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Acute and Sub-acute Toxicity Studies of the Aqueous Leaf Extract of *Lippia multiflora*

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ABSTRACT

Background and Objective: *Lippia multiflora* is used by sub-saharan African populations as tea, beverage and pharmaceutical preparations to treat various diseases. This study was conducted to identify the chemical groups in the leaves of *L. multiflora* harvested in Toumodi (Côte d'Ivoire) and to evaluate the toxicity of the aqueous extract (LiMAE) in two mammals.

Materials and Methods: After identification of the phytochemicals, 4 Swiss mice and 18 female Wistar rats were used for the acute toxicity and sub-acute toxicity studies, respectively, according to the OECD guidelines (423 and 425). At 28th day, rats were anaesthetized and sacrificed. Blood samples were collected by cardiac puncture and biochemical metabolites and hematological parameters were monitored.

Results: Phytochemical screening revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechin tannins, saponosides and alkaloids in *L. multiflora* leaves. Administered orally, LiMAE was not toxic. LiMAE did not significantly modify ($p>0.5$) the quantities of food and water consumed by the treated rats in comparison with the controls. Biochemical and hematological parameters were not also influenced by LiMAE-treatment. In contrast, a significant increase in body weight was observed in treated animals compared to controls.

Conclusion: The results of this study showed that *Lippia multiflora* aqueous leaf extract has no apparent toxic effect on hematological and biochemical parameters, weight and food intake in animals. The non-toxicity of the aqueous extract and the chemical compounds highlighted in the leaves would partly justify the folkloric use of this plant to treat various diseases.

INTRODUCTION

Plants are the main sources of therapeutic preparations in Africa[1]. They are used by more than 80% of populations[2] . However, their empirical use raises real concerns[3]. The prescription and use of traditional medicines are not regulated. And because of this, patients are more exposed to risks of intoxication[4]. Authors revealed that the use of plants is not always safe for the populations. This traditional use of plants exposes people to real risks of therapeutic accidents that can sometimes be tragic. Indeed, it has been shown that some medicinal plants are potentially toxic[5]. Pharmacological and toxicological studies must be done to better use of African pharmacopoeia plants[6].

Lippia multiflora is one of the medicinal plants, commonly called "Tea of Gambia". *Lippia multiflora* is a plant that grows spontaneously in the savanna zones of West and Central Africa. It is used by people in sub-saharan Africa for its pesticidal, medicinal and nutritional properties. *L. multiflora* possesses fatigue relieving and diuretic properties[7].

Its leaves are softened over low heat to relieve stress and improve sleep[8]. Leaf infusion serves as a drink to fight fever, enteritis, cough and flu[7]. *L. multiflora* is also used in many other traditional medicines to treat bronchial inflammation, gastrointestinal disorders and colds[9]. In Côte d'Ivoire, this plant is commonly used. It is marketed in the form of instant tea and packaged leaves are exported. This makes *L. multoiflora* an export crop[10,11]. Thus, it is clear that knowledge of the doses, biochemical, pharmacological and toxicological properties of *L. multiflora* is important for its rational and efficient use.

Many scientific works have been carried out on the pharmacological properties and toxicity of this medicinal plant in order to verify the therapeutic virtues advanced by populations. They demonstrated the antihypertensive[12], hypotensive[12,13], cardio-moderator[14,15], hepatoprotective[16,17] and psychotropic effects of leaf extract of *L. multiflora*. Analgesic, antipyretic, tranquilizers and anti-inflammatory[18], antibacterial[19,20], antifungal[19], ovicidal and larvicidal[21] effects of the essential oil of the dry leaves of this plant have demonstrated. *Lippia multiflora* is known to have a good antioxidant activity[22,23]. *Lippia multiflora* is also used to treat many diseases[24]. These pharmacological properties mentioned would be subtended by the secondary metabolites (flavonoids, leucoanthocyanins, steroids or terpenoids, tannins, alkaloids) and the volatile compounds contained in the leaves of this plant species[8,25]. Finally, Hondi-Assah[26] showed that *L. multiflora* aqueous leaf extract is well tolerated in rats.

