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Acute and Sub-acute Oral Toxicity Studies of an Aqueous Extract of *Ocimum gratissimum* (Lamiaceae) in the Mouse *Mus Musculus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ocimum gratissimum is a medicinal plant that is widely used in the world, and has multiple uses in traditional medicine, including the treatment of epilepsy in children. The objective of this study was to evaluate the acute and subacute toxicity of decoction of *Ocimum gratissimum* leaves in *Mus musculus* mice. For the acute toxicity test, a single dose of 50, 300 and 2000 mg/kg of extract was administered orally to randomly selected to mice; then they were observed for 14 days. In the subacute study, the extract was administered orally daily to mice of both sexes at doses of 250, 500 and 1000 mg/kg body weight during the 28 days of the experiment. finally, Body weight was measured weekly, haematological, biochemical and histological analysis were performed. The aqueous extract of *Ocimum gratissimum* did not cause any mortality or significant changes in relative animal and organ weights during the acute and subacute tests at all doses. Few significant changes in food and water intake were observed at the dose of 1000 mg/kg. The highest dose of aqueous extract (2000 mg/kg) administered orally in the acute toxicity did not induce mortality. There were no significant changes in haematological parameters in the different batches treated. Therefore, the oral aqueous extract of *Ocimum gratissimum* did not produce any adverse side effects compared to the control group (distiller water) in the acute and subacute study. We can therefore conclude that *Ocimum gratissimum* can be considered safe in oral administration at the dose tested since it did not cause lethality or undesirable effects in general behaviour in white mice.

Keywords: Acute toxicity; aqueous extract; Ocimum gratissimum; mice; sub-chronic toxicity.

1. INTRODUCTION

In Africa, the therapeutic attributes of plants were known to populations empirically [1]. For a long time, they have played a very important role for humanity, as they can synthesize a large number of complex organic molecules, often with potential biological activities. To this end, Cameroon has an undisputed potential due to the diversity and richness of its flora. Plants provide natural resources that are essential for the survival and development of populations. Allabi et al. [2]; as well as WHO reports [3] rightly report, therefore, that plants contribute to food security and primary health care in nearly 80% of the population in developing countries. However, despite the advent of generic drugs in these countries, many treatments remain inaccessible to financially disadvantaged populations. Associated with this economic failure, the side effects of synthetic molecules have accentuated the use of medicinal plants for therapeutic purposes in recent years [4]. In addition, since a significant number of these plants can be toxic [5], a good knowledge of their metabolite constituents as well as toxicity is necessary for adequate use in the formulation of traditionally improved drugs.

Ocimum gratissimum is a plant that is very common in several ecosystems, with very little demand in climatic conditions. It is a pantropical species found in the Americas and tropical Africa, India, and South Asia thanks to its ease of adaptation [6,7]. It is used in the traditional system as a condiment for culinary, ornamental, ritualistic purposes; in Cameroon and northern Brazil [6,8]. People in Benin use it to treat diarrhea, dysentery, hypertension, candidiasis, diabetes in pregnant women, and Buruli ulcer [9,10,11]. Its flowers and leaves are rich in essential oil and are used for the preparation of tea and infusions [12].

Previous pharmacological work on *O. gratissimum* has highlighted certain chemical constituents of its leaves: Eugenol, linalool, methyl cinnamate, camphor and thymol [13]. The eugenol contained in the essential oil of *O. gratissimum* justifies its use in the control of *Herpetomonas samuelpessoai* [14]. Researchers have highlighted its antimicrobial and antifungal properties [8], antioxidant capacity [15], immune-stimulating [16], anti-tumor [17] and hepato-protective effects [12]. In addition, there are very few studies on its toxic effects. The aim of this work was therefore be to study the acute and subacute toxicity of the aqueous extract of *O. gratissimum*.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extracts

The leaves of *O. gratissimum* were harvested in the morning in the city of Yaoundé (Adamaoua-Cameroon), and were dried at room temperature

of 25 ° C for a week. A sample of the plant was deposited at the National Herbarium of Yaoundé and identified under the number 73616HNC. The leaves of *O. gratissimum* were crushed with a mortar and sieved to obtain a powder. A decoction was prepared by introducing 10g of dry powder of *O. gratissimum* into a tube containing 50 ml of distilled water. The set was brought to a boil for 20 minutes on a hot plate (at 60 °C). After cooling, the obtain mixture was filtered using No 1 filter paper. The resulting aqueous extract (decoction) were then administered orally to mice in a volume of 10 mL/kg. The process made it possible to obtain 0.83 gram of dry extract of *O. gratissimum*, giving a yield of 8.3%, stored in the refrigerator and used for futures studies.

2.2 Phytochemical Analysis of *Ocimum gratissimum*

Chemicals tests were carried out on the extract using standard procedure to identify the constituents as described by Pessoa et al.; Soforwa and Trease and Evans [18,19,20].

2.3 Animal

The mice (*Mus musculus*) used were obtained from the animal the National Veterinary Laboratory, Garoua, Cameroon, and used throughout these experiments. They were housed in standard Plexiglas cages with food and water *ad libitum*. All these animals came from the animal facility of the Faculty of Sciences of the University of Ngaoundere (Cameroon). The animal house was maintained constantly at 25^0C on a 12-hour/12- hour light/dark cycle. The experiments were also carried out in accordance with the national guidelines (reg. No.FWA-IRB00001954). Fifteen (15) white mice (*Mus musculus*) ten (10) week old of both sexes were used respectively for the assessment of acute toxicity. Fourty (40) white mice weighing (18 ± 6g) of both sexes were used for the subacute toxicity test.

2.4 Chemicals Used

All biochemical assays were performed using the "CRESCENT DIAGNOSTICS" kits for proteins, transaminases, and creatinine, and the "Human" (Germany) kits for the lipid.

2.5 Acute Toxicity Evaluation of LD_{50}

The acute oral toxicity study of the aqueous extract (decoction of *O. gratissimum* leaves) was evaluated on six white Mus musculus mice in accordance with the OECD (Organization for Economic Co-operation and Development) Test Guideline No. 423 for the Testing of Chemicals adopted on 17 December 2001. An acute toxicity experiment was conducted in accordance with guideline 423 of the OECD Protocol. Ten-week-old mice of both sexes were fasted for 12 hours. Three (3) batches of five (5) mice were administered the aqueous extract of *O. gratissimum, per os,* at doses of 50, 300 and 2000 mg/kg body weight, respectively. The control batch was given distilled water. After treatment, the animals were observed individually at least once during the first 30 minutes and regularly for the first 24 hours after treatment. Then, they were fed and observed after 4, 8 and then 14 days during the whole study period (mortality, physical appearance, behaviour, pain and signs of illness etc).

