; MCHC: Mean Corpuscle Concentration in Hemoglobin; PLT: Platelets; N: Neutrophils; L: Lymphocytes.The differences were significant for p <0.05.

DISCUSSION

The aqueous extracts of both plants were prepared from 200 g of fruit powder of *Combretum micranthum* and 200 g of bark powder of *Musanga cecropioides*. The masses obtained after drying in an oven at 50 °C were 24 g and 7.2 g respectively for *Combretum micranthum* and *Musanga cecropioides.* The extraction yields (RE) were low respectively of 12% and 3.6% as that obtained by Ekossono [13] and Etame *et al*., [14].

The phytochemical screening of the extracts of the two plants made it possible to highlight the presence of various phytochemical groups. Tests of alkaloids, sterols, terpenes, phenols, flavonoids, saponins, coumarins, reducing sugars, tannins were positive with at least one solvent, while those of anthraquinones and anthocyanins were negative, with all the solvents, whatever the species considered. All tests were negative with the *combretum micranthum* ethyl acetate extract [15, 14, 16].

The results of the study revealed that the average masses of male and female rats generally fluctuated in all groups during the period of experiment [14].

The acute toxicity study focused on the observation of physiological changes in albino rats of Wistar strain. At the end of the fourteen days of observation, no abnormality of the studied parameters was observed except for the aggressiveness of the rats during the first five days. No death was recorded. The LD_{50} was therefore greater than 5000 mg/Kg [17, 15, 18].

The combinated extract of *M. cecropioides* and *C. micranthum* in proportions of 50% - 50% was administered to the rats and then the observation was made during to the subacute toxicity. During the third and fourth week, salivary secretions, nasal excretions, and sneezes (rats 1, 2, 3) were noted [15, 19, 18].

The analysis of the blood biochemical parameters of rats after subacute toxicity study showed that the average value of the creatinine level of the control group was lower than that of groups 2 and 3 with significant differences. The average uric acid, cholesterol, triglyceride and blood glucose levels of the control group rats were higher than those of the rats in groups 2 and 3 with significant differences. The average HDL value of the control group rats was higher than that of the groups 2 and 3 rats, with a significant difference in group 3 and not significant in group 2. The GOT and GPT values of the control group were higher those of groups 2 and 3, with very significant differences. Etame *et al.*, [14], found the same results in their work.

Analysis of urea and creatinine revealed that the administration of the extract did not cause any significant changes. Serum urea and creatinine are

considered the major markers of nephrotoxicity, although serum urea is often considered a more reliable predictor of renal function than serum creatinine [20].

The values of hematological parameters of the rats were, except for Neutrophils and Lymphocytes higher in the control group than in group 2 and lower in the control group than in group 3. The level of white blood cells was higher in males in group 1 while in groups 2 and 3, it was more in females. Red blood cell and hemoglobin levels were higher in males in all three groups. Hematocrits were higher in males in groups 1 and 2, while in group 3 they were higher in females. Blood cell volume and hemoglobin content were higher in females in group 1, but higher in males than in groups 2 and 3. The mean corpuscular concentration in hemoglobin were higher in groups 1 and 3, with insignificant differences whereas in group 2, in the males. Blood platelet counts were higher in males of groups 1 and 2, and higher in females in group 3. Etame *et al.*, [14] found the same results in their work.

Transaminases or amino transferases are tissue enzymes catalyzing the transport of alpha-amino radicals from alanine and aspartic acid to alpha-ketoglutaric acid. They are present in the liver, muscle, kidney, pancreas, and other tissues. They are synthesized at the level of the cytoplasm of the cells of these organs and discharged into the circulation when these cells are damaged [21, 22].

CONCLUSION

The general objective of the work was to evaluate the acute and subacute toxicity of the combination of aqueous extracts of the bark of the trunk of *Musanga cecropioides* and the fruit of *Combretum micranthum*. The respective extraction yields of 12% and 3.6% were obtained for *Combretum micranthum* and *Musanga cecropioides*. Tests of alkaloids, sterols, terpenes, phenols, flavonoids, saponins, coumarins, reducing sugars, tannins were positive with at least one solvent while those of anthraquinones and anthocyanins were negative, with all solvents whatever the species considered. At the end of the fourteen days of study of the acute toxicity, no abnormality of the studied parameters was observed except for the aggressiveness of the rats, during the first five days. Most parameters varied in different groups during the subacute toxicity study. The values of blood biochemical and hematological parameters of the rats fluctuated sometimes. The study pharmacological activities *in vitro* and *in vivo* would allow the development of medicinal products based on these medicinal plants.

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