Many studies have already been done on the biological properties of *L. multiflora*. However, Soro[20] encouraged further research on these pharmacological properties and justified the chemical profiles of its leaves. Study of three samples from three distinct regions showed a variation in the chemical profile. This variation of chemical profile could therefore have an influence on the biological properties in general and pharmacological in particular.

The present study, in the general theme of the safety of the use of medicinal plants, concerns the safety of *L. multiflora* harvested in one of the regions of Côte d'Ivoire. More specifically, the aim was to evaluate the acute and sub-acute toxicity of the aqueous leaf extract respectively in Swiss mice and Wistar rats. Also, toxicology tests and the examination of biochemical and hematological parameters of the animals treated were made.

MATERIALS AND METHODS

Study period: This study took place over a period of 3 months. It started in mid February 2018 with the collection of fresh leaves of plant. It ended in mid-May after the hematological and biochemical assays.

Plant material and extraction: Fresh leaves of *L. multiflora* (Verbenaceae) were harvested in February 2018 in Assouvoué, village of Toumodi Commune (Région du Bélier, Côte d'Ivoire). The fresh leaves were identified and authenticated by a Botany expert, Dr ASSI Rose-Monde of the "Centre National de Floristique", UFR-Biosciences, Université Félix HOUPHOUËT-BOIGNY (Abidjan, Côte d'Ivoire). The plant extract was obtained according to the method used by Nene-Bi *et al.*[27] and Koko *et al.*[28]. The fresh leaves were washed and dried at room temperature (28±2°C). Dried leaves were pulverized to powder with the use of a laboratory blender-About 100 g of powder were macerated during 24 hrs in distilled water (1L), thereafter filtered. An oven at a temperature of 50°C was used to concentrate the filtrate and the extract obtained (LiMAE) was re-dissolved extemporaneously in normal saline (NaCl 0.9%) for the experiment[29].

Experimental animals: About 4 female Swiss mice (19-25 g) aged 12 weeks and 18 Wistar rats (*Rattus norvegicus*) weighing 200-250 g and aged 8-12-week-old were used for the acute and subacute oral toxicity tests, respectively. They were purchased from the animal house of UGRA-IPCI (Unité de Gestion des Ressources Animales, Institut Pasteur de Côte d'Ivoire). Animals were allowed to acclimatize for 2 weeks before being used for the experiment. They were kept in temperature controlled environment (25±2°C) with a 12 hrs light-dark cycle and were allowed free access to water and feed *ad libitum* throughout the acclimation period. Experimental procedures and protocols used in this study were in accordance with the guide for the care and use of laboratory procedures animals (European Council Legislation 87/607/EEC for the protection of experimental animals).

Phytochemical analysis: Five grams of *L. multiflora* powder were added to boiling water (50 mL) and allowed to infuse for 15 min. The mixture was filtered. The filtrate was subjected to phytochemical screening for the presence of alkaloids, flavonoids, tannins, sterols, polyterpenes, saponins, and quinones using standard procedures[30].

Acute toxicity study: The LD_{50} determination was conducted in mice using the limit test at 3000 mg kg^{-1} b.w. of the OECD 425 protocol based on the "dose adjustment" method[31]. The limit test at 3000 mg kg^{-1} b.w. was performed after the preliminary test at 1000 mg kg^{-1} b.w. Four mice (M_1, M_2, M_3, M_4) were used separately at intervals of 48 h. Before oral administration of LiMAE (2 mL/100 g b.w.), each mouse was starved but received water for 3 hrs, then weighed. After administration of the test substance, the animals were also starved for 1 h. The treated animals were observed for 14 days.