2.6 Determination of Subacute Toxicity

The experimental protocol used for the assessment of subacute toxicity was that described by the modified OECD 407 Guidelines for the Testing of Chemicals, adopted on 3 October 2008 [21]. To do this, 40 animals, i.e. 20 males and 20 females previously fasted for 12 hours, were divided into 04 batches composed of 5 males and 5 females each. The mice were then treated as follows: batch 1, receiving distilled water at a dose of 1 mL/100 mg body weight (control batch); Batches 2, 3 and 4 receiving a solution of the extract at doses of 250, 500, 1000 mg/kg body weight respectively. Two batches 5 and 6 farming the satellite batches composed of male and female animals respectively, and receiving a 1000mg/kg dose solution of the extract were also constituted. Administration of the extracts was carried out by gavage using an esophageal tube daily, over a period of 28 days. At the end of the treatment, mice from batches 1, 2, 3, and 4 were fasted for 24 hours, then anesthetized by inhalation of Diethyl ether and the blood was collected in dry tubes. This blood was centrifuged at 4900 rpm, the supernatant was collected and stored at -20 °C for biochemical analysis. After dissection, the brain, liver and kidneys were removed and preserved for biochemical and histological analysis. Animals from satellite batches (batches 5 and 6) were observed an additional 14 days after discontinuation of treatment and sacrificed on day 42. Organs and blood were also collected for biochemical analyses.

2.7 Biochemical Analyses

2.7.1 Protein assay

Serum proteins were measured by the Biuret method [22].

*Principle: In a basic medium, tartrate forms a soluble complex with cupric salt. The addition of one protein displaces the copper in the complex to form another violet-colored complex. The intensity of the staining is proportional to the concentration of proteins in the solution to be measured.

*Procedure: The tubes used for protein assay were prepared and supplemented with the various reagents in the order shown in Table 1.

The tubes were stirred and incubated between 20 and 30°C for 20 min. Optical densities were read at 540 nm using a white. The concentration (in g/dl) of the proteins in sample tubes is determined from the following formula:

$$[\text{Protein}]\ (\text{g/dl}) = \frac{\text{DOsample}}{\text{DOstandard}} \times\times\ \text{Standard Concentration}$$

2.7.2 Creatinine assay

Creatinine was measured by Jaffé's colorimetric method, using the "CRESCENT DIAGNOSTICS" kit.

***Principle:** Creatinine reacts with picric acid in an alkaline environment to form an intense yellow complex. The amount of complex formed is directly proportional to the creatine level in the sample.

*Procedure: Testing was performed in the test tubes as shown in Table 2.

The preparations were homogenized. Then, the absorbance of the standards and samples was at 492 nm after 30 seconds (A1) and again after exactly 90 seconds (A2). The concentration of creatinine is obtained by the formula:

$$[Creatinine]\left(\frac{mg}{dL}\right) =$$

$$\frac{(A_2 - A_1)_{Sample}}{(A_2 - A_1)_{Standard}} \times [Standard]$$

2.7.3 Transaminase determination

Determination of transaminasic activities (ALT and AST) was performed according to the method described by Gella et al., [23] using the "CRESCENT DIAGNOSTICS" kit.

*Principle: Transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) catalyze the following reactions, respectively:

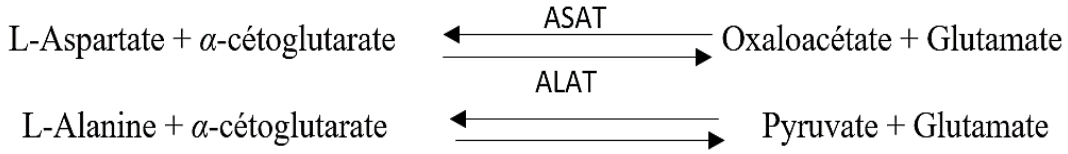


Table 1. Protein assay protocol

Constituents of reagents	White	Standard Tube	Sample Tubes
Biuret reagent (mL)	1	1	1
Standard (µL)	-	25	-
Sérum (µL)	-	-	25

Table 2. Creatinine testing protocol

Reagent	White Tube	Standard tube	Sample tubes
Standard (µL)	-	100	-
Sample (µL)	-	-	100
Reagent Mixture (mL)	1	1	1

Table 3. Transaminase assay

Sample (µL)	200
Reagent 1 (µL)	1000
Homogenized and incubated for 1 minute at 37°C	
Reagent 2 (µL)	250

ALT and AST catalyze the transfer of the amino group of alanine to α-ketoglutarate to form pyruvate and glutamate, and from aspartate to α-ketoglutarate to form oxaloacetate and glutamate, respectively. The pyruvate or oxaloacetate formed reacts with 2,4-dinitrophenylhydrazine (DNPH) to give the brown pyruvate hydrazone or oxaloacetate hydrazone complex that absorbs in the presence of NaOH at 340 nm. The intensity of the staining is proportional to the amount of pyruvate or oxaloacetate in the medium, i.e. related to the activity of ALT and AST.

*Procedure: Serum transaminase level was assessed according to the protocol in Table 3.

Once all solutions were introduced, the tubes were agitated and the absorbance (A) was read at 340 nm every minute for 03 minutes. The enzymatic activity of transaminases was obtained from the following formula:

Activity (U/L) = ∆A/min x 1746

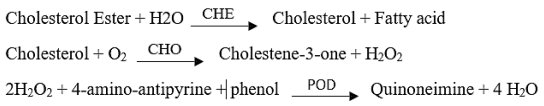
∆A = change in absorbance between 2 times intervals;

∆A/min = change in sample absorbance per minute; 1746 = multiplication factor.

2.7.4 Total cholesterol determination

Total cholesterol (TC) was measured in serum using the CHOD-PAP method with Lipid Clarifying Factor (LCF) using the CHOLESTEROL liquicolor kit.

*Principle: Cholesterol is determined after oxidation and enzymatic hydrolysis. In the presence of phenol and peroxidase, the indicator quinoneimine is formed from hydrogen peroxide and 4-amoniantipyrine.



