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(RESEARCH ARTICLE)

Acute toxicity and effect of subacute administration of *"4 heures du matin"* bitters on anthropometric and hematological parameters in *Wistar rats*

Gérard Kouassi Kouassi [1, 2, *], Léandre Kouakou Kouakou [1], Jean-Baptiste N'Guessan Oussou [1], François Djah Malan [2, 3] and Paul Angoué Yapo [1]

[1] *Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.*
[2] *Laboratory of Botany and Valorization of Plants Diversity, Sciences of Nature Department, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.*
[3] *Aké-Assi Botanical Institute of Andokoi, 02 BP 172, Abidjan 02, Côte d'Ivoire.*

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Abstract

"Bitters" are macerations of medicinal plants in alcohol, widely consumed for various reasons. Despite their high consumption, there is little scientific data on their safety/toxicity profile. Therefore, this study aims to assess the acute toxicity and the effect of subacute administration of "*4 heures du matin*" bitters, consumed to correct erectile dysfunction. The acute toxicity tests were conducted on female rats with the equivalent of 17 or 22 cups corresponding to doses of 1190 and 1540 ml/kg bw. Clinical signs of toxicity and animal death were assessed. For the subacute toxicity study, 70 rats including 35 females and 35 males divided into 3 groups were used. The group 1 rats received orally distilled water. Those in groups 2 and 3, subdivided into 3 subgroups each, received the respective doses of 140; 280 and 420 ml/kg bw of "*koutoukou"* and "*4 heures du matin"* bitters daily for 28 days. The body weight of the rats was weekly assessed. Blood samples were taken for hematological analysis. The results of acute toxicity study showed clinical signs of toxicity in rats. "*4 heures du matin*" bitters caused mortalities ranging from 40 to 60% of the total number of rats. The anthropometric study showed a non-significant increase in the weight of experimental rats. At the haematological level, a significant increase ($p<0.001$) in MCHC levels and a significant drop in hematocrit percentages was highlighted in male rats gavaged with "*koutoukou*". On the leukocyte, the solutions did not induce significant changes in both sexes of rats. The "*4 heures du matin*" bitters can be considered relatively safe for low-dose consumption in a short period. However, the long-term and high-dose consumption of this bitter poses a huge risk and harmful effect that could compromise the health of consumers.

Keywords: Toxicity; *"4 heures du matin"* Bitters; Anthropometry; Hematology; Rats

1. Introduction

Alcoholic bitters are preparations obtained by macerating various parts of fresh or dried plant species or animal parts in alcohol [1; 2]. They are widely sold in bistros, consumed in several West African countries [3; 4] and are known under different names including "*atikédy*" in Togo [3], "*pitess*", in Côte d'Ivoire derived from "bitters" as it is known in Ghana and Nigeria [2;5]. Alcoholic bitters are widely consumed all over in Abidjan [4]. This high consumption is linked to many diseases that it supposed to treat. Since its General Assembly, the World Health Organization (WHO), through its programs, has encouraged research on herbal products to verify their safety and efficacy [6]. Medicinal plants constitute a precious heritage for humanity and more particularly for the majority of poor communities in developing countries

* Corresponding author: Gérard Kouassi Kouassi
Laboratory of Physiology, Pharmacology and Pharmacopoeia, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.