Sub-acute toxicity study: The experiment was conducted according to OECD 423 guidelines[32]. Eighteen female rats were distributed randomly into six groups. Three animals were placed in each group. Group 1 served as normal control, while groups 2, 3, 4, 5 and 6 served as test animals. Group 1 received distilled water. The other groups (2, 3, 4, 5, 6) were treated with LiMAE at 200, 400, 600, 800 and 1000 mg kg^{-1} b.w. respectively. A volume of plant extract (2 mL/100 g b.w.) was administered daily to the animals in a single dose for 28 days. Clinical signs and toxicity were observed daily in all animals, before administration, immediately after administration and 3h after administration of LiMAE. Rats are observed daily to record any apparent physiological and/or behavioral changes. Consumptions of water and food were determined daily. Animals are also weighed weekly to appreciate the impact of LiMAE on weight gain.

Blood collection: After 28 days administration of LiMAE to the experimental animals, they were starved overnight, anaesthetized with Isoflurane (Forene®) and sacrificed. A thoracotomy was performed. Blood samples were collected from the animals through cardiac puncture.

Hematological studies: Eighteen blood samples were collected in Ethylene Diamine Tetra-Acetic acid (EDTA) coated bottles**.** Samples were analyzed for the assessment of the number of Red Blood Cells (RBCs), White Blood Cells (WBCs), platelets, lymphocytes, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) according to standard methods using the HITACHI® 704R auto-analyser.

Biochemical estimation: Eighteen blood samples collected in non-heparinized tubes were allowed to clot for about 15 min and centrifuged at 3000 rpm for 5 min. Serum was separated from the clot with pasteur pipette and dispensed into clean tube for the measurement of the biochemical indices. Analysis of the selected serum biochemical indices were carried out on each sample. Except glycemia, all parameters were measured using Chemistry Analyzer (Humanlyser 2000 auto-analyser). Glycemia was determined at the time of blood collection using the glucose oxidase method[33]. An Accu-Chek® Active glucometer was used for this purpose.

Statistical analysis: All the data were expressed as mean±S.E.M (standard error of means). Statistical analyses were performed by one way analysis of variance ANOVA and differences between means were determined by Turkey's Multiple Comparison test using GraphPad Prism 5.0 program.

RESULTS

Phytochemical screening: Table 1 summarizes the results of different tests used to characterize the chemical groups present in the *L. multiflora* aqueous leaf extract. According to these results, it contains sterols, polyterpenes, polyphenols, flavonoids, catechin tannins, saponosides and alkaloids.

Acute toxicity: No signs of toxicity were observed in mice after administration of LiMAE at 3000 mg kg^{-1} b.w. All animals survived after 14 days of observation (Table 2). This showed that the LD_{50} of LiMAE is greater than 3000 mg kg^{-1} b.w.

Sub-acute toxicity: In terms of subacute toxicity, there was no change in the general appearance of rats (hair, skin, eyes, ears and mouth). The animals had no diarrhea, hematuria, uncoordinated movements, or respiratory distress during the study period. The rats of the control groups are much more alert and vigilant. No symptoms of illness were observed during the 28 days of treatment. However, 10-15 min after administration of LiMAE, the rats lengthened and slept 30-60 min.

Food and water intake have not been significantly modified (Table 3). Controls consumed 2521.68±0.03 g of food during the 28 days of experimentation. In rats treated with LiMAE 200 and 1000 mg kg^{-1} b.w., the quantity of food ingested was estimated at 2481.36±4.56 g and 2550.24±0.03 g, respectively. Food quantities ingested by the treated rats were not statistically different from that of the controls at $p>0.05$. Regarding the water consumption by animals, slight decreases were recorded

Table 1: Phytochemical screening of *Lippia multiflora* leaves

Chemical Groups		*Lippia multiflora extracts*
Sterols and polyterpenes		+
Polyphenols		+
Flavonoids		+
Tannins	Galic	-
	Catechin	+
Quinones		-
Alkaloids	D	+
	B	+
Saponins		+

- : absence; +: presence; D: Dragendorff; B: Bouchardat

Table 2: Acute toxicity of *L. multiflora* leaves aqueous extract at 3000 mg kg^{-1}b.w. in Swiss mice

	Mortality	
Mices treated with LiMAE 3000 mg kg^{-1} b.w.	24 hrs (Day 0 – Day 2)	Day 3 – Day 28
M_1	No	No
M_2	No	No
M_3	No	No
M_4	No	No