***Procedure:**

Once all solutions had been introduced, the tubes were agitated and then left to incubate for 10 min at 25°C, the absorbance (θʌ) was read at 500 nm after 60 min. The cholesterol concentration was obtained by the following formula:

$$[Cholesterol] = [STD]\ \mathrm{x}\frac{\theta\Lambda}{\theta\Lambda STD}\,[\mathrm{mg/dl}]$$

[Cholesterol]= Concentration of cholesterol in the sample θʌ= Absorbance of the sample

[STD] = Standard concentration (210 mg/dl) θʌSTD = Standard Absorbance

2.7.5 Triglyceride determination

*Method: Triglyceride (TG) was measured in serum using the CHOLESTEROL liquicolor test kit.

***Principle:** Triglycerides are determined after enzymatic degradation by lipoprotein lipase (LPL). The indicator quinoneimine is formed from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

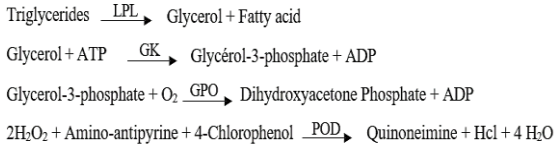


Table 4. Cholesterol measurement

Insert into tubes	White reagent	Sample or Standard (*STD*)
Sample or Standard	---	10 µl
RGT/R1	1000 µl	1000 µl

Table 5. Triglyceride determination

Insert into tubes	White	Standard/Calibrator	Sample
Reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard/Calibrator	-	10 µl	-
distilled Water	10µl	-	-

***Procedure:**

Once all the solutions had been introduced, the tubes were shaken and then incubated for 10 min at 37°C, the absorbance of the samples (θʌsample) and the standard (θʌSTD) was read at 500 nm against the reactive blank after 60 min. The enzymatic activity of triglycerides was obtained from the following formula:

$$[Triglycerides](mg/dL) = \frac{(\theta\Lambda)_{Sample}}{(\theta\Lambda)_{STD}} \times [STD]$$

[Triglycerides] = Concentration of triglycerides in the sample (θʌ)sample = absorbance of the sample
[STD] = Standard concentration (200 mg/dl) (θʌ) STD = absorbance of the standard

2.7.6 HDL and LDL cholesterol determination

HDL cholesterol was measured in serum by the precipitation method; using the CHOLESTEROL liquicolor test kit.

***Method:** Chylomicrons, VLDL (Very Low Density Lipoprotein) and LDL (low density lipoprotein) are precipitated by adding phosphotungstic acid and magnesium chloride After centrifugation, the supernatant contains the HDL (high-density lipoprotein) fraction which is analysed for the presence of HDL cholesterol using the CHOLESTEROL liquicolor test kit.

***Procedure:**

Precipitation: 200 µL of our sample and 500 µL of PREC were pipetted and fed differently into centrifuge tubes. Once all solutions were introduced, the tubes were stirred and then allowed to incubate for 10 minutes at room temperature, and the solutions were subsequently centrifuged for 2 minutes at 10000g.

Determination of cholesterol:

Once all solutions were introduced, the tubes were shaken and then incubated for 5 minutes at 37°C, the absorbance of the sample (θʌ) and the standard (θʌ)STD) was read at 546 nm against the reactive blank after 60 min. The concentration of HDL Cholesterol was determined by the following formula:

$$[HDL\ Cholesterol](mg/dL) = \frac{3{,}5\ \text{x}\ (\theta\Lambda)_{Sample}}{(\theta\Lambda)_{STD}} \times [STD]$$

[HDL Cholesterol] = HDL Cholesterol Concentration (θʌ)sample = absorbance of the sample (θʌ) STD = absorbance of the standard [STD] = Standard concentration (210 mg/dl).

LDL cholesterol concentration or low-density cholesterol is calculated from total cholesterol (TC) concentration, HDL cholesterol concentration, and triglyceride (TG) concentration according to Friedewal et al*.,* [24] according to the formula below:

$$[LDL\ Cholesterol]\ (mg/dl)\ = CT - \left(\frac{TG}{5} + HDL\right)$$

[LDL] = LDL cholesterol Concentration
CT = Total Cholesterol TG = Triglycerides
HDL = HDL Cholesterol

Table 6. Biochemical estimation of cholesterol

Pipette in the bowls	White reagent	Standard *(STD)*	Sample
Distilled Water	100 µl	--	--
Standard *(STD)*	--	100 µl	--
HDL Supernatant	--	--	100 µl
Reagent	1000 µl	1000 µl	1000 µl

2.8 Statistical Analyses

The results obtained were analyzed using GraphPad Prism 8.0.1 software and expressed as an average ± standard error of the mean (S.E.M). A one-way analysis of variance (ANOVA) followed by Dunnet's test was performed to determine the difference between the batches. P values less than 0.05 were considered as significant.

3. RESULTS

3.1 Phytochemical Screening of *O. gratissimum*

Chart 1 shows the main groups of chemical molecules contained in the aqueous extract of *O. gratissimum* leaves. It appears that this extract contains alkaloids, anthraquinones, coumarins, anthocyanins, flavonoids, tannins, triterpenes, sterols, glucosides, saponins and polyphenols.

3.2 Acute Toxicity of the Decoction of *O. gratissimum*

Oral administration of the decoction of *O. gratissimum* did not result in any deaths in animals for each of the test doses (50; 300; 2000 mg/kg). No sign of toxicity was observed during the 14 days observation period (Table 7). As stated in OECD guideline number 425 [25], decoction has been classified according to the Globally Harmonized System of Classification of Chemicals (GHS) in category-5 (2000 mg/kg≤LD50≤5000 mg/kg), i.e. chemicals with very low acute toxicity.

3.3 *In vivo* Evaluation of the Subacute Toxicity of the *O. gratissimum* Decoction

3.3.1 Behavioural response

Mice treated daily with 250, 500 and 1000 mg/kg of *O. gratissimum* decoction showed no evidence of behavioural disturbances during the four weeks of treatment. No mortality was recorded at the different doses of the *O. gratissimum* decoctate. The mice's stool did not change in appearance, and the social interaction remained the same.

3.3.2 Effects of *O. gratissimum* on physical and nutritional parameters in mice at subacute toxicity

3.3.2.1 Effects of O. gratissimum on animal weight growth

Fig. 1 A and B shows that the evolution of the body weights of the animals is identical for the different test groups compared to the normal control group, in both males and females. *O. gratissimum* did not influence weight gain in the animals compared to the control group.

Each bar represents the average ± SEM. CN: Normal control, D250: dose 250 mg/kg *O. gratissimum*, D500: dose 500 mg/kg *O. gratissimum*, D1000: dose 1000 mg/kg *O. gratissimum*; Sem 1, 2, 3, 4: Weeks 1, 2, 3, 4.

