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SUBCHRONIC TOXICITY OF TIKONI TEA FROM *VITEX MADIENSIS* OLIV. LEAVES (LAMIACEAE) AQUEOUS EXTRACT IN THE WISTAR RAT

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ABSTRACT: *Vitex madiensis*, consumed daily as a tea named Tikoni, is widely used in traditional medicine in Congo Brazzaville to treat fever, pain, inflammation, malaria, dysentery, female infertility, mental illness and epilepsy. Its aqueous leaf extract does not exhibit acute toxicity. Its pharmacological effects against pain, inflammation, insomnia, oxidative stress, and *Plasmodium falciparum* are very promising. The objective of this study was to investigate the subchronic toxicity of its aqueous extract at a dose of 200 mg/kg orally in Wistar rats over 42 days. Toxicity assessments were conducted on days 14, 28, and 42. The results show that the Tikoni tea aqueous extract does not significantly reduce body weight gain and the growth of certain vital organs. However, liver and lung weights in males, and liver and spleen weights in females, decreased significantly. This extract decreased platelet counts in both sexes and significantly increased leukocyte counts in males. ALT and ALP activities increased significantly in males, while in females, AST, ALT, and ALP activities decreased significantly, and blood glucose levels increased. No significant changes were observed in cholesterol, urea, triglycerides, creatinine, total protein, direct bilirubin, hemoglobin, hematocrit, MCV, MCH, and MCHC levels.

INTRODUCTION: Medicinal plants constitute a group of plants of great socio-economic importance because they contain active components used in the treatment of various diseases. However, studies have shown that not all medicinal plants are safe for direct human use. Although the overall number of deaths due to toxic plants is low, they are considered a significant cause of morbidity and mortality, plant toxicity deserves greater attention [1].

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Studies conducted on approximately 1,500,000 plants have shown that most of these plants contain toxic substances [18]. *Vitex madiensi* Oliv. is pharmacologically effective and widely used in traditional medicine in Congo in particular and in Africa, Asia, and Latin America in general [5]. In Africa, the medicinal properties of the plant are known and vary among different populations [21].

For example, in Cameroon, the roots and aerial parts are used to treat headaches, indigestion, and stomach aches. In the Democratic Republic of Congo, the bark is used to treat hemorrhoids; the leaves for coughs, stomach aches, and the flu; and the entire plant for malaria [20, 25]. In the republic of Congo, the bark pulp and the inner part of the root

bark are used to treat dysentery-like diarrhea, female infertility, and the mentally ill or epileptics, respectively [16]. Some ethnic groups in the Congo drink an aqueous decoction of the bark for spiritual deliverance. The juice of the leaves is used in ear drops to treat ear infections; applied to the gums for toothaches [6]. A decoction or fumigation of these leaves relieves headaches. A cold gargle of the leaf decoction is a good remedy for bleeding after a tooth extraction. An ointment made from the roots of *Vitex madiensis* is used to treat pinworm infections [16]. Phytochemical studies of leaf extracts revealed the presence of tannins, anthocyanins, flavones, saponins, mucilage, sterols, and triterpenes. Pharmacological and toxicological studies conducted by our predecessors on the Congo sample showed that the aqueous extract of *Vitex madiensis* leaves does not exhibit acute or cellular toxicity in rodents and displays interesting analgesic, anti inflammatory, sedative, and antioxidant activities [24, 28]. An absence of cytotoxicity in human cells and a promising antiplasmodial effect were demonstrated in the Gabonese sample in the Central African sub-region [32]. Despite its medicinal properties, it is also consumed daily as tea by some farmers [24]. To determine whether the abundant and continuous use of this species by local populations has long term consequences, this study aimed to investigate chronic toxicity in rats.

MATERIALS AND METHODS:

Tikoni Tea Preparation: Tikoni tea is made with the leaves of *Vitex madiensis* Oliv. (Lamiaceae). Plant material were collected in the savanna of south of Brazzaville in Ngangalingolo in May 2019. Botanical identification was carried out by Professor Jean Marie MOUTSAMBOTE of the National Institute for Research in Exact and Natural Sciences (IRSEN), and a specimen was deposited in the National Herbarium and registered under number 7658 [25]. The plant leaves were dried for 7 days at room temperature, approximately 28°C, in the dark. The dried plant material was ground using an IKA-WERKE GmbH-CO-KG, D-79219, Staufen, machine equipped with a 0.25 mm sieve. The obtained powder stocked in a bottle hermatically closed is Tikoni tea.

Animal Material: The animal material consisted of 48 male and female Wistar rats weighing between 150 and 250 g, provided by the animal facility of the National Institute of Health Sciences Research (IRSSA) in Brazzaville, Republic of Congo. These animals were kept under standard conditions (12 hours of light and 12 hours of darkness at an ambient temperature of 26 ± 1°C) and had free access to standard food and drinking water.

Tikoni Tea's Aqueous Extract Preparation: One hundred (100) g of Tikoni mass were placed in a glass flask containing 1000 mL of distilled water and heated to a boil for thirty (30) minutes on a heating mantle at a temperature of 100°C. The decoction was filtered through absorbent cotton. The resulting filtrate was then placed in an oven at a reduced temperature of 60°C. The dry extract obtained was used to prepare the test solution.

Dose Selection: A dose of 200 mg/kg was chosen based on previous studies [27].

Subchronic Toxicity Study: For the determination of subchronic toxicity, the experimental protocol used was that described by Nsonde Ntandou *et al.* (2015) [27, 29].

Grouping of Mice [43]: 48 rats, male and female were divided into 4 groups of 12 rats each. Groups 1 and 3 (male and female) served as the control group and received distilled water at a dose of 10 mL/kg orally. Groups 2 and 4 (male and female) were treated with an aqueous extract of Tikoni tea at a dose of 200 mg/kg orally. Treatments were administered daily at regular intervals until the evaluation days.