who depend on them for their primary health care and their livelihoods [7]. This important role of plants in the health of populations confirms the dependence in certain Asian and African countries on traditional medicine for primary health care. This is due to many positive aspects such as its diversity, availability, low cost, efficacy and low side effects [8]. However, in low-income countries, traditional healers most often use plant extracts in the long and short term without knowledge of the toxic effects that these could cause [9]. This situation could most often lead to toxic effects especially on the liver [9; 10], or even to death [11]. As an example, the toxicity studies conducted by [12], showed that the administration of the aqueous extract of the stem bark of *Cassia sieberiana* D.C. (Caesalpiniaceae), can cause morphological abnormalities of the hepatic tissue causing necrosis in the centrilobular vein in female rats. In Côte d'Ivoire, *Uvaria afzelii* Scott-Elliot (Annonaceae) is a medicinal plant commonly macered mostly alone local alcohol *"koutoukou*" for the production of "a famous beverage known "*4 heures du matin* "bitters*""*, in relation to its aphrodisiac effects. Indeed, this macerate is used to treat gastric pain, sexual asthenia and infections [2]. This practice of mixing alcohol "*Koutoukou*" with medicinal plants for therapeutic purposes is therefore an integral part of traditional medicine [3]. However, the toxicological effects of this plant in alcohol are unknown. Studies carried out on this species have concerned its antiparasitic activity [13], its hepatoprotective activity [14], its anti-tuberculous and anti-microbial activities [15] etc. ... For the "*koutoukou*", many studies have been done on its effect on the health of consumers [16; 17]. However, few toxicological data exist on the alcoholic macerations. In view of all the above, it is necessary to assess the safety of "*4 heures du matin*" bitters through toxicity studies. So, this work aimed to evaluate the acute and subacute toxicities of the "*4 heures du matin* bitters" made with roots of *Uvaria afzelii* "in rats.

2. Material and methods

2.1. Material

2.1.1. Plant

The plant material used were composed of the root of *Uvaria afzelii* collected in the city of Adiaké (Côte d'Ivoire). The species has been identified at the National Floristic Centre of the Félix Houphouët-Boigny University (Abidjan), under the following herbarium number "Devinean 1185 dated 28/05/1975.

2.1.2. Animal

Albino *Wistar* rats (*Rattus norvegicus*) of 4 to 5 weeks old and body weights between 80 and 113 g were used. These rats were from the animal facility of the Laboratory of Physiology, Pharmacology and Pharmacopoeia of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). They were fed with pellets from the company IVOGRAIN® (Abidjan, Côte d'Ivoire). The house's daily temperature was around 22°C and a 12-hour dark/light cycle. The various experimental protocols were followed in accordance with the protocol for the protection of experimental animals of the European Council of Legislation 2012/707/EU. [18]

2.2. Methods

2.2.1. Preparation of the "4 heures du matin" bitters

The method of preparation was inspired by the method of preparation of the practitioners of this activity. It consisted of macerating *Uvaria afzelii* roots in local alcohol named "*koutoukou*" for 24 hours. The roots of *U. afzelii* were carefully cleaned, cut and dried in the Laboratory for one week at room temperature (22 ± 2°C). After drying, the organs were put in a bottle. The degree of the "*koutoukou*" was measured before it was added to the bottle using the centesimal alcoholometer from Gay Lussac (China). One liter of alcohol was added to the bottle containing the cut organs and left to macerate for 24 hours. The yellow-orange maceration obtained was named "*4 heures du matin*" bitters.

2.2.2. Acute toxicity study of "4 heures du matin" bitters in rats

The acute oral toxicity study was conducted based on the OECD guideline 425 [19]. Fifteen female rats were divided into 3 groups of 5 rats each for each experiment. After 12 h fasting, the rats of the control group (group 1) received, orally, distilled water at the rate of 1 ml/100 g of body weight (bw). Those in groups 2 and 3 received orally a single dose equivalent to 17 or 22 glasses corresponding respectively to 1190 ml/kg bw and 1540 ml/kg bw of "*koutoukou"* and "*4 heures du matin*" bitters. After the gavage of the solutions, the rats were observed for the first 30 minutes and the first 4 hours. In addition, daily observation was made for 14 days. During this period, signs of toxicity including fur and eye changes and some other signs such as tremors, convulsions, salivation, diarrhea, lethargy, sleepiness and even mortality were noted.

2.2.3. Subacute toxicity study of "4 heures du matin" biters in rats

The method used to study the subacute toxicity of the bitters was that described by [20] with a slight modification. This study was conducted on 70 rats, including 35 females and 35 males divided into 3 groups of 10 rats each. The rats of group 1 (control) received distilled water by oral route. Those in groups 2 and 3, subdivided into 3 subgroups, received the respective doses of 140; 280 and 420 ml/kg bw of "*koutoukou*" and "*4 heures du matin" bitters* daily for 28 days. These doses were based on an unpublised ethnobotanical survey carried out. The high proportion was observed only for those who took [3-5 glasses] and [6-9 glasses], i.e., 46.18% and 32.84% respectively, hence the choice of 2 glasses (low dose), 4 glasses (medium dose) and 6 glasses (high dose).