Chart 1. Phytochemical screening of leaf extract of *Ocimum gratissimum*

Phytochemical	Extract content
Alkaloids	++
Anthraquinones	++
Coumarins	++
Anthocyanins	++
Flavonoids	+
Tannins	+++
Triterpenes	-
Sterols	-
Glucosides	+++
Saponins	+++
Polyphenols	+++

- = Amount absent ; + = Amount present ; ++ = Moderate amount present ; +++ = Appreciate amount present

Table 7. Effect of aqueous extract of O. gratissimum on selected clinical signs of acute toxicity

Observed parameters	Study Period																	
	1h	2h	4h	8h	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14
Mobility	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Appearance of the stool	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Aggressiveness	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Drowsiness	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Noise Sensitivity	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

Legend: N = Normal; A = Absence; D = Day; 0 = No fatalities

Table 8. Effets de *O. gratissimum* sur les taux sériques de protéines, créatinine, AST et ALT

Traitements/Doses (en mg/kg)	Paramètres							
	Protéine (mg/ml)		Créatinine (mg/dl)		AST (IU/L)		ALT (IU/L)	
	Mâles	Femelles	Mâles	Femelles	Mâles	Femelles	Mâles	Femelles
CN	6.27±0.74	6.76±1.44	1.85±0.29	1.55±0.47	19.72±0.44	30.39±8.9	17.46±0.56	12.36±0.18
O. gratissimum 250	5.04±0.67	5.70±1.78	1.57±0.34	2.10±0.48	24.49±0.38	35.24±5.61	24.80±0.43	15.46±0.19
O. gratissimum 500	6.19±0.81	6.01±0.51	0.50±0.04	1.24±0.72	23.57±1.04	22.63±6.4	22.86±0.87	14.08±0.66
O. gratissimum 1000	6.15±0.59	8.97±2.26	0.65±0.45	1.28±0.49	20.26±1.31 *	20.69±5.72 *	18.46±0.44	13.00±0.19
C. Sat	6.41±0.91	6.55±0.87	1.89±0.68	1.62±0.09	18.27±0.59	29.02±5.57	16.92±0.45	12.21±0.79

*Values are expressed as mean ± standard error over the mean, n= 5 mice per batch. *p≤0.05;*
significant differences compared to normal controls. CN: Normal Control; O. gratissimum 250-1000: Doses 250, 500 and 1000 mg/kg of O. gratissimum; C. Sat: Satellite Control; ALT: alanine amino transferase, AST: aspartate aminotransferase

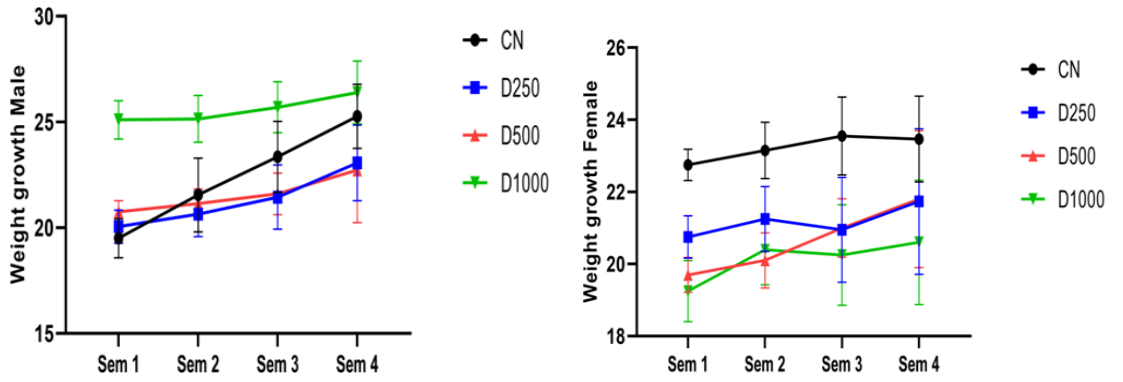


Fig. 1. A: Effects of *O. gratissimum* on the relative weight of male mice: B: Effect of *O. gratissimum* on the weight of female mice

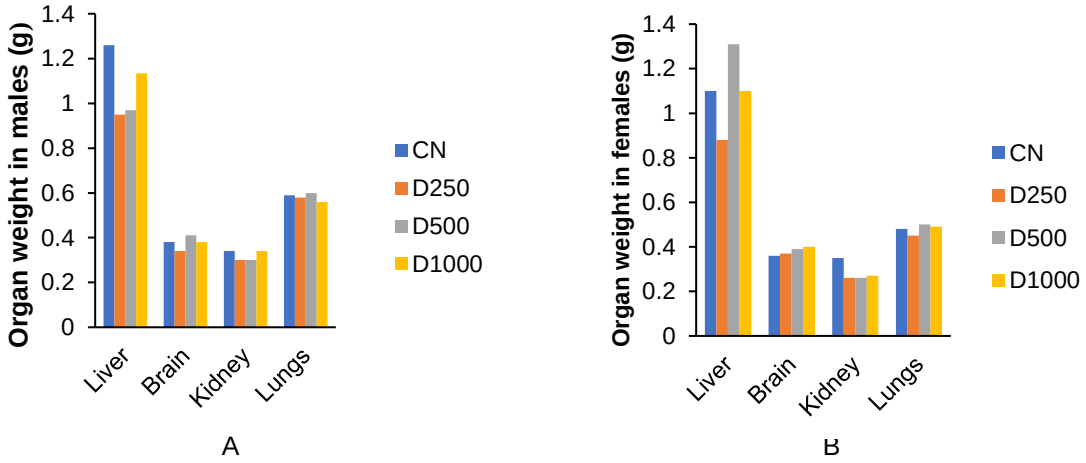


Fig. 2. Effect of *Ocimum gratissimum* on relative organ weights: A: Effects of *O. gratissimum* on relative organ weights in males. B: Effect of *O. gratissimum* on organ weights in females. n = 5 animals per lot

The data was analysed by the one-way ANOVA, followed by the Dunnet test. CN : Normal control, D250 : dose 250 mg/kg O. gratissimum, D500 : dose 500 mg/kg O. gratissimum, D1000 : dose 1000 mg/kg O. gratissimum

3.3.2.2 Effects of O. gratissimum on the relative weight of some organs

Administration of the decoction of *O. gratissimum* (250, 500 and 1000 mg/kg) to male and female animals in daily doses for 28 days did not cause any significant variation ($p \leq 0.05$) in the relative weights of liver, kidney, brain and lungs compared to the normal control batch (Fig. 2). It should be noted that, compared to the control batch, macroscopic observation of the different organs of mice treated at different doses of the decoctate revealed no macroscopic signs of abnormality with regard to their shape or coloration.