Measurement of Water intake and Food Consumption [31]: The volume of water and the amount of food consumed were measured daily for 42 days by weighing the food remaining in the trough and the volume of water remaining in the water bottles.

Study of Rat Weight Gain: The body weight of the rats used in this study was measured before administration of the extract and then every 2 days after administration.

Evaluation of Hematological and Biochemical Parameters [28, 45]: Toxicity assessments were performed on days 14, 28, and 42. Rats were fasted

overnight before each assessment. On day 0 (before treatment) and on days 14, 28, and 42 (after treatment with Tikoni tea aqueous extract), four rats from each group were anesthetized with ether. Blood was then collected from the jugular vein using EDTA and dry tubes for hematological and biochemical testing respectively. Blood samples collected in tubes containing an anticoagulant (EDTA) were immediately used to determine white blood cell count, red blood cell count, hematocrit, hemoglobin, MCV, MCHC, MCHC, and platelet count. Blood samples in dry tubes were centrifuged at 4000 rpm for 30 minutes after coagulation to separate the serum from the red blood cell pellet. The decanted serum was used for the analysis of direct bilirubin, alkaline phosphatase (ALP), transaminases (ALT; AST), urea, creatinine, triglycerides, total protein, cholesterol, and blood glucose. The measurement of the enzymatic activity of the two transaminases was based on the kinetic enzyme method.

A colorimetric kinetic assay, in accordance with the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine), was used to analyze phosphatase activity. Bilirubin, in the presence of diazero sulfanilic acid and in an alkaline medium, forms a red colored compound. Of the two dyes presented in serum, bilirubin gucuromide and free bilirubin loosely bound to albumin, only bilirubin-gucuromide reacts directly in an aqueous solution (direct bilirubin). Creatinine forms a red complex in a basic picrate solution. The absorbance, at predetermined times during the conversion is proportional to the creatinine concentration in the sample. Triglycerides are enzymatically hydrolyzed into glycerol and free fatty acids.

The released glycerol is first phosphorylated by glycerol kinase and then oxidized by glycerol-3 phosphate oxidase, releasing an equivalent amount of hydrogen peroxide (H_2O_2). The H_2O_2 participates in a Trinder reaction, which leads to the formation of a red quinoneimine dye. The intensity of the color formed is proportional to the triglyceride concentration in the sample. In the presence of glucose oxidase (GOD), β-D-glucose is oxidized to gluconic acid and peroxide. Since glucose exists in both α and β forms in solution, complete conversion of glucose requires mutarotation of α-D-glucose to β-D glucose. This latter reaction is accelerated in the presence of the mutarotase enzyme (MRO). After glucose oxidation, the hydrogen peroxide (H_2O_2) formed is measured by oxidative coupling of 4 aminopantipyrine (AAP) to 4-chlorophenol in the presence of peroxidase (POD), yielding a quinonimine red dye. Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyzes the esters, and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol oxidase according to the following equations. In the last reaction, a red quinoneimine dye is formed, the intensity of which is proportional to the concentration of cholesterol in the sample. Urea is hydrolyzed to ammonia ($NH4+$) and CO_2. Ammonia reacts with α-ketoglutarate to form L-glutamate and NAD+ from NADH7. This reaction is catalyzed by glutamate dehydrogenase (GLDH). The decrease in NADH is proportional to the urea concentration. Proteins in a basic copper sulfate medium containing tartate (biuret reagent) form a complex colored blue-violet. The intensity of the color formed is proportional to the total protein concentration in the sample.

Organ Collection**:** After blood sampling at each evaluation, the rats were subsequently sacrificed. The vital organs (liver, heart, lungs, spleen, and kidneys) were collected, weighed, and macroscopically examined. We looked for changes in size, color, and shape, as well as the presence of malformations in the outer layer of the skin [10].

Statistical Analyses: The values presented in the tables correspond to calculations expressed as means plus or minus (±) standard error (MSE). Means were compared using Student's t-test. The significance level was set at $p < 0.05$. The various statistical analyses of the results were performed using Excel software.

RESULTS:

Effect of Tikoni Tea's Aqueous Extract on Body Weight Gain: The subacute toxicity study yielded results shown in **Fig. 1.** This figure illustrates the effect of the extract on the weight gain of male and female rats after 42 days of treatment with a single dose of 200 mg/kg and distilled water. A non-significant decrease in body weight was observed in both males and females of the animals treated

with the aqueous extract compared to the controls treated with distilled water. On day 28, a highly significant decrease ($p < 0.001$) in weight was observed in males, and a significant decrease ($p < 0.05$) in females.

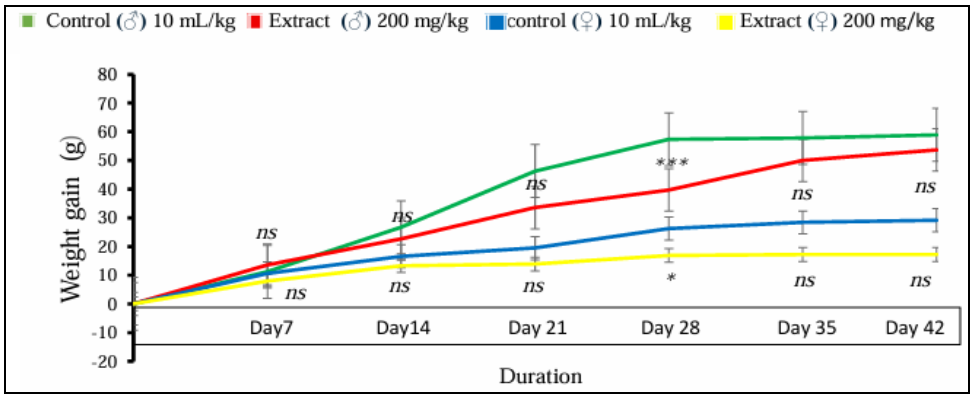

FIG. 1: EFFECT OF AQUEOUS EXTRACT OF TIKONI TEA ON BODY WEIGHT GAIN IN WISTAR RATS. Results are expressed as mean ± standard error, n = 4 rats per group. *p < 0.05; ***p < 0.001 significant difference compared to controls.

