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In-vitro and in-vivo toxicological studies of hydroethanolic leaf extract of *Ocimum gratissimum* Linn. (Lamiaceae) in Wistar rats

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ABSTRACT

Herbal medicines have been used for human health management, prevention, and cure of human diseases since ancient civilizations. In recent days, the use of herbal medicines has increased significantly in various forms such as herbal formulations, dietary supplements, and nutraceuticals in the global market. This growing demand undoubtedly improves the therapeutic claims of herbal medicines as biomedicines and/or functional foods. However, the safe use of herbal products and herbal drugs remains a challenge due to toxicity and regulatory issues. This study aims to evaluate the cytotoxicity of hydroethanolic leaf extract of *Ocimum gratissimum* on the larvae of *Artemia salina in vitro* and *in-vivo* acute and subchronic toxicity study in Wistar rats. The test on *A. salina* showed that the hydroalcoholic extract of *O. gratissimum* did not show any toxicity. The LC50 values of *O. gratissimum* on brine shrimp were 0.60 ± 0.19 mg/ml. The acute toxicity study revealed no behavioral disturbances or death in rats. The extract lethal dose (LD50) is greater than 5000 mg/kg body weight. Subchronic toxicity results showed a significant increase in abdominal fat at 1000 mg/kg. As for the hematological parameters, there was a significant decrease in the number of platelets at doses of 500 and 1000 mg/kg. Some biochemical parameters were affected by repeated administration of *O. gratissimum* extract for 28 days. Alkaline phosphatase (ALP) activity was increased at 500 and 1000 mg/kg. Creatine phosphokinase (CPK) and urea decreased at 500 mg/kg and 1000 mg/kg, respectively. Blood electrolytes showed no significant change. Histological sections showed no organ damage. In conclusion, aqueous extract of *O. gratissimum* caused no adverse effects in rats after acute and subchronic treatment at 500 and 1000 mg/kg. However, specific hematological and biochemical parameters should be monitored during chronic use.

Keywords: *Ocimum gratissimum*, cytotoxicity, toxicity, Wistar rats.

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Abbreviations: ALP: Alkaline Phosphatase; **ANOVA:** analysis of variance; **Ca++:** Calcemia; **Cl-:** Chloraemia; **CPK:** Creatine phosphokinase; **CRP:** C-reactive Protein; **DL_{50}:** Lethal Dose; **EDTA:** disodium salt of ethylenediaminetetraacetic acid; **HB:** Hemoglobin; **HCT:** Hematocrit; **K+:** Kaliemia; **LC_{50}:** Lethal Concentration; **MCH:** mean corpuscular hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **MCV:** Mean Corpuscular Volume; **Na+:** Natremia; **OECD:** Organization for Economic Cooperation and Development; **OG:** *Ocimum gratissimum*; **PLT:** Platelets; **RBCs:** Red Blood Cells; **SEM:** standard error of the mean; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamic pyruvic transaminase; **TG:** Triglycerides; **TOTAL CHOL:** Total Cholesterol; **WBC:** White Blood Cells.

INTRODUCTION

The therapeutic potential of plants, which was empirically known by the populations (N'guessan et al., 2009), is now recognized by the World Health Organization (WHO) as an essential component of primary health care (Onayade et al., 1990). According to the WHO, more than 80% of the world's population still relies on herbal medicines as their primary source of health care (Adeyemi et al., 2009). *Ocimum gratissimum* (OG) is one such plant belonging to this plant biodiversity.

Popularly known as African basil in English, OG is also called "Azèou" or "Éssrou" by the Kabyè and Ewe peoples in Togo respectively. It is a medicinal plant used as a spice widely cultivated in tropical and subtropical regions (Ojo et al., 2019). The leaves prepared by maceration, decoction, or infusion in water or alcohol are generally taken in folk medicine to treat various ailments such as rheumatism, hemorrhoids, bronchitis, stomatitis (Iwu,1993; Ede et al., 2012), cardiovascular diseases (Kpètèhoto et al., 2017), diabetes, cancer, inflammation, anaemia, diarrhoea, pains, and fungal and bacterial infections (Ugbogu et al., 2021).

In addition, it had been shown that OG possesses many pharmacological properties hence its use in traditional or alternative medicine. These properties include antioxidant (Akinmoladun et al., 2007; Ojo et al., 2013a, 2013b, 2014a; Dubey et al., 2000); anti-anemic (Ojo et al., 2014b); anti-diarrheal (Offiah et al., 1999) and protective effects on hepato-renal indices (Ojo et al., 2014c).