3.3.2.3 Effects of O. gratissimum on food and water intake

Figs. 3 A, B shows that food (3A) and water (3B) consumption do not varied significantly in male mice treated with the different doses of the decoction (250, 500 and/or 1000 mg/kg) compared to mice treated with distilled water during subacute toxicity. In addition, *O. gratissimum* induced a small decrease in food consumption at 250 and 500 mg/kg and moderately significant at 1000 mg/kg in the first week in females, but this consumption did not vary in the batches treated compared to the normal control during weeks 2, 3 and 4. However, it induced a significant decrease in fluid consumption during treatment (Fig. 4).

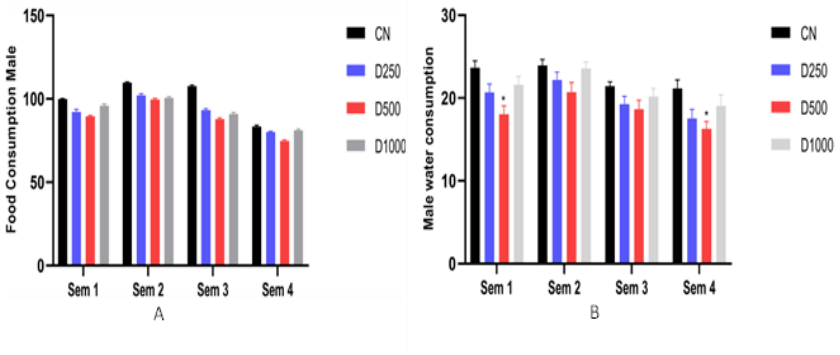


Fig. 3. Effect of *Ocimum gratissimum* on food (A) and water (B) consumption during subacute toxicity in males: A: Effects of *O. gratissimum* on food consumption in male mice. B: Effect of *O. gratissimum* on water consumption in male mice

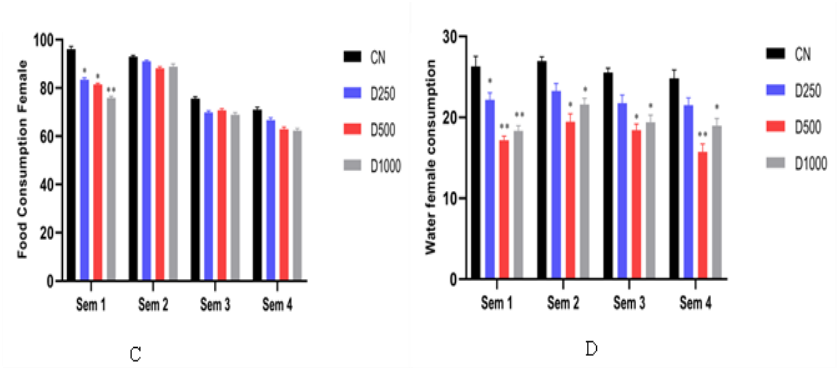


Fig. 4. Effect of *Ocimum gratissimum* on food (C) and water (D) consumption during subacute toxicity in females: C: Effects of *O. gratissimum* on food consumption in female mice. B: Effect of *O. gratissimum* on water consumption in female mice

Each bar represents the mean: ± standard error over the mean. CN: Normal control, D250: dose 250 mg/kg *O. gratissimum*, D500: dose 500 mg/kg *O. gratissimum*, D1000: dose 1000 mg/kg *O. gratissimum*; Sem 1, 2, 3, 4: Weeks 1, 2,3, 4. No significativity at P≤0.05.

Each bar represents the mean ± standard error over the mean. *P≤0.05, **P≤0.01: significant differences compared to the normal control. CN: Normal control, D250: dose 250 mg/kg *O. gratissimum*, D500: dose 500 mg/kg *O. gratissimum*, D1000: dose 1000 mg/kg *O. gratissimum*; Sem 1, 2, 3, 4 and Weeks 1, 2, 3, 4.

3.3.3 Effects of *O. gratissimum* on biochemical parameters

3.3.3.1Effects of O. gratissimum on serum levels of protein, creatinine, AST, and ALT

Table 8 shows the levels of protein, creatinine, and transaminases ALT and AST in the serum of animals after administration of the decoction of *O. gratissimum* for 28 days. It was found that *O. gratissimum* did not alter the levels of protein and creatinine in the serum of the animals in either males or females. On the other hand, the decoctate of *O. gratissimum* induced a significant increase in the concentration of AST in males, and a decrease in this concentration in females. In addition, *O. gratissimum* induced an inversely dose-dependent

increase in ALT concentration in males, and a dose-dependent increase in females. In addition, this rate returned to normal in the satellite control group after 42 days.

3.3.3.2 Effects of O. gratissimum on hepatic and renal protein levels, and hepatic transaminase levels

Tables 9A and 9B show the hepatic and renal protein levels in mice of both sexes (Table 4), as well as their hepatic ALT and AST transaminase levels (Table 5) after administration of the *O. gratissimum* decoction for 28 days. It was found that *O. gratissimum* did not significantly alter

protein levels in the liver and kidney of animals in either males or females, or in the satellite group (Table 4). On the other hand, a significant decrease in hepatic AST was observed at 1000 mg/kg in males (p≤0.001); and a non-significant decrease in hepatic ALT levels was observed at different doses in both males and females (Table 5). In addition, these levels returned to normal in animals in the satellite control group sacrificed 14 days after discontinuation of treatment (Table 5).

3.3.3.3 Effect of O. gratissimum on lipid profile

Table 10 shows the effect of *O. gratissimum* on Triglycerides, Total Cholesterol (TC), Hight Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) levels and in mice of both sexes after administration of the *O. gratissimum* decoction for 28 days. It was found that *O. gratissimum* induced a moderately significant increase (p≤0.05) in triglyceride levels at different doses of the decoctate (250, 500 and 1000 mg/kg) in both males and females, without altering the CT level. In addition, the decoction significantly increased HDL levels at doses 250 (p≤0.05); 500 (p≤0.01) and 1000 (p≤0.001), and induced a drop in LDL. A return to normal of these levels was observed in animals in the satellite control group sacrificed 14 days after discontinuation of treatment.

3.3.4 Effects of *O. gratissimum* on haematological parameters

Table 11 shows the effect of *O. gratissimum* on haematological parameters. It was found that the administration of the decoction did not significantly alter haematological parameters in animals treated with different doses of *O. gratissimum* during the 28 days of treatment, and even in the satellite control sacrificed 14 days after discontinuation of treatment, in both males and females.