Cumulative Effect of the Aqueous Extract on Food Intake: The **Fig. 2** shows the cumulative effect of the aqueous extract of Tikoni tea on the food intake in rats (males and females) subjected to subchronic treatment of 200 mg/kg for 42 days. A decrease in food consumption is observed in the treated rats (males and females) compared to the control group (males and females).

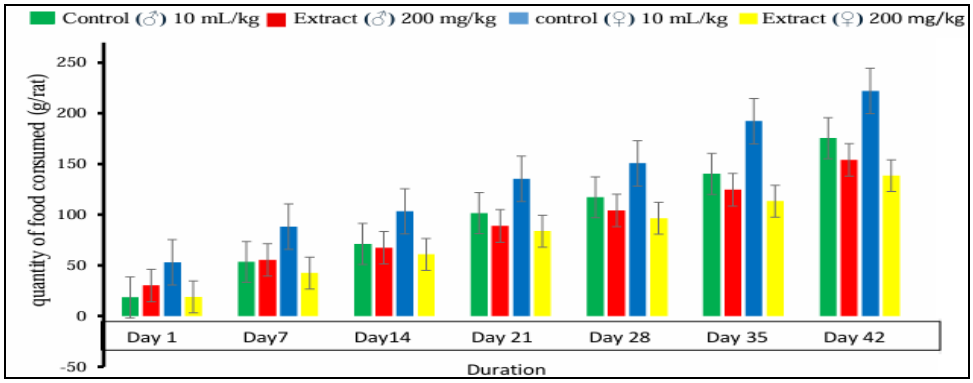

FIG. 2: CUMULATIVE EFFECT OF FOOD INTAKE IN TREATED RATS (MALE AND FEMALE); N = 4 RATS PER GROUP. Results are expressed as mean ± standard error, n = 4 rats per group.

Cumulative Effect of the Aqueous Extract of Tikoni Tea on Water Intake: The **Fig. 3** shows the cumulative effect of the aqueous extract of Tikoni tea on the water intake of rats (males and females) subjected to subchronic treatment with 200 mg/kg for 42 days. A decrease in water intake is observed in the treated rats (males and females) compared to the control group (males and females).

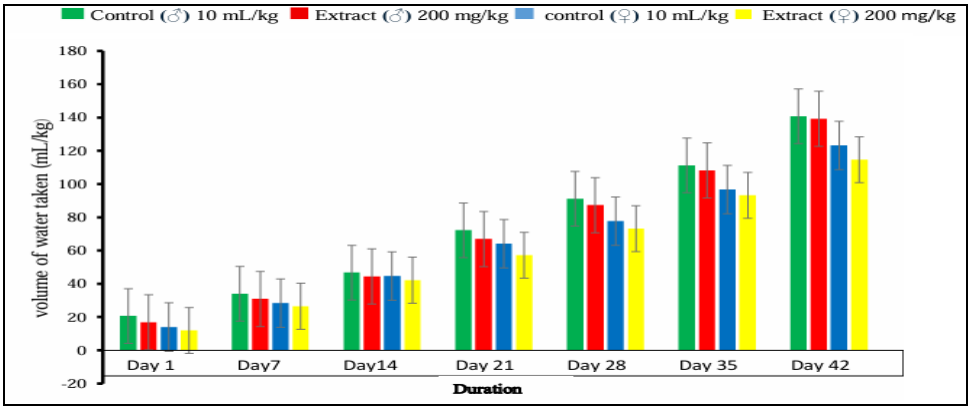

FIG. 3: CUMULATIVE EFFECT OF WATER INTAKE IN CONTROL (MALES AND FEMALES) AND TREATED (MALES AND FEMALES) RATS, N = 4 RATS PER GROUP. (E.D. M) DISTILLED WATER (MALE), (EXT. AQ. M) AQUEOUS EXTRACT (MALE), (E.D. F) DISTILLED WATER (FEMALE), (EXT. AQ. F) AQUEOUS EXTRACT (FEMALE)

Effect of the Aqueous Extract of Tikoni Tea on Organ Weight: Table 1 shows the effect of the aqueous extract of Tikoni tea on the organ weight of male rats subjected to subchronic treatment with 200 mg/kg for 42 days. A significant decrease ($p<0.01$) in liver weight was observed after 42 days of treatment, and a significant decrease ($p<0.05$) in lung weight was observed after 14, 28, and 42 days of treatment, respectively, compared to controls. Results are expressed as mean ± standard error, n = 4 rats per batch, significant for *$p < 0.05$; **$p < 0.01$; ns: not significant compared to control rat.