Phytochemical screening of OG shows the presence of phenolic compounds such as tannins (Yémoa et al., 2008), flavonoids (N'guessan et al., 2009; Kpètèhoto et al., 2017); nitrogenous compounds; steroids; terpenoids; reducing compounds; nutrients (proteins; nitrogen; iron; zinc; copper) and heavy metals (lead; cadmium) (Kpètèhoto et al., 2017). The leaves contain an essential oil composed of monoterpenes, sesquiterpenes, aromatic compounds, and oxygenated aromatic compounds (Sanda et al., 1998). The presence of all these compounds in general and more specifically of the essential oil and heavy metals determines the toxicity considering the intensive use of this plant. The almost daily use of this plant for the preparation of sauce but also in the treatment of the mentioned diseases raises questions about its safety.

This study aims to evaluate the cytotoxicity of the larvae of *Artemia salina* in vitro; the acute and subchronic toxicity by oral administration, of the leaf, extract of *O. gratissimum* in female Wistar rats.

METHODS

Collection and extraction of plant material

The fresh leaves harvested in the morning of *O. gratissimum* were obtained at the Nukafou market located in the second district of the city of Lomé (TOGO) from medicinal plant sellers. These leaves were then identified by the Department of Botany and Ecology of the University of Lomé. A reference specimen was recorded in the herbarium under the number TOGO15572.

Leaves were cleaned and dried under an air conditioner (20°C). Then 1000 g of dried and pulverized leaves were soaked in 15 L of ethanol-water (50-50) for 72 h with intermittent agitation for extraction. After double filtration with cotton and Whatman paper, the filtrate was evaporated using a rotary evaporator (Rotavapor IKA RV 10, Germany). The yield of this extraction was calculated according to the formula: R = (weight of extract/weight of dry leaves) × 100.

Animals

Twelve-week-old female Wistar rats (150 to 200 g) were used for the acute and subchronic experiments on the one hand. They were provided by the Animal Physiology Department's animal facility and were acclimated at least one week before the start of the experimentation. The animals were fed standard rodent food and water ad libitum. Animal care and handling were in accordance with accepted guidelines (Mlingi et al., 2011; Vandegeer et al., 2013). Ethical approval was obtained from the Institutional Ethics Committee for Teaching and Research under the number (ref. CNCB- CEER 2801/2010). On the other hand, Artemia salina eggs were used for the cytotoxicity study.

Phytochemical screening

Qualitative

The screening was conducted to assess the presence of certain chemical groups such as alkaloids, saponins, total phenols, hydrolyzable tannins, condensed tannins, sterols, terpenes, anthracenes, cardiotonic heterosides, coumarins, and flavonoids. Saponins, total phenols, hydrolyzable tannins, condensed tannins, sterols, terpenes, anthracenes, cardiotonic heterosides, coumarins, and flavonoids. These groups were checked as described by Karumi et al. (2004) and Edeoga et al. (2005). For alkaloids, Bourchadat, Dragendorff, and Mayer reagents were used to determine their presence as described by Evans and Trease (1989).

Quantitative

Cardiotonic glycoside content was determined from a calibration range with digoxin (10 to 100 µg/ml) and results were expressed as mg digoxin equivalent per gram of dry extract (mg EqD / g). Three assays were performed for the sample (Tofighi et al., 2016).

Assessment of larval toxicity of the substance

The bioactivity of the extract on the larvae was monitored by the brine shrimp lethality test (Meyer et *al*., 1982). Different concentrations of extract were prepared. Using a conical micropipette, a colony of 16 live larvae was contacted with a series of solutions at graded concentrations (from 25 to 0.049 mg/mL) of plant drug extracts. These media and controls were allowed to stir and live larvae were counted 24 hours after incubation. The mean percent mortality was plotted as a function of the logarithm of

concentrations. The concentration (LC_{50}), at which 50% of the larvae were killed, was determined from the graph (Meyer et al., 1982; Diallo et al., 2020; Dossou-Yovo et al., 2020).

Acute toxicity test

The limit dose of 5000 mg/kg was applied to three female Wistar rats by Organization for Economic Cooperation and Development (OECD) guidelines (OECD, 2002). Each rat received sequential doses at an interval of 48 hours. Animals were observed individually for signs of acute toxicity or behavioral changes 30 minutes after dosing for the first 24 hours. Rats were observed at least once daily for 14 days.

Sub-chronic toxicity

Repeated-dose oral toxicity was performed according to OECD guideline 407 (OECD, 2008). Animals were divided into three groups of 6 individuals each. The first group (group 1) received distilled water and served as a control group. The second (group 