3.3.5 Effects of *O. gratissimum* decoction on liver, brain and kidney histology

Fig. 5 shows the effects of the aqueous extract of *O. gratissimum* on the microarchitecture of the brain in male (A) and female (B) mice, as well as the kidneys and liver (C). Histological sections of the liver of animals in the control group and in the groups treated with the aqueous extract at different doses show normal architecture of the hepatic parenchyma with a distinct centro-lobular vein and hepatocytes. Histology of the kidney showed a normal parenchyma with a distinct glomerulus and urinary space. Histology of the brain shows normal structure of neurons in the dentate gyrus, hippocampal CA1 and CA3 regions, and cerebral cortex. These neurons have a nucleus surrounded by a cytoplasmic membrane.

Table 9A. Effects of *O. gratissimum* on hepatic and renal protein levels

Treatments/Doses (mg/kg)	Parameters			
	Protein Hp (g/g)		Proteine Re (g/g)	
	Males	Females	Males	Females
CN	51.02±0.98	42.13±0.48	26.75±0.88	17.33±0.74
***O. gratissimum* 250**	43.63±0.63	38.65±1.01	27.33±0.39	19.28±0.35
***O. gratissimum* 500**	48.72±0.41	44.76±0.57	23.47±0.9	15.77±0.21
***O. gratissimum* 1000**	42.13±0.52	31.34±0.9	20.12±0.39	22.25±0.61
C. Sat	49.96±0.84	43.85±0.93	26.02±0.56	18.00±0.35

Values are expressed as mean ± standard error over the mean, n= 5 mice per batch
CN: Normal Control; O. gratissimum 250-1000: Doses 250, 500 and 1000 mg/kg of O. gratissimum; C. Sat: Satellite Control; Hp protein: Liver protein; Re protein: Renal protein

Table 9B. Effects of O. gratissimum on hepatic AST and ALT Levels

Treatement/Doses (mg/kg)	Parameters			
	AST (IU/L)		ALT (IU/L)	
	Males	Females	Males	Females
CN	13.38±0.74	5.82±0.84	15.9±0.86	15.90±0.6
***O. gratissimum* 250**	10.47±0.97	6.59±0.78	9.5±0.56	11.33±0.43
***O. gratissimum* 500**	9.31±0.92	8,34±0.38	12.02±0.31	12.61±0.52
***O. gratissimum* 1000**	6.79±0.52***	6.4±1	9.5±0.03	9.70±0.07
C. Sat	12.16±0.6	5.76±0.2	14.98±0.26	14.51±0.5

*Values are expressed as mean ± standard error over the mean, n= 5 mice per batch. ***p≤0.001; significant differences compared to normal controls. CN: Normal Control; O. gratissimum 250-1000: Doses 250, 500 and 1000 mg/kg of O. gratissimum; C. Sat: Satellite Control*

Table 10. Effect of *O. gratissimum* on lipid profile

Traitement/Dose (mg/kg)	Parametres							
	Triglycérides (g/l)		Cholestérol Total (g/l)		HDL (g/l)		LDL (g/l)	
	Mâles	Femelles	Mâles	Femelles	Mâles	Femelles	Mâles	Femelles
CN	76.67±0.63	75.21±0.52	107.64±0.71	135.7±0.56	28.18±0.99	27.34±0.67	44.63±0.83	42.11±0.89
***O. gratissimum* 250**	94.6±0.44**	73.32±0.06	121.54±1.01	134.13±0.64	67.72±0.55** *	38±0.38*	31.56±0.66	31.88±0.58
***O. gratissimum* 500**	91.39±0.92* *	75.03±0.56	126.04±1	133.94±0.88	42.81±0.39**	41.75±0.72 *	36.81±0.77	32.83±0.37
***O. gratissimum* 1000**	96.03±0.70* *	74.94±0.87	105.33±0.47	134.19±0.64	93.18±0.56** *	46.89±0.21 *	33.39±0.7	36.05±0.38
C. Sat.	78.05±0.42	75.33±0.79	108.58±0.61	135.48±0.17	36.32±0.87	42.11±0.82 *	37.45±0.81	41.20±0.33

*Values are expressed as mean ± standard error over the mean. *p≤0.05; **p≤0.01,*
****p≤0.001; significant differences from normal controls. CN: Normal Control; O. gratissimum 250-1000: Doses 250, 500 and 1000 mg/kg of O. gratissimum; C. Sat: Satellite Control*

Table 11. Effect of *O. gratissimum* on haematological parameters

Parameters	Doses of *O. gratissimum*									
	CN		250		500		1000		C. SAT	
	Mâle	Female	Mâle	Female	Mâle	Female	Mâle	Female	Mâle	Female
WBC (10^9/L)	1.83±0.28	2.46±0.38	2.93±1.02	3.43±0.86	3.4±0.94	3.03±0.81	2.56±0.83	3.6±0.5	1.56±0.41	2.33±0.69
LYM %	35.9±0.61	44.83±0.62	36.56±0.82	47.36±0.44	37.5±0.37	40.3±0.57	42±0.21	40.63±0.82	36.7±1.79	44.1±0.63
MID%	37.26±0.53	32.36±0.51	31.1±0.49	30.043±0.43	33.2±0.9	32.26±0.91	28.93±0.26	32.26±0.91	38.47±0.48	31.4±0.43
GRAN (%)	24.36±0.89	30.33±0.47	24.33±0.73	31.36±0.44	25.3±0.94	33.66±0.47	23.06±0.54	32,6±0,48	25.86±0.83	29.66±0.47
RBC (10^12/L)	7.08±0.66	5.77±0.51	7.91±0.17	6.24±0.5	7.56±0.16	6.46±0.68	7.89±0.71	6.31±0.62	7.39±0.91	6.27±0.48
HGB (g/dL)	13.46±0.44	12.04±0.74	14.26±0.12	13.12±0.81	13.56±0.87	12.94±0.94	14.56±0.84	12.94±0.33	13.87±0.91	12,2±0,53
HCT (%)	35.23±0.71	33.66±0.62	36.33±0.47	31.23±1.11	35.63±0.44	29.68±0.49	38.56±0.63	32.3±0.66	34.28±0.72	32.76±0.26
MCV (fL)	50±0.7	47.93±073	46±0.69	49.1±0,5	46.95±0.2	46.56±0.61	49.1±0.64	46.56±0.61	49.66±0.96	46.4±0.48
MCH (pg)	19.25±1.26	25,03±0,7	18±0.43	24.9±0.16	18.6±0	26.23±0.94	18.2±0.57	25.16±0.54	19.9±0.82	24.36±0.49
RDW_SD (fL)	29.35±0.24	33.2±1.12	29±0.32	32.96±0.24	27.95±0.33	33.16±0.12	26.13±0.37	30.91±0.49	28.5±0.92	33.3±0.75
PLT (10^9/L)	599.5±0.4	610.53±0.41	556.66±0.47	594±0.81	407.33±0.47	616±0.81	541.66±0.47	609.46±1.11	595.33±0.47	614.73±0.89
MPV (Fl)	5.35±0.2	6.73±0.48	5.86±0.16	7.73±0.94	6.2±0.32	6.23±0.41	5.56±0.04	6.36±0.46	5.36±0.54	6.16±0.30
PCT (%)	0.32±0.06	0.54±0.39	0.26±0.1	0.56±0.5	0.15±0.01	0.31±0.07	0.296±0.04	0.42±0.07	0.30±0.04	0.4±0.1
P_LCR (%)	3.35±0.33	5.86±0.73	6.63±2.47	7.3±0.72	4.75±0.67	6.73±1.35	4.6±0.32	6.54±0.49	3.51±0.69	6.8±0.58
PDW (fL)	6.7±0.37	7.93±0.24	7.06±2.24	8.2±0.86	9.13±0.98	8.03±0.2	6.9±0.14	7.46±0.38	6.23±0.59	7.23±0.32