TABLE 1: EFFECT OF TIKONI TEA'S AQUEOUS EXTRACT ON ORGAN WEIGHT IN MALE RATS

Organs	Day 0	Products	Day 14	Day 28	Day 42
		Control	6,93±0,05	7,51±0,05	7,52±0,03
Liver	8,61±0,26				
		Extract	6,33±0,10ns	7,27±0,10ns	5,52±0,30**
		Control	1,11±0,02	1,03±0,02	1,02±0,02
Heart	1,03±0,02				
		Extract	0,99±0,07ns	0,84±0,003ns	0,95±0,05ns
		Control	1,31±0,17	1,94±0,02	1,31±0,17
lungs	1,50±0,02				
		Extract	0,93±0,01*	1,36±0,06*	0,93±0,005*
		Control	0,48±0,03	0,51±0,005	0,67±0,10
Spleen	0,54±0,04				
		Extract	0,49±0,02ns	0,48±0,03ns	0,40±0,05ns
		Control	0,77±0,01	0,80±0,01	0,72±0,01
Left kidney	0,81±0,03				
		Extract	0,66±0,04ns	0,65±0,01ns	0,61±0,01ns
		Control	0,82±0,01	0,83±0,01	0,73±0,01
Right kidney	0,64±0,008				
		Extract	0,71±0,06ns	0,68±0,006ns	0,64±0,02ns

Subjected to subchronic treatment with 200 mg/kg for 42 days. A significant decrease ($p < 0.001$) in liver weight was observed after 42 days of treatment, and a significant decrease ($p < 0.05$) in spleen weight was observed after 14, 28, and 42 days of treatment compared to controls.

TABLE 2: EFFECT OF AQUEOUS EXTRACT OF ON ORGAN WEIGHT IN FEMALE RATS

Organs	Day 0	Products	Day 14	Day 28	Day 42
		Control	6,04±0,37	6,65±0,37	5,91±0,01
Liver	5,9±0,12				
		Extract	6,75±0,25ns	6,29±0,25ns	4,31±0,01***
		Control	0,99±0,07	0,74±0,02	0,72±0,01
Heart	0,77±0,02				
		Extract	0,91±0,06ns	0,71±0,02ns	0,64±0,01ns
Lungs	1,38±0,09	Control	0,95±0,02	1,33±0,16	0,95±0,02
	Extract		1,09±0,15ns	1,24±0,21ns	1,09±0,15ns
	Control		0,97±0,05	0,91±0,09	0,98±0,09
Spleen	0,5±0,01				
	Extract		0,65±0,03*	0,60±0,06*	0,45±0,04*
	Control		0,65±0,05	0,59±0,005	0,56±0,02
Left kidney	0,44±0,01				
	Extract		0,66±0,09ns	0,52±0,17ns	0,44±0,01ns
	Control		0,63±0,03	0,65±0,02	0,52±0,01
Right kidney	0,48±0,01				
	Extract		0,64±0,06ns	0,55±0,02ns	0,45±0,03ns

Results are expressed as mean ± standard error, n = 4 rats per group, significant for *$p < 0.05$; ***$p < 0.001$; ns : not significant compared to control rats.

Effect of the Aqueous Extract of Tikoni Tea on Organ Morphology: After macroscopic observation of the different organs collected, their sizes, shapes, and colors were found to be normal **Fig. 4 (a, b, c, d).** The liver, heart, spleen, and kidneys appear reddish-brown or burgundy, and the lungs are pale pink with variable typical morphologies.