Rats were fed and hydrated *ad libitum* and then weighed weekly. The weight of each rat was measured before the administration of the different alcoholic solutions and weekly until the 28th day.

The formula below was used to calculate the percentage of weight gain by the rats [21].

$$WG\ (\%)\frac{FW(g) - IW(g)}{IW\ (g)} \times 100$$

WG: Weight gain; FW: Final weight; IW: Initial weight

At the end of the experiment, the rats were fasted for 24 hours and blood samples were taken for hematological tests.

2.3. Dosage of hematological parameters

The blood count (NFS) was performed directly in order to obtain reliable results. The automatic counter (Sysmex XT-2000 I, Japan) was used for this purpose. The following hematological parameters: the levels of erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCHC), mean corpuscular hemoglobin concentration (MCHC) and the rate of thrombocytes were determined [22].

2.4. Data analysis

The results were expressed as a mean followed by the standard error on the mean (M ± SEM). The comparisons between the values of the parameters of the rats treated with "*Koutoukou*" and "*4 heures du matin bitters*" and those of the control was made with thanks to the one-factor variance analysis (ANOVA 1) and as a post-hoc test, the Tukey-Kramer test at using Graph Pad Prism 5.01 software (San Diego, California, USA).

3. Results

3.1. Acute toxicity of "*4 heures du matin*" bitters

3.1.1. Effect of the "4 heures du matin" bitters on the rats behavior

The results of the study showed no change in coat appearance, mobility, respiration, salivation, diarrhea, lethargy, and sleep in control rats (distilled water). Regarding the rats fed with "*koutoukou*" and "*4 heures du matin*" bitters results showed a change in the appearance and mobility of the rats during the observation period. Progressive loss of mobility, change in breathing and piloerection were observed. All the animals were dispersed in the cage sometime after the administration of the alcoholic solution. In addition, manifestations of sleep were observed from the first 30 minutes for the rats fed with "*koutoukou*". On the other hand, those fed with "*4 heures du matin*" bitters presented a state of sleep after 25 minutes. This sleep time was very long for 95% of the animals fed with the alcoholic solutions ("*koutoukou*" and "*4 heures du matin*" bitters) with a sleep time of approximately 13 hours.

3.1.2. Effect of "4 heures du matin" bitters on rats' mortality rate

The results indicating the mortality rate of the animals were recorded in Table 1. They indicated that no mortality was noted in the control rats (distilled water) and those fed with the doses of 1190 and 1540 ml/kg of "*koutoukou*". As for the rats fed with the "*4 heures du matin*" bitters, 40% and 60% mortality of the rats were recorded with the doses of 1190 and 1540 ml/kg bw respectively.

Table 1 Mortality rate of rats treated with "*4 heures du matin*" bitters

Groups	Dose (ml/kg bw)	Number of rats	Number of rats dead	Mortality rate (%)
Control		05	00	00.00
« Koutoukou »	1190	05	00	00.00
« 4 heures du matin» bitters		05	02	40.00
« Koutoukou »	1540	05	00	00.00
«4 heures du matin» bitters		05	03	60.00

3.2. Subacute toxicity studies of the "*4 heures du matin*" bitters in rats

3.2.1. Effect of "4 heures du matin" bitters on rats weight gain

Statistical analysis of the results showed no significant weight gain (p>0.05) neither in female nor in male rats fed with the studied doses of "*koutoukou*" and "*4 heures du matin*" bitters compared to those of control group (Figure 2a and 2b).