Values are expressed as mean ± standard error over the mean, n= 5 mice per batch. No significant difference at p≤0.05; from normal controls. CN: Normal Control; O. gratissimum 250-1000: Doses 250, 500 and 1000 mg/kg of O. gratissimum; C. Sat: Satellite Control. WBC: White Blood Cells; LYM: lymphocytes; MID: Monocytes; GRAN: granulocytes; RBC: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: mean corpuscular volume; MCH: Mean haemoglobin concentration; RDW_SD: Size distribution of red blood cells; PLT: Platelets; MPV: Mean platelet volume; PCT: Mean Platelet Count; P_LCR: Platelet cell count; PDW: Platelet size distribution

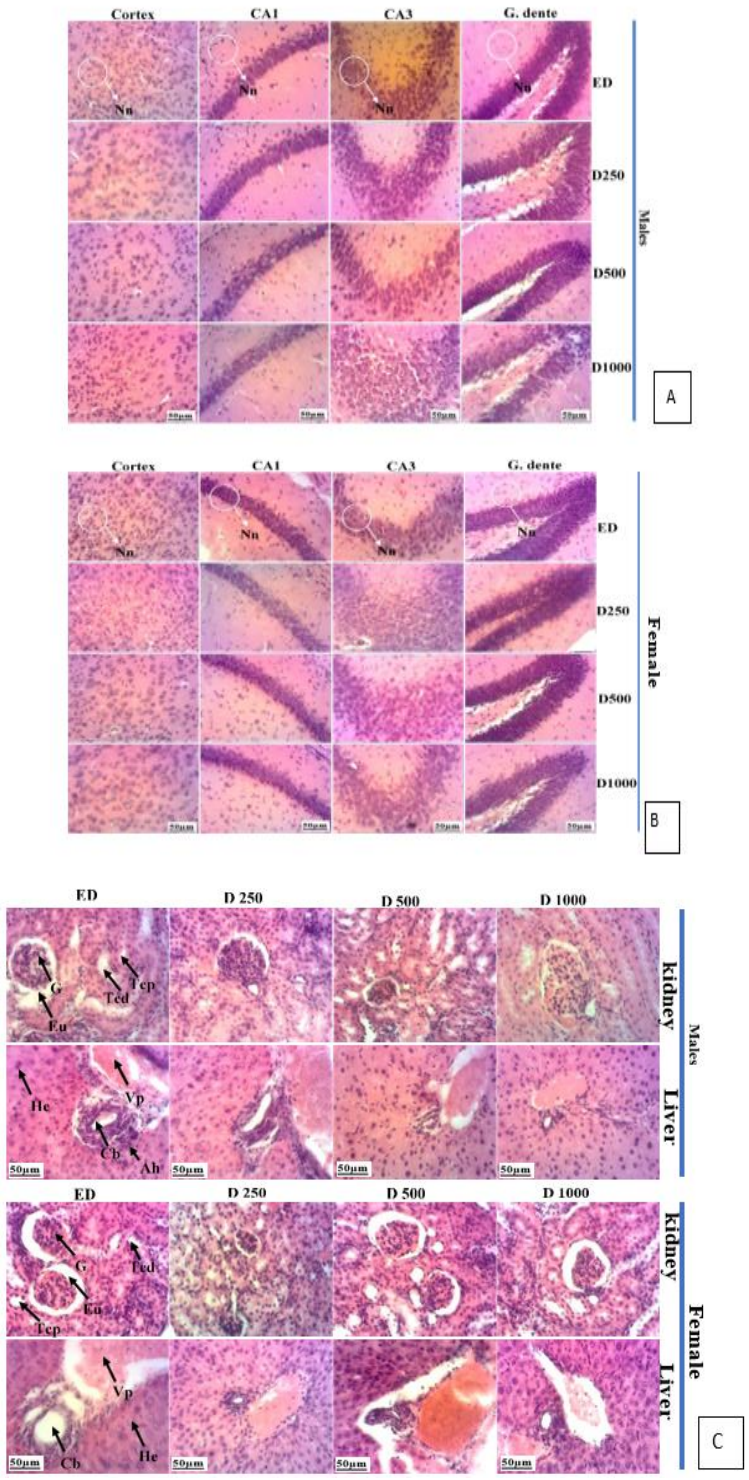


Fig. 5. Microphotograph of histology of the brain, liver and kidneys (50µm): A: Microarchitecture of the male brain; B: Microarchitecture of the female brain; C: Microarchitecture of the kidneys and liver in males and females

Nn: Normal neuron; He: Hepatocytes; Vp: portal vein; Eu: Urinary space; Cb: Gall Canaliculus; Ah: Hepatic artery; G: Glomerulus; Tcd: Distal convoluted tubule; Tcp: Proximal convoluted tubule; G. dentate: Gyrus Dentate; AC1-CA3: Horn of Amun 1-3. ED: Distilled water, animals of the normal control group treated with distilled water (10ml/kg); D250, D500, D1000: Doses 250, 500 and 1000 mg/kg of the decoction of O. gratissimum

4. DISCUSSION

Phytochemical analysis of the *O. gratissimum* decoction did not show a significant difference with previous studies. A content of phenolic compounds, tannins, glucosides, alkaloids, anthraquinones, coumarins, and anthocyanins, flavonoids, and saponins has been revealed [14,26]. Secondary metabolites are responsible for the biological activities of plants [27]. Tannins have been reported to act on proteins to form protective layer on mucus membranes [28]. Flavonoids have been found to have membrane stabilizing properties and also affect some process of intermediary metabolism and inhibit lipid peroxidation in different systems [29]. Phenols have antioxidant properties which carry out their protective activity on cells either by preventing the production of free radicals or by scavenging free radicals produced in the body [30,31]. Almost all the photo constituents of *O. gratissimum* confirmed in this study are known to influence biological system activities.