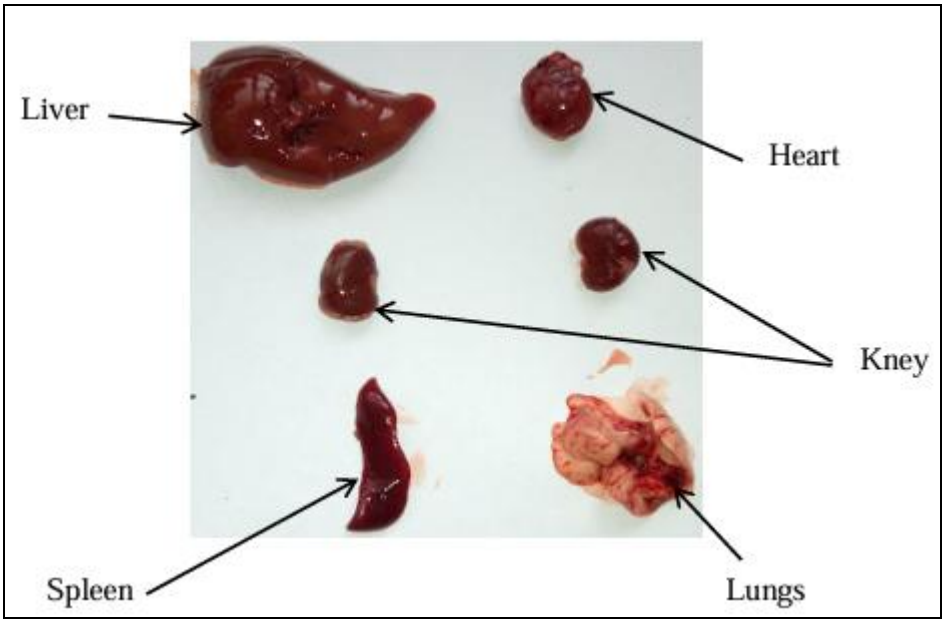

FIG. 4A: PHOTOGRAPH OF THE VITAL ORGANS BEFORE TREATMENT

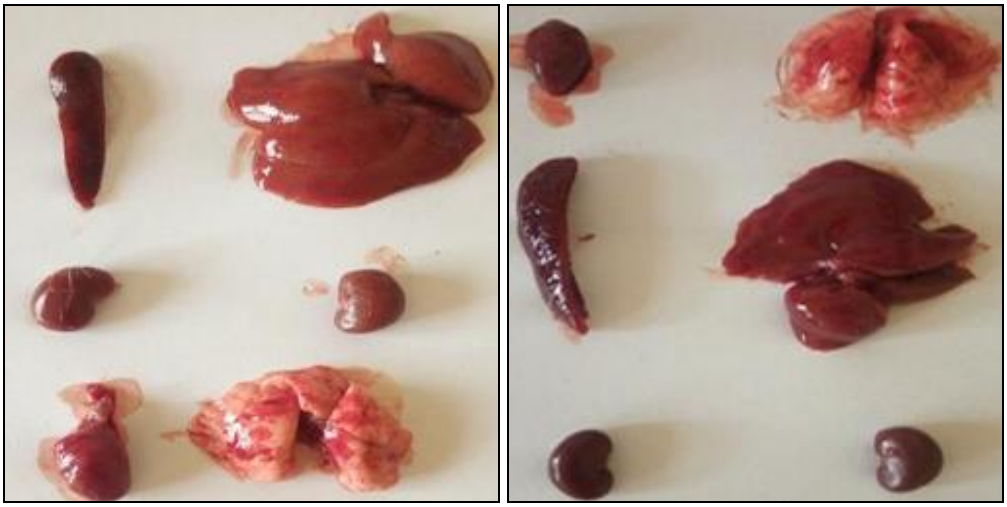

FIG. 4B: PHOTOGRAPH OF THE TREATED ORGANS WITH CONTROL (LEFT) AND AQUEOUS EXTRACT OF TIKONI (RIGHT) AFTER 14 DAYS OF TREATMENT

FIG. 4C: PHOTOGRAPH OF THE TREATED ORGANS WITH CONTROL (LEFT) AND AQUEOUS EXTRACT OF TIKONI (RIGHT) AFTER 28 DAYS OF TREATMENT

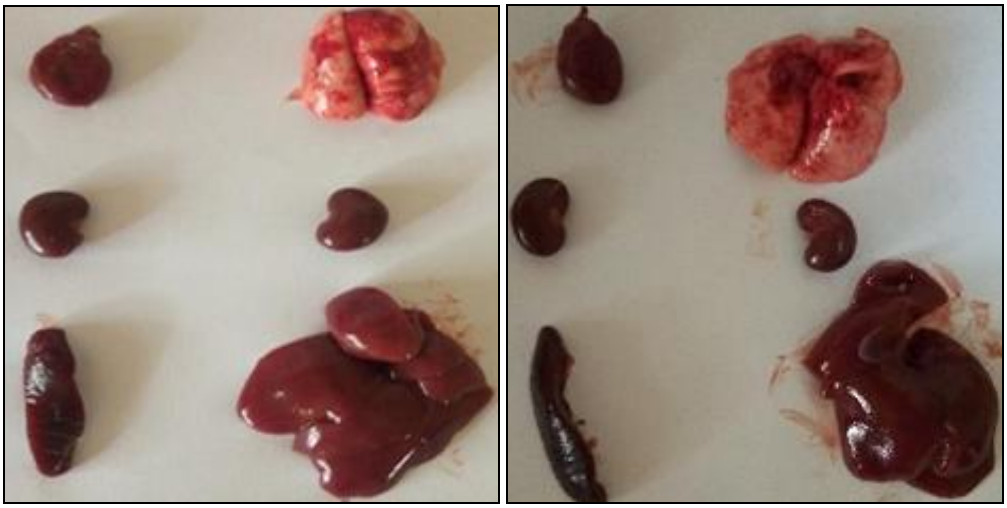

FIG. 4D: PHOTOGRAPH OF THE TREATED ORGANS WITH CONTROL (LEFT) AND AQUEOUS EXTRACT OF TIKONI (RIGHT) AFTER 42 DAYS OF TREATMENT

Effect of the aqueous extract of *V. madiensis* Oliv leaves on hematological parameters The **Table 3** shows the effect of the aqueous extract of Tikoni tea on the hematological parameters of male rats subjected to subchronic treatment with 200 mg/kg for 42 days.

A significant increase ($p < 0.01$) in white blood cell count was observed after 14, 28, and 42 days of treatment, and a decrease in platelet count ($p < 0.01$) was observed after 14, 28, and 42 days of treatment compared to controls 15.86 ± 0.40.

TABLE 3: TIKONI TEA'S AQUEOUS EXTRACT EFFECTS ON HEMATOLOGICAL PARAMETERS IN MALE RATS

HP	Day 0	Products	Day 14	Day 28	Day 42
		Control	8,73±1,42	9,45±1,17	10,72±0,42
WBC 109/l	13,63±1,24				
		Extract	12,17±1,45**	14,56±4,40**	15,86±0,40**
		Control	7,66±0,48	8,56±0,08	8,56±0,01
RBC1012/l	7,67±0,48				
		Extract	7,73±0,43ns	9,18±0,37ns	9,6±0,25ns
		Control	15,1±0,69	16,8±0,17	15,16±0,14
HGBg/dl	14,56±0,87				
		Extract	15,5±0,51ns	16,95±0,66ns	16,53±0,14ns
		Control	48,34±1,88	50,28±0,46	49,27±0,01
HCT %	42,56±1,34				
		Extract	47,96±2,03ns	53,70±1,86ns	54,95±1,18ns
		Control	63,4±1,55	59,5±0,42	57,66±0,33
MCVfl	63,66±1,58				
		Extract	62,2±0,86ns	58,5±0,28ns	57±0,57ns
		Control	17,7±0,17	19,73±0,54	20,15±0,49
MCHpg	20,46±0,57				
		Extract	17,26±0,31ns	18,4±0,57ns	19,8±0,34ns
		Control	30,8±0,28	31,4±0,75	33,63±0,95
MCHCg/dl	32,46±1,42				
		Extract	30,1±0,40ns	31,23±0,26ns	32,33±0,31ns
		Control	821±52,53	886±72,74	934±10,39
PLT.109/l	859,33±74,02				
		Extract	759,6±26,84**	752±93,21**	729±5,77**

(HP) Hematological parameters. Results are expressed as mean ± standard error, n = 4 rats per group, significant for *$p < 0.05$; **$p < 0.01$; ns: not significant compared to control rats.