Table 2a Evolution of weight gains in female rats

		Time (weeks)				
Parameters	**Groups**	**Dose (ml/kg bw)**	**1**	**2**	**3**	**4**
Weight gain (%)	Control		17.9 ± 0.49	8.89 ± 0.27	8.23± 2.25	7.07 ± 1.84
	« *Koutoukou* »	140	16.8 ± 3.53	14.9 ± 1.89	8.77 ± 0.69	7.25 ± 0.69
		280	13.1 ± 2	10.4 ± 1.79	10.9± 2.03	7.76 ± 1.19
		420	17.5± 2.17	9.62 ± 1.16	7.57 ± 1.11	7.54 ± 1.49
	« *4 heures du matin* » bitters	140	16.9 ± 3.19	10.2 ± 1.68	7.3 ± 1.15	7.6 ± 1.08
		280	8.59 ± 0.56	8.82 ± 2.44	7.64 ± 2.04	7.85 ± 0.58
		420	10.8 ± 1.94	12.6 ± 2.19	9.11 ± 1.33	8.5 ± 1.12

The values are expressed as means ± standard error on the mean. n = 5. p>0.05: Not significant

Table 2b Evolution of weight gains in male rats

		Time (weeks)				
Parameters	**Groups**	**Dose (ml/kg bw)**	**1**	**2**	**3**	**4**
Weigt gain (%)	Control		25.3 ± 2.15	21.5 ± 3.25	7.92 ± 0.72	5.96 ± 0.41
	« Koutoukou »	140	24.3 ± 5.04	14.3 ± 1.16	7.06 ± 0.49	10 ± 0.92
		280	12.4 ± 5.67	12 ± 1.27	9.68 ± 1.79	7.84 ± 1.2
		420	20.4 ± 1.31	8.59 ± 1.66	7.75 ± 2	4.21 ± 0.59
	« 4 heures du matin » bitters	140	13.3 ± 2.28	12.6 ± 1.22	11 ± 1.93	6.89 ± 0.98
		280	10.9 ± 2.32	12.7 ± 2.2	11.2 ± 1.66	5.94 ± 0.57
		420	13.3 ± 2.28	12.6 ± 1.22	11 ± 1.93	6.89 ± 0.98

The values are expressed as means ± standard error on the mean. n = 5. p>0.05: Not significant

3.2.2. Evaluation of the effect of "4 heures du matin" bitters on hematological parameters in rats

Effect of "4 heures du matin" bitters on erythrocyte parameters

Table 3a Erythrocyte parameters in female rats treated with "*4 heures du matin*" bitters

Parameters							
Groups	**Dose (ml/kg bw)**	**Red Blood Cells (10^6/mm^3)**	**Hematocrit (%)**	**MCH (Pg)**	**MCHC (g/dL)**	**MCV (fL)**	**Hemoglobin (g/dL**
Control		6.42 ± 0.08	39.7 ± 0.41	23.3 ± 1.56	34.3 ± 0.67	62.2 ± 1.12	13.6 ± 0.28
« Koutoukou »	140	6.11 ± 0.13	37.3 ± 0.75	20.9 ± 0.48	34.1 ± 0.45	61.1 ± 0.73	12.7 ± 0.25
« 4 heures du matin » bitters		5.87 ± 0.3	39.1 ± 1.16	21.9 ± 0.63	34.2 ± 0.71	61.3 ± 1.12	11.6 ± 0.70
« Koutoukou »	280	5.58 ± 0.37	38.1 ± 0.69	25.1 ± 2.54	34 ± 0.77	64.7 ± 0.56	13.2 ± 0.29
« *4 heures du matin* » bitters		6.13 ± 0.04	38.3 ± 0.27	21.8 ± 0.39	35.8 ± 0.75	59.7 ± 0.19	13.4 ± 0.19
« Koutoukou »	420	6.30 ± 0.49	37.3 ± 2.5	22.8 ± 0.89	33.9 ± 0.27	62.3 ± 0.78	13.2 ± 0.29
« 4 heures du matin » bitters		5.73 ± 0.22	35.4 ± 0.77	24 ± 1.59	35.3 ± 0.28	59.5 ± 0.68	13.4 ± 0.19

The values are expressed as means ± standard error on the mean. n = 5. p>0.05: Not significant. MCH: Mean Corpuscular Hemoglobin ; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean corpuscular Volume

Table 3b Erythrocyte parameters in male rats treated with "*4 heures du matin*" bitters