Sub-acute toxicity: In terms of subacute toxicity, there was n

Table 3: Effect of LiMAE-treatment on food and water quantities consumed by Wistar rats

	Total quantities ingested by rats during the 28 days of experimentation	
Treatment of animals	Water (mL)	Aliments (g)
Control	2367.96±5.28[a]	2521.68±0.03[a]
LiMAE 200 mg kg^{-1} b.w.	2179.80±9.66[b]	2481.36±4.56[a]
LiMAE 400 mg kg^{-1} b.w.	2380±5.88[a]	2497.88±2.40[a]
LiMAE 600 mg kg^{-1} b.w.	2212±13.01[b]	2524.2±0.02[a]
LiMAE 800 mg kg^{-1} b.w.	2347.80±5.24[a]	2523.64±0.01[a]
LiMAE 1000 mg kg^{-1} b.w.	2464±5.88[a]	2550.24±0.03[a]

LiMAE: *L. multiflora* aqueous extract. The values of weight are expressed as Mean±S.E.M for three rats (n = 3). In the same column values, same letters are not significantly different (p>0.05)

at doses of 200 and 800 mg kg^{-1} b.w. compared to controls. Water quantity consumed by the control rats was 2367.96±5.28 mL. Rats treated with LiMAE 200 mg kg^{-1} b.w. consumed 2179.80±9.66 mL of water. With LiMAE 1000 mg kg^{-1} b.w., a water quantity of 2464±5.88 mL was recorded.

Lippia multiflora aqueous extract (LiMAE, 200-1000 mg kg^{-1} b.w.) did not negatively affect the weight of the treated animals. On the contrary, a positive trend in body weight was recorded (Table 4). Animal weight increased in a dose-dependent manner after administration of LiMAE. The weight of controls increased from 225.68±0.00 g (Day 0) to 232.88±0.54 g (Day 28). When rats were treated with LiMAE 200 mg kg^{-1} b.w., the initial weight of 225.38±0.27 g (Day 0) was increased to 255.70±2.17 g (Day 28). The increase in weight was greater when the high dose of LiMAE 1000 mg kg^{-1} b.w. was administered to the rats. The initial weight of the animals weight (225.38±0.27 g) was estimated at 336.18±54.81 g at day 28. These values correspond to respective significant increases of 2.92% ($p<0.05$), 13.45% ($p<0.0$ 1) and 49.16% ($p<0.001$). Weights of LiMAE treated-animals were greater than that of controls throughout the experiment.

Hematological and biochemical studies: The results on the hematological parameters have been recorded in Table 5. Data shows that LiMAE did not cause any change in the hematological parameters studied. Its administration did not modify the hematogram of the treated animals at $p>0.05$. Similar results have been obtained for blood biochemical parameters. In comparison with the values measured in the control rats, the urea and creatinine concentrations in treated animals did not vary significantly at $p>0.05$. It was the same for transaminases (glutamic transaminases, oxaloacetic and pyruvic). Glucose and total protein levels were not disturbed at $p>0.05$ and variations in triglyceride and total cholesterol levels were also not significant (Table 6).

Table 4: Body weight variation in LiMAE-treated Wistar rats

	Body weight of rats (g)				
Treatment of animals	Day 0	Day 7	Day 14	Day 21	Day 28
Control	225.68±0.00[a]	226.40±0.05[a]	228.30±0.60[a]	229.26±0.57[a]	232.88±0.54[a]
LiMAE 200 mg kg^{-1} b.w.	225.38±0.27[a]	227.20± 1.10[a]	235.52±5.09[a]	243.74±2.67[c]	255.70±2.17[a]
LiMAE 400 mg kg^{-1} b.w.	225.38±0.27[a]	229.45±0.79[ab]	242.28±7.55[ab]	249.93±0.94[d]	265.78±1.43[a]
LiMAE 600 mg kg^{-1} b.w.	225.38±0.27[a]	233.90±2.60[b]	250.45±7.97[b]	263.63±0.83[a]	271.89±2.96[a]
LiMAE 800 mg kg^{-1} b.w.	225.38±0.27[a]	242.10±2.82[c]	252.87±3.92[b]	261.84±2.04[a]	269.27±1.85[a]
LiMAE 1000 mg kg^{-1} b.w.	225.38±0.27[a]	255.07±2.70[d]	270.57±0.81[c]	293.10±1.47[e]	336.18±54.81[b]