The **Table 4** shows the effect of the aqueous extract of Tikoni tea on the hematological parameters of female rats subjected to subchronic treatment with 200 mg/kg for 42 days.

A significant decrease in platelets ($p < 0.01$) was observed after 14, 28, and 42 days of treatment compared to controls.

TABLE 4: TIKONI TEA'S AQUEOUS EXTRACT EFFECTS ON HEMATOLOGICAL PARAMETERS IN FEMALE RATS

HP	Day 0	Treatment	Day 14	Day 28	Day 42
		Control	9,87±3,49	10,55±1,07	12,88±0,19
WBC 109/l	11,10±1,19				
		Extract	10,39±3,42ns	10,59±0,32ns	13,89±0,34ns
		Control	6,86±0,64	7,77±0,30	8,42±0,07
RBC1012/l	6,3±0,47				
		Extract	7,38±0,25ns	8,15±0,42ns	8,48±0,10ns
		Control	13,82±0,24	14,06±0,08	14,9±0,15
HGBg/dl	13,03±0,44				
		Extract	14,07±0,36ns	14±0,86ns	14,83±0,06ns
		Control	39,62±0,88	44,35±1,01	47,88±0,22
HCT %	39,90±1,58				
		Extract	44,43±1,86ns	47,85±1,31ns	47,98±0,68ns
		Control	46,33±0,88	60±0,57	62,4±1,27
MCVfl	63,8±2,57				
		Extract	40,6±2,59ns	56,66±1,45ns	61,5±0,28ns
		Control	17,9±0,23	18,93±0,45	21,1±0,28
MCHpg	20,83±0,81				
		Extract	17,63±0,20ns	18,83±0,20ns	20,9±0,34ns
		Control	30,93±0,26	32,1±0,34	32,73±0,37
MCHCg/dl	32,73±0,37				
		Extract	30,73±0,66ns	31,16±0,43ns	31,83±0,20ns
		Control	901,33±49,94	914±12,70	996±46,76
PLT.109/l	850,33±76,17				
		Extract	680±97,28**	777,66±30,31**	780,33±20,49**

HP: Hematological parameters. Results are expressed as mean ± standard error, n= 4 rats per group, significant for *p < 0.05; **p < 0.01; ns: not significant compared to control rats.

Tikoni Tea's Aqueous Extract of on Biochemical Parameters: A **Table 5** shows the effect of the aqueous extract of Tikoni tea on the biochemical parameters of male rats subjected to subchronic treatment with 200 mg/kg for 42 days. A significant increase (p < 0.05; p < 0.01) in ALT activity was observed after 14, 28, and 42 days, respectively, and a significant increase (p < 0.05; p < 0.01) in ALP activity was observed after 28 and 42 days of treatment, respectively, compared to controls. A **Table 6** shows the effect of the aqueous extract of Tikoni tea on the biochemical parameters of male rats subjected to subchronic treatment with 200 mg/kg for 42 days. A significant decrease in AST activity (p<0.05) and ALP activity (p<0.01) was observed after 42 days of treatment, a significant decrease (p < 0.05; p < 0.01) in ALT activity after 14, 28, and 42 days of treatment, and a significant increase (p<0.01) compared to controls.

TABLE 5: EFFECT OF AQUEOUS EXTRACT OF TIKONI TEA ON HEMATOLOGICAL PARAMETERS IN MALE RATS

BP	Day 0	Products	Day 14	Day 28	Day 2
		Control	138,13±8,96	202,70±28,35	244,7±53,61
AST(U/L)	273,89±115,33				
		Extract	136,80±13,45ns	254,44±85,95ns	268,68±74,93ns
		Control	35,59±4,61	40,68±8,01	41,32±1,70
ALT(U/L)	65,91±1,30				
		Extract	59,24±12,28*	64,08±18,58**	70,26±17,37**
		Control	136,80±13,45	173,80±39,42	175,28±0,59
ALP (U/L)	207,35±5,46				
		Extract	138,13±8,96ns	237,75±11,64*	275,70±16,67**
		Control	0,15±0,04	0,22±0,04	0,13±0,01
Bilirubin (mg/L)	0,2±0,02				
		Extract	0,1±0,03ns	1,41±0,56ns	0,18±0,30ns
		Control	87,81±2,64	109,33±4,85	108,21±33,44
Cholesterol (mg/dL)	116,61±22,60				

		Extract	100,35±20,18ns	112,10±2,85ns	113,74±5,17ns
		Control	40,79±8,02	47,63±0,022	56,81±21,18
Protein(g/L)	115,33±1,29				
		Extract	56,81±21,18ns	60,66±0,37ns	82,73±31,70ns
		Control	0,57±0,07	0,61±0,02	0,58±0,08
Creatinine (mg/dL)	0,61±0,005				
		Extract	0,47±0,03ns	0,49±0,06ns	0,57±0,04ns
		Control	40,8±3,64	47,41±0,16	62,06±2,39
Urea (mg/dL)	38,78±0,82				
		Extract	55,74±1,99ns	61,10±7,77ns	61,51±7,29ns
		Control	58,16±1,75	79,65±7,46	96,06±7,47
Triglicerides (mg/dL)	81,41±36,53				
		Extract	86,39±6,98ns	108,48±12,06ns	109,42±23,85ns
Blood glucose (mg/dL)	113,82±55,21	Control	76,32±24,62	88,94±6,55	110,31±22,13
		Extract	88,52±3,66ns	91,64±18,01ns	104,81±10,67ns

HP: Hematological parameters. Results are expressed as mean ± standard error, n = 4 rats per batch, significant for *p < 0.05; **p < 0.01 ***p < 0.001; ns: not significant compared to control rats.