	Parameters						
Groups	**Dose (ml/kg bw)**	**Red Blood Cell (10^6/mm^3)**	**Hematocrit (%)**	**MCH (Pg)**	**MCHC g/dL**	**MCV (fL)**	**Hemoglobin g/dL**
Control		7.01 ± 0.24	42.8 ± 1.28	23.3 ± 1.56	31.7± 0.3	61.1± 0.61	13.6 ± 0.34
« Koutoukou »	140	6 ± 0.27	37.3 ± 1.38*	20.9 ± 0.48	36 ± 1.39*	59.5 ± 1.31	13.5 ± 0.55
«*4 heures du matin* » bitters		5.52 ± 0.79	40.7 ± 0.61	21.9 ± 0.63	32.4 ± 0.84	61.3 ± 1.12	12.9 ± 0.44
Koutoukou	280	6.97 ± 0.23	39.1 ± 0.43*	25.1 ± 2.54	35 ± 0.46**	59. 2 ± 0.39	14.4 ± 0.42
«*4 heures du matin* » bitters		6.80 ± 0.18	40 ± 0.11	21.8 ± 0.39	33.7 ± 0.5*	56.8 ± 0.67	13.5 ± 0.20
« Koutoukou »	420	6.56 ± 0.29	37.7 ± 1.45*	22.8 ± 0.89	33.8 ± 0.74*	57.4 ± 0.63**	12.7 ± 0.27
«*4 heures du matin* » bitters		6.57 ± 0.23	39.5 ± 1.08	24 ± 1.59	32.3± 0.38	60.2 ± 0.62	12.7 ± 0.21

The values are expressed as means ± standard error on the mean. n = 5. * p<0.05; ** p<0.01: Significant difference as compared to control. MCH: Mean Corpuscular Hemoglobin ; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean corpuscular Volume

Table 3 presents the effects of "*4 heures du matin*" bitters on hematological parameters. The results did not indicate any significant changes (p>0.05) in the levels of red blood cells, hematocrit, MCV, TMCH, MCHC and hemoglobin in female rats fed with "*koutoukou"* and "*4 heures du matin*" bitters compared to control rats throughout the experimental period. However, no significant difference was observed between the rats fed with "*koutoukou"* and those with "*4 heures du matin*" bitters (Table 3a). In male rats, the bitter did not cause any significant change in the levels of red blood cells compared to control rats. However, "*koutoukou*" lead to a significant reduction of the hematocrit levels in comparison with those of the control rats. There was no significant variation of this parameter between the rats treated with "*koutoukou"* and those treated with "*4 heures du matin*" bitters. The administration of "*koutoukou*" to rats at doses of

140; 280; 420 ml/kg bw, promoted a significant increase in MCHC levels compared to those of the controls (Table 3b). As for the "*4 heures du matin* bitters" its intake by the rats at a dose of 280 ml/kg bw, significantly increased the level of MCHC compared to those of the controls. However, no significant difference in MCHC levels was observed between rats fed with "*koutoukou*" and those of "*4 heures du matin*" bitters

Effect of "4 heures du matin" bitters on rats leukocyte parameters

The oral administration of "*koutoukou*" and "*4 heures du matin"* bitters in female or male rats didn't cause any significant variation ($p>0.05$) in the levels of leukocyte parameters compared to control rats. Also, this same observation was observed between the rats fed with the "*4 heures du matin*" bitters and those which consumed the vehicle, "*koutoukou*" (Table 4a, 4b).

Table 4a Leukocytes parameters in female rats treated with "*4 heures du matin*" bitters

		Parameters			
Groups	**Dose (ml/kg bw)**	**WBC (10^3 /mm^3)**	**Lymphocytes (10^3 /mm^3)**	**Granulocytes (10^3 /mm^3)**	**Monocytes (10^3 /mm^3)**
Control		12.5 ± 1.20	10.8 ± 1.24	1.02 ± 0.22	0.86 ± 0.08
« Koutoukou »	140	11.6 ± 0.32	9.58 ± 0.42	0.82 ± 0.21	1.15 ± 0.17
«*4 heures du matin* » bitters		8.83 ± 1.68	7.40 ± 1.47	0.67 ± 0.171	0.75 ± 0.11
« Koutoukou »	280	10.9 ± 0.61	9.58 ± 0.57	0.60 ± 0.13	0.75 ± 0.12
«*4 heures du matin* » bitters		10 ± 0.5	8.56 ± 0.32	0.46 ± 0.09	0.90 ± 0.23
« Koutoukou »	420	11.0 ± 1.02	8.72 ± 0.59	0.98 ± 0.29	1.12 ± 0.27
«*4 heures du matin* » bitters		8.64 ± 1.67	7.17 ± 0.71	0.52 ± 0.08	0.67 ± 0.07