LiMAE: *L. multiflora* aqueous extract. The values of weight are expressed as Mean±S.E.M for three rats (n = 3). In the same line values, same letters are not significantly different (p>0.05)

Table 5: Hematological indices in LiMAE-treated Wistar rats at day 28

	Treatment of animals					
Parameters	Control	LiMAE 200 mg kg^{-1} b.w.	LiMAE 400 mg kg^{-1} b.w.	LiMAE 600 mg kg^{-1} b.w.	LiMAE 800 mg kg^{-1} b.w.	LiMAE 1000 mg kg^{-1} b.w.
WBCs (x10^6/mm^3)	4.59±0.04^{a}	4.74±0.03^{ab}	5.06±0.07^{ab}	4.75±0.67^{ab}	5.45±0.02^{ab}	5.75±0.12^{b}
RBCs (x10^6/mm^3)	6.92±0.07^{a}	6.72±0.28^{a}	6.89±0.02^{a}	7.01±0.03^{a}	6.69±0.16^{a}	6.91±0.09^{a}
Hemoglobin (g/dL)	10.69±0.49^{b}	12.78±0.50^{a}	12.71±0.09^{a}	13.02±0.056^{a}	13.20±0.084^{a}	13.57±0.14^{a}
Platelets (x10^6/mm^3)	223.00±7.07^{c}	233.00±5.65^{cd}	251.50±3.53^{d}	259.00±2.82^{ab}	264.50±3.53^{ab}	273.50±6.36^{b}
Lymphocytes (%)	77.34±6.78^{ab}	66.42±4.87^{b}	82.72±0.36^{a}	81.69±0.63^{a}	83.35±0.86^{a}	79.53±0.15^{ab}
Hematocrit (%)	29.73±3.40^{a}	28.99±0.48^{a}	31.29±0.48^{a}	36.84±1.83^{ab}	45.14±3.67^{bc}	50.38±1.04^{c}
MCV (μ^3)	56.39±1.02^{a}	57.29±2.58^{a}	57.23±0.35^{a}	57.89±0.34^{a}	57.22±0.36^{a}	54.60±1.35^{a}
MCHC (%)	23.12±0.40^{a}	23.26±0.55^{a}	22.26±0.86^{a}	26.14±3.53^{a}	27.79±1.87^{a}	25.43±2.67^{a}

LiMAE: *L. multiflora* aqueous extract; WBCs: White blood cells; RBCs: Red blood cells; MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration. The values of weight are expressed as Mean±S.E.M for three rats (n = 3). In the same column values, same letters are not significantly different (p>0.05)