TABLE 6: BIOCHEMICAL PARAMETERS OF TIKONI TEA'S AQUEOUS EXTRACT IN FEMALE RATS

BP	Day 0	Products	Day 14	Day 28	Day 42
		Control	144,76±2,80	246,92±2,22	245,17±13,46
AST(U/L)	292,71±5,65				
		Extract	193,67±17,61ns	257,50±3,49ns	277,44±7,01*
		Control	44,77±0,21	54,15±1,97	55,19±0,60
ALT(U/L)	71,82±3,81				
		Extract	59,86±3,43*	70,08±6,88*	88,44±0,10*
		Control	128,44±0,66ns	130,74±25,30ns	184,1±10,04
APL(U/L)	164,53±23,77				
		Extract	129,1±9,41	144,76±2,80	193,67±17,61 **
		Control	0,51±0,15	0,4±0,03	0,32±0,40
Bilirubin (mg/L)	0,51±0,44				
		Extract	0,27±0,03ns	0,31±0,005ns	0,22±0,01ns
Cholesterol (mg/dL)	143,65±12,76	Control	95,41±2,13	112,76±7,29	118,7±10,14
	Extract		92,32±16,12ns	111,9±8,13ns	111,81±0,47ns
	Control		83,29±14,57	95,40±29,60	168,62±12,99
Protein (g/L)	110,15±1,36				
	Extract		77,30±3,08ns	86,95±4,47ns	167,9±14,21ns
	Control		0,44±0,07	0,51±0,03	0,59±0,08
Creatinin (mg/dL)	0,63±0,02				
	Extract		0,32±0,08ns	0,36±0,06ns	0,56±0,14ns
	Control		55,86±3,05	57,26±2,85	63,28±1,48
Urea (mg/dL)	43,77±10,92				
	Extract		48,68±0,49ns	54,01±3,31ns	59,5±4,14ns
	Control		83,43±10,27	93,70±7,19	114,51±20,48
Triglicerides (mg/dL)	111,32±5,12				
	Extract		70,21±0,95ns	81,60±12,26ns	99,32±2,79ns
	Control		84,16±3,22	100,19±1,68	138,83±4,27
Blood glucose (mg/dL)	120,63±17,54				
	Extract		87,25±2,52ns	144,51±2,39**	161,65±4,47**

BP: Biochemical parameters. Results are expressed as mean ± standard error, n = 4 rats per batch, significant for *p < 0.05; **p < 0.01; ***p < 0.001; ns: not significant compared to control rats.

DISCUSSION: The aim of this study was to investigate the subchronic toxicity of an aqueous extract Tikoni tea from *Vitex madiensis* Oliv. leaves in Wistar rats. This study is of great importance because this species is widely used in rural areas to treat several ailments, including malaria, inflammation, pain, and fever. It is also the basis of the traditional tea known locally as tikoni. Changes in body weight are used as an indicator of the adverse effects of chemical compounds [17]. Weight loss is correlated with the animal's physiological state. The results obtained showed a non-significant decrease in body weight in animals treated with aqueous Tikoni extract, compared to

controls treated with distilled water, in both males and females. This decrease, although not significant, could be explained either by a reduction in water and food intake due to decreased appetite, or by malabsorption reducing the amount of food absorbed [31, 35]. Food intake is regulated by the hypothalamus and depends on the interaction between two regions: the "appetite center," located laterally in the nuclear region of the medial prosencephalic fasciculus at its junction with the pallidohypothalamic fibers, and a medial "satiety center" located in the ventromedial nucleus. Stimulation of the appetite center triggers feeding behavior in conscious animals, and its destruction in healthy animals induces severe and potentially fatal anorexia. Stimulation of the ventromedial nucleus leads to the cessation of food intake [13].

Our results corroborate those of Ntabaza *et al.* (2024), who also studied the subacute toxicity of an aqueous extract of *Vitex madiensis* from the Democratic Republic of Congo after oral administration in another mammalian animal model, the guinea pig, and also observed a significant decrease in weight on day 28 24. The decreased water intake is likely due to stress factors and the husbandry conditions of the rats. Similar findings were reported by Otmani and Yahiaoui [33], who studied the acute and subacute toxicity of *Fumaria officinalis* in albino Wistar mice. Organ weight is an important indicator of pathology and physiological status in animals [36].

After macroscopic observation of the various organs collected, their sizes, shapes, and colors were found to be normal. However, a significant decrease in liver weight was observed after 42 days of treatment in both sexes, and a significant decrease in lung and spleen weight was also observed during all assessments in male and female rats, respectively. A decrease in spleen weight, or splenic atrophy, may be due to a reduction in demand or function of the organ; it is often a sign of exhaustion or chronic organ distress. The decrease in lung weight, often linked to atrophy or destruction of the lung parenchyma, could be caused by a nutritional deficiency which itself would be due to a decrease in water and food consumption [37]. A decrease in liver weight would be due to a poor diet marked by a decrease in water and food consumption or in a liver metabolism dysfionnement [17]. Our results are similar to those of Etame *et al.* (2017), who demonstrated metabolic disturbance of the liver in a study of acute and subacute toxicity of *Fumaria officinalis* in albino mice [12]. The hematopoietic system is one of the most sensitive targets of toxic compounds and an important indicator of physiological and pathological status in humans and animals [23]. Changes in the hematopoietic system have a higher predictive value for human toxicity when the data come from animal studies [11, 30]. In this regard, bone marrow activity and intravascular effects were monitored by hematological examinations. The results indicate a significant increase in white blood cell (WBC) count after 14, 28, and 42 days of treatment in males but not in females; however, platelet levels in both sexes decreased significantly after 14, 28, and 42 days of treatment.