The values are expressed as means ± standard error on the mean. n = 5. p > 0.05. No significant. n=5. WBC = White Blood Cells

Table 4 b Leukocytes parameters in male rats treated with "*4 heures du matin*" bitters

	Parameters				
Groups	**Dose (ml/kg bw)**	**WBC (10^3 /mm^3)**	**Lymphocytes (10^3 /mm^3)**	**Monocytes (10^3 /mm^3)**	**Granulocytes (10^3 /mm^3)**
Control		10.5 ± 0.97	8.38 ± 0.55	1.06 ± 0.17	1.10 ± 0.33
Koutoukou «4 heures du matin » bitters	140	11.7 ± 1.94	9.52 ± 1.87	1.10 ± 0.29	1.04 ± 0.43
		12.4 ± 0.45	10.5 ± 0.4	1.10 ± 0.13	1.38 ± 0.25
Koutoukou «*4 heures du matin* » bitters	280	10.8 ± 0.43	9.93 ± 0.25	0.75 ± 0.07	0.30 ± 0.05
		10.3 ± 0.52	8.90 ± 0.38	0.76 ± 0.12	0.66 ± 0.11
Koutoukou «*4 heures du matin* » bitters	420	12.4 ± 1.33	11.1 ± 1.68	1.63 ± 0.34	1.70 ± 0.08
		12 ±1.72	11.2 ± 1.93	1.33 ± 0.17	1.65 0.20

The values are expressed as means ± standard error on the mean. n = 5. p > 0.05. No significant. n=5. WBC = White Blood Cells

Effect of "4 heures du matin" bitters on rats blood platelets

Table 5 shows the result of the effect of "*koutoukou*" and "*4 heures du matin*" bitters on the platelet levels of female and male rats. They showed no significant variation ($p>0.05$) in the average levels of the platelets of the rats treated with these alcoholic solutions compared to those of the control rats.

Table 5 Blood platelets in female and male rats treated with "*4 heures du matin*" bitters

		Parameter	
		Blood platelets (10^3/mm^3)	
Groups	**Dose (ml/kg bw)**	**Female**	**Male**
Control		478 ± 124	354 ± 116
« Koutoukou » «*4 heures du matin* » bitters	140	316 ± 86.4	359 ± 38.2
		346 ± 22.4	289 ± 37.2
« Koutoukou » «*4 heures du matin* » bitters	280	490 ± 89.5	604 ± 116
		325 ± 37.3	527 ± 95.6
« Koutoukou » «*4 heures du matin* » bitters	420	326 ± 43.9	333 ± 36.4
		441 ± 85.2	208 ± 23.4

The values are expressed as means ± standard error on the mean. n = 5. p > 0.05. No significant with control