The toxicity study focuses on research on substances with potential therapeutic effects, in order to assess their level of safety, and to determine the appropriate maximum doses for treatments with No Observed Adverse Effects (NOAELs) [32,33].

The acute toxicity study of *O. gratissimum* did not report any deaths; No significant changes in animal behaviour were observed following administration of the decoction. This suggests that the leaves of *O. gratissimum* do not contain metabolites that can damage the body. This result is consistent with that of Ojo et al., [26], who confirms that this herb has clinical safety at high doses, especially during acute administration of the extract.

Subacute toxicity was assessed on several endpoints, including mortality, behavioural changes, weight growth, food and water intake, biochemistry, haematology, and histology of animals. Concerning mortality, behavioural changes and weight growth, a reduction in body mass following drug administration has been shown to be indicative of toxicity. Changes in food and water intake, and in the mass of vital organs reflect the impact that a substance might have on animals [33,34,35]. No deaths or behavioural changes were recorded in animals during the treatment days, including in the satellite groups sacrificed 14 days after discontinuation of treatment. In addition, no loss of body weight and vital organ mass compared to the normal control group. This suggests that the decoction of *O. gratissimum* leaves would not be toxic in subacute administration and would not affect the appetite of animals or the functioning of major vital organs. These results are consistent with those of Ojo et al., [26], who demonstrated that the aqueous extract of *O. gratissimum* was not toxic in subacute administration.

Biochemically, the liver and kidneys are the organs that play a very critical role in the metabolism, detoxification, storage, and excretion of chemicals and their metabolites [35]. It is necessary to check the impact that the decoction of *O. gratissimum* leaves could have on these organs. Aminotransferases are non-functional plasma enzymes that are normally located in cells of the liver, heart, kidneys, and muscles. Their presence in serum can provide information about tissue damage or organ dysfunction [36]. Blood and tissue levels of ALT and AST can therefore be used to assess the toxic impact of a chemical compound. We assessed these indicators of liver function in addition to protein levels. In addition, we profiled the lipid profile and assessed the condition of the kidney by measuring serum creatinine and renal protein levels.

No significant differences were observed in serum or hepatic protein levels in either males or females in the extract-fed groups, as well as in the satellite groups sacrificed 14 days after discontinuation of treatment; compared to control groups that received only distilled water. Similarly, there was no significant difference in serum or hepatic ALT and AST levels in animals of both sexes at 250 and 500 mg/kg; however, a very significant decrease ($p \leq 0.001$) in specific AST was observed at 1000 mg/kg in males (Table 9B). This rate also returned to normal in the satellite groups when treatment was discontinued. Therefore, *O. gratissimum* would not affect liver function. This finding is consistent with those of Ojo et al., [26], who state that *O. gratissimum* may not pose a toxicological threat to the liver when used in traditional medicine at lower doses.

Impaired fatty acid metabolism is characterized by increased cholesterol and triglyceride levels [37]. However, an abnormal increase in cholesterol levels, especially LDL cholesterol, creates a risk for the cardiovascular system; where it may contribute to the development of high blood pressure and stroke [38,39]. Prolonged administration of the aqueous extract

of *O. gratissimum* induced an increase in triglyceride levels without affecting total cholesterol levels. In addition, a significant increase in HDL levels was observed in males at all doses. This specific increase in HDL without any change in total cholesterol levels therefore reflects the fact that the aqueous extract of *O. gratissimum* leaves would promote the synthesis of HDL to the detriment of LDL [40]. It could thus protect against metabolic diseases.

When it comes to kidney function, creatinine is one of the key markers of kidney function. The creatinine produced is released into the bloodstream where it is filtered by the

kidneys and excreted in the urine [41]. Thus, an increase in serum creatinine would reflect kidney damage, resulting from an inability of the kidneys to filter creatinine from the blood. No differences were observed in serum creatinine levels for all groups, including satellite groups; compared to normal groups fed with distilled water. The extract would therefore not affect the functioning of the kidneys.

On the hematologic level, the hematopoietic system reveals the physiological and pathological state of organisms because it is a sensitive target for toxic compounds. The impact of a drug on this system is therefore one of the major indicators of toxicity [32,42,43]. No significant changes were observed in red blood cells, lymphocytes, granulocytes, mean corpuscular volume and blood platelets. In addition, no significant changes were observed in white blood cells, monocytes and lymphocytes. O. gratissimum therefore does not affect erythropoiesis, morphology, or osmotic fragility of red blood cells [43], and does not deteriorate the normal haematological profile as a whole. These observations support the non- toxic nature of the aqueous extract of O. gratissimum.

With respect to animal histology, histopathological studies of the liver and kidneys are very useful for confirming haematological and biochemical analyses in toxicity studies [32,33,35]. Analysis of histological sections revealed no liver and kidney alterations in animals administered the aqueous extract from the leaves of O. gratissimum. These results corroborate the biochemical findings, and confirm that the aqueous extract from the leaves of *O. gratissimum* does not present any danger to the organism in acute and subacute administration.

5. CONCLUSION

In conclusion, the oral administration of different doses of *O. gratissimum* aqueous extract in the acute toxicity test did not lethaly or adverse changes in the general behaviour. The oral toxicity test in mice at the respective doses classified this plant as very low-toxicity substance, as no signs of treatment related intoxication were throughout the test period; and the sub-acute oral toxicity test at the various doses did not result in any treatment-related deaths*. O. gratissimum* did not affect the weight of the animals., It did not alter lipid profile, liver and renal function parameters, and its administration maintained good preservation of haematological parameters. Its use in traditional medicine would therefore be safe for the population.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

STATEMENT OF ETHICAL APPROVAL

The protocols were performed in concordance with the International Guide for the Care and Use of Laboratory Animal (National Institute of Health; publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee, Yaoundé (No. FW-IRB00001954).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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