The significant thrombocytopenia observed could be due to bone marrow and liver disorders. The liver produces thrombopoietin, a hormone that stimulates platelet production in the bone marrow. Liver dysfunction can therefore decrease this production. Thrombocytopenia would thus be linked to liver atrophy, characterized by weight loss, and to liver dysfunction, characterized by the significant elevation of transaminases observed [37]. The increase in leukocyte count may be due to an overproduction of hematopoietic regulatory elements such as CSF (colony-stimulating factor), EPO (erythropoietin), and TPO (thrombopoietin) by macrophages and bone marrow stromal cells, thus creating a local environment favorable to hematopoiesis [9, 44].

Hyperleukocytosis may be due either to hematopoietic activity of the aqueous extract of Tikoni tea, as observed with certain plant extracts, or to an inhibitory effect on leukocyte migration out of the blood, as observed with corticosteroids, such as steroidal anti-inflammatory drugs like prednisone [4]. The decrease in platelet count in treated rats compared to controls indicates that the aqueous extract affects platelet production or induces thrombocytopenia (a reduction in the number of platelets in the blood). This effect is among the evidence of toxic effects on hematopoiesis. Furthermore, with a decrease in platelet count, there is an increased risk of bleeding [42]. These results corroborate those of James *et al.*

(2010), who reported that platelet counts decreased in female rats and mice after injection of the aqueous extract of *Polygala fruticosa* [19]. The liver is the primary target of toxicity and the first organ exposed to everything absorbed in the small intestine; it metabolizes foreign substances into compounds that can be hepatotoxic [38]. The liver works in conjunction with the kidneys to remove toxic substances from the blood [43]. A study of renal and hepatic function can therefore be useful in assessing the toxic effects of medicinal plants [35].

These tests primarily include the determination of AST, ALT, CREAT, and others, and any necrosis of liver cells leads to a significant increase in AST and ALT enzymes in the blood serum [2, 14]. Serum studies performed on rats treated with aqueous extract of Tikoni tea show a significant increase in ALT activity after 14, 28, and 42 days of treatment, an increase in ALP activity after 28 and 42 days in male rats, and a significant decrease in AST and ALP activity after 42 days of treatment, a decrease in ALT activity after 14, 28, and 42 days of treatment, as well as an increase in blood glucose in females. Transaminases (AST, ALT) are plasma enzymes of hepatic origin that allow for the assessment of liver function [22]. AST is considered a good indicator of liver function and a biomarker for predicting potential toxicity [17]. ALT is located exclusively in the cytoplasm of hepatocytes. It can be released into the bloodstream as a consequence of increased membrane permeability or membrane necrosis, secondary to hepatocyte damage [41].

Elevated levels of this enzyme often indicate hepatocellular injury. Measuring its activity is more specific for liver damage than measuring AST [26]. ALP is generally measured to indicate bile duct obstruction. Elevated ALP levels are found in rapidly dividing cells or those in which metabolism is active [37]. These cells include the epithelium of the bile ducts and liver, circulating granulocytes, intestinal epithelium, renal proximal tubule, placenta, and lactating mammary glands. ALP elevations occur in primary biliary cirrhosis; in conditions of disorganization of the liver architecture; and in diseases characterized by inflammation, regeneration, and obstruction of the intrahepatic bile canaliculi [39]. These results are consistent with those of Ntabaza *et al.* (2023), who noted a significant increase in ALP with the same plant in Guinea pig toxicity study [24]. In our study, the activity of ALP and ALT increased significantly in male rats, suggesting that the aqueous extract of Tikoni tea has direct effects on the liver and kidneys of male rats. A histological study is needed for more precise information on the organ's condition; in addition, a chronic toxicity study is recommended [15]. These results are consistent with those of Witthawaskul *et al.* (2003), who noted a significant increase in ALP activity in rats injected with *Schefflera leucantha* extract [45].

Analysis of urea and creatinine levels revealed that administration of the extract did not result in any significant changes. Serum urea and creatinine are considered the main markers of nephrotoxicity, although serum urea is often considered a more reliable indicator of renal function than serum creatinine [34]. Our results do not agree with those of Ntabaza *et al.* (2023), who showed a significant increase in urea with the same type of study and extract in guinea pigs. This difference may be explained by the doses or the animal model; therefore, the 200 mg/kg dose is better suited for daily use. In cases of hyperglycemia, the pancreas releases insulin, which stimulates the liver (and muscles) to store glucose as glycogen. In cases of hypoglycemia, the pancreas releases glucagon to stimulate the liver to release glucose and maintain normal blood glucose levels [14]. In our context, we lack data to assess pancreatic function, but we know that the liver has shown dysfunction characterized by elevated serum transaminase levels and atrophy. It is likely that the hyperglycemia is linked to impaired liver function and metabolism [40].

The elevated blood glucose level may be due to damage induced by the aqueous extract of Tikoni tea in the liver [8]. These results are consistent with those of Baliga *et al.* (2004), who administered the *Alstonia scholaris* extract orally to mice and rats [3]. *Vitex madiensis* is very rich in primary and secondary metabolite compounds, mineral elements and vitamins; this constitutes a major advantage and explains the pharmacological and toxicological effects observed with this extract [24, 25, 27, 28].

CONCLUSION: Consumption of aqueous extract of *Vitex madiensis* as tikoni tea is encouraged in

immunocompromised or ill individuals, but prohibited in diabetics.

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