Table 6: Serum biochemical levels in LiMAE-treated Wistar rats at day 28

	Treatment of animals					
Parameters	Control	LiMAE 200 mg kg^{-1} b.w.	LiMAE 400 mg kg^{-1} b.w.	LiMAE 600 mg kg^{-1} b.w.	LiMAE 800 mg kg^{-1} b.w.	LiMAE 1000 mg kg^{-1} b.w.
Glyc (g L^{-1})	0.90±0.03^{e}	0.80±0.03^{d}	0.63±0.01^{c}	0.56±0.03^{bc}	0.50±0.01^{ab}	0.43±0.01^{a}
Urea (mg L^{-1})	0.31±0.04^{ab}	0.29±0.04^{ab}	0.26±0.01^{ab}	0.22±0.01^{a}	0.35±0.035^{b}	0.31±0.02^{ab}
Crea	12.94±0.42^{a}	12.67±0.43^{a}	12.90±1.06^{a}	12.52±0.06^{a}	12.60±0.04^{a}	12.83±0.20^{a}
AST (IU L^{-1})	78.85±3.49^{a}	77.81±1.18^{a}	77.15±2.12^{a}	79.87±0.37^{a}	73.75±2.68^{a}	81.18±2.071^{a}
ALT (IU L^{-1})	33.89±3.42^{a}	39.42±2.66^{a}	37.23±0.35^{a}	42.11±1.08^{a}	37.18±1.32^{a}	33.71±2.22^{a}
ALP (IU L^{-1})	159.30±2.80^{a}	158.39±2.75^{a}	151.07±0.28^{a}	156.80±1.66^{a}	158.30±0.93^{a}	155.52±4.02^{a}
LDL-C (mg dL^{-1})	43.51±3.06^{a}	42.17±0.27^{a}	43.97±3.67^{a}	39.95±0.58^{a}	42.95±2.40^{a}	41.65±1.44^{a}
HDL-C (mg dL^{-1})	71.51±1.17^{a}	68.86±0.169^{a}	70.50±1.49^{a}	70.96±0.39^{a}	71.01±0.94^{a}	72.44±0.70^{a}
Chol-T (mg dL^{-1})	115.82±4.44^{a}	111.01±0.94^{a}	121.48±4.01^{a}	114.04±2.2^{a}	120.78±1.69^{a}	112.87±0.47^{a}
P.T	69.88±2.49^{a}	71.51±1.65^{a}	65.65±0.46^{a}	64.74±3.95^{a}	67.92±5.73^{a}	67.09±1.61^{a}
TG (mg dL^{-1})	55.35±2.27^{a}	54.39±3.85^{a}	56.25±0.87^{a}	49.36±1.47^{a}	53.06±1.76^{a}	53.30±2.92^{a}
CPK (IU L^{-1})	152.37±4.28^{a}	151.30±4.18^{a}	153.53±2.99^{a}	155.31±3.76^{a}	149.41±1.07^{a}	151±2.12^{a}

LiMAE: *L. multiflora* aqueous extract; Gly : Glycemia ; AST: Aspartate aminotransferase ; ALT: Alanine transaminase ; ALP: Alkaline phosphatase; LDL-C: Light density lipoprotein cholesterols ; HDL-C: High density lipoprotein cholesterols ; Chol-T: Total cholesterol ; TG: Triglycerides ; CPK: Phosphokinase creatine; PT: Prothrombin time; Crea: Creatinine. The values of weight are expressed as Mean±S.E.M for three rats (n = 3). In the same column values, same letters are not significantly different (p>0.05)

DISCUSSION

The aqueous extract of *L. multiflora* was tolerated by animals. All mice treated with single doses of LiMAE survived after 14 days of observation. No change in behavior of the animals was recorded. Daily treatment of rats for 28 days did not cause death and animals showed no clinical sign of suffering. LiMAE is a non-toxic substance. These results are in agreement with those of previous works[5,13,16,24,34]. In Mali, researchers have used the leaves of this plant as tea for more than 25 years without observing adverse side effects[5]. Pelissier et *al.*[34] conducted a study on 50 people who consume the leaves of this plant. No side effects and no toxicity were recorded Authors concluded that consumption of *L. multiflora* would be safe for consumers and non-toxicity of this vegetable will allow to promote their use in food for nutritional purposes and in traditional medicine for the therapeutic properties. Hondi-Assah *et al.*[16] and Gandonou *et al.*[24] also showed that the aqueous extract of *L. multiflora* is not toxic in mammals. Djengue *et al.*[13] have obtained the same results with samples collected in six regions of Benin.