4. Discussion

The results of the acute toxicity study carried out on the "*4 heures du matin*" bitters showed signs of toxicities such as convulsions, erect hairs, jerky breathing, progressive loss of mobility and drowsy states in rats. In addition, mortalities ranging from 40 to 60% were recorded with doses of 1190 and 1540 ml/kg bw respectively. These changes in the animals' behavior and deaths occurred could be due to the concentration of active ingredients in the "*4 heures du matin*" bitters. The extract become more toxic when the doses were increased. These results corroborate those of [23], who showed behavioral changes and animal death rates ranging from 0 to 100% with doses of 0.5; 1; 2 and 4 ml/kg of Alomo Bitters in a single dose. On the other hand, the work carried out by [24], showed no change in behavior and no mortality in rats treated with doses of 300 or 5000 mg/kg bw of Bitters Cure Nature for 24 h. That was the case of [25], who mentioned that no signs of toxicity were reported upon acute ingestion of "Alomo bitters" to rats at a dose of 0.2 ml/kg bw for 28 days. The acute toxicity study is of limited clinical significance since cumulative toxic effects may occur even at very low doses. Hence the need for a subacute toxicity study. The results showed that the alcoholic solutions ("*koutoukou*" and "*4 heures du matin*" bitters) lead to a non-significant weight gains in both female and male rats. This weight gain in rats may be due either to a stimulation of the effect of alcoholic solutions on the central nervous system, which includes the center of appetite management, or to the presence of active ingredients likely to promote the appetite of animals. These results agreed with those of [20], who showed that the administration of "Super Bitters" to rats at a dose of 6.2 10^{-4} ml/kg bw promoted weight gain in rats for 28 days. The same observations were made by [24] with "plain bitters" at a dose of 200 mg/kg bw.

The analysis of the haematological parameters of the animals showed a variation in the erythrocyte lineage in the rats treated with the alcoholic solutions compared to the control rats. Indeed, a non-significant decrease in red blood cell levels was observed in rats consuming "*4 heures du matin*" bitters and "*Koutoukou*". The decrease in red blood cell production may be due to the bone marrow suppressing effect of the ethanol contained in the various alcoholic solutions. It could also be the result of lysis of blood cells and destruction of erythrocytes [23]. This result suggests that the consumption of large doses of "*4 heures du matin*" bitters over a long period could lead to anemia due to the destruction or reduction of red blood cell production. These results are similar to those of [23] and [26], who showed a decrease in the level of red blood cells in rats treated respectively with doses of 0.5 and 2.68ml/kg of Alomo bitters. A significant increase in MCHC was observed for "*koutoukou*" at all doses and a decrease in MCV at the dose of 140 ml/kg bw. The presence of significant effects of these alcoholic solutions on certain parameters of the erythrocyte lineage of rats could translate that these alcoholic solutions would not cause anemia in rats. The decrease in erythrocyte lineage could mean that the content, morphology and osmotic fragility of erythrocytes would have been altered at this dose of alcoholic solution. According to [27], these markers are used to determine mild anemia in alcoholic conditions. These results are supported by those of [28] and [16], who showed a decrease in certain parameters of the erythrocyte lineage in male rats.

The results on the leukocyte lineage showed significant variations in rats. A decrease in lymphocyte and white blood cell levels was noted with 420 ml/kg bw for rats treated with "*4 heures du matin*" bitters. This decrease in white blood

cells was probably due to the effect of the "*4 heures du matin*" bitters, which caused massive destruction of blood cells, which could lead to anemia and a weakening of the immune system [29]. This result was in line with those obtained by [26], who also showed a decrease in white blood cells in rats administered the different bitters (Action Bitters, Alomo Bitters; Orign Bitters) in Nigeria.

The results of this study suggest that "*koutoukou*" and "*4 heures du matin*" bitters abuse can induce a wide range of adverse effects, as evidenced by the observed indicators of erythrocytopenia, thrombocytopenia and leukopenia.

5. Conclusion

This study focused on the evaluation of the toxicities of an alcoholic solution ("*4 heures du matin* bitters"). Behavioral signs of toxicity and animal death were assessed for acute toxicity and the weight and the hematological parameters were measured for subacute toxicity. At the level of the erythrocyte lineage, consumption of "*koutoukou*" led to a decrease in red blood cell counts, hematocrit in female rats and Mean Corpuscular Volume (MCV) in males. In contrast, a significant increase in Mean Corpuscular Hemoglobin (TMHC) and Mean Corpuscular Hemoglobin Concentrations (MCHC) were reported at 120 doses; 280; and 420 ml/kg bw for "*Koutoukou*" in male rats. At the leukocyte levels, a decrease in the levels of lymphocytes and white blood cells at the dose of 420 ml/kg bw in female rats consuming "*4 heures du matin* bitters" was noted. The "*4 heures du matin* bitters" was toxic in medium and high doses. The low dose would be recommended to the consumers of this alcoholic extract.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have not declared any conflict of interests.

Statement of ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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