Observations of present study on toxicity are consistent with results from blood samples taken at the end of treatment in rats (Day 28). Examination of hematological and biochemical blood parameters did not reveal any significant difference between LiMAE-treated and control groups. This observation suggested that *L. multiflora* did not change the levels of the hematological and biochemical parameters studied ($p>0.05$). These results are different from those of Bouagnon *et al.*[17]. Levels of RBCs and platelets counts were increased while those of ALT, AST, CGT, WBCs and MCV were decreased in treated rats compared with ethanol intoxicated animals. This difference would be related to the health status of animals used. This research was conducted on healthy rats while Bouagnon *et al.*[17] used animals with ethanol (15%)-induced liver toxicity. However, these authors have shown that variations in these parameters allowed to correct the anomalies caused by intoxication with ethanol 15%. *Lippia multiflora* has a hepatoprotective effect.

An increasing evolution of body weight was observed in the treated rats. Bouagnon *et al.*[17], for their part, did not observe a significant change between the weights of the animals treated with the aqueous extract of *L. multiflora* and those of the untreated intoxicated rats (Controls). This positive evolution of body weight showed that the rats supported the different doses of the plant extract. LiMAE did not affect the health of the animals. According to many authors, the increasing body weight of treated rats is a good indicator of their physical health, while weight loss is the first indicator of toxicity. However, present results corroborate those of Mpofu *et al.*[35] and Matshogo *et al.*[36] They incorporated *Lippia javanica* of Verbenaceae family in the diet of broilers. Mpofu *et al.*[35] showed that the use of *L. javanica* at 5 g kg^{-1} b.w. in the diet had positive effects on growth performance and carcass characteristics of broilers.

The increase in chicken weight would result from an adaptive mechanism to manage increasing amounts of fiber and phytochemicals that ultimately optimize digestion and uptake[35]. Also, the weight gain of the treated rats could be attributed to the good digestibility of the foods in the treated rats. LiMAE would promote digestibility following analysis of food and water quantities consumed by rats. Indeed, the comparison of the quantities showed that there was no significant difference ($p>0.05$) between the quantities (food and water) taken by the control rats and those of the rats which received the increasing doses of LiMAE. Thus, it is clear that the treated rats better potentiated the food they ingested. This would justify the dose-dependent increase in weight gain of treated rats compared to control animals. These results are consistent with those of Ekissi *et al.*[37]. According to these authors, *L. multiflora* (savanna tea) would facilitate digestion, increase appetite and stimulate urinary secretion.

Used as tea, *L. multiflora* would not be slimming. LiMAE effect on animal weight is contrary to that of slimming teas. These promote weight loss and are recommended for slimming diets. Their consumption is often accompanied by quantitative changes in food intake and a decrease of food digestibility[38]. Decrease in the absorption of nutrients and increase energy expenditure may contribute to slimming effects of green teas[39,40]. This is not the case of LiMAE, the aqueous extract of *L. multiflora* (savanna tea).

The effects of *L. multiflora* observed are due to the different chemical compounds contained in the aqueous leaf extract (LiMAE). According to Kapepulaa *et al.*[23], *Lippia multiflora* has various medicinal virtues that can be explained by its phytochemical composition. Phytochemical screening revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechins tannins, saponins and alkaloids. These chemical compounds have also been highlighted by numerous studies[3,23,41]. *Lippia multiflora* contains several phytochemical compounds that are known to have interesting biologic properties such as those obtained in present study[13].

CONCLUSION

The aqueous extract of leaves of *L. multiflora* (savanna tea) is not toxic. Study of hematological and biochemical parameters showed that *L. multiflora* extract is tolerated by animals. The aqueous extract of leaves (LiMAE) would also facilitate the digestion revealed by the increase in body weight of the treated animals which consumed food and water quantities substantially equal to those of the controls.

SIGNIFICANCE STATEMENT

These results militate in favor of its use as slimming tea. The use of sophisticated and more advanced methods for the phytochemical screening and the cytotoxicity tests would be of high important to improve the beneficial effects of *Lippia multiflora* from Toumodi (Région du Bélier, Côte d'Ivoire). This study shows the beneficial effects of *Lippia multiflora* on health. These effects supported by an increase of body weight and not associated with toxicity at very high doses opens an interesting field of research. This study discovers the possible use of *Lippia multiflora* leaves for the preparation of fattening diets and not for slimming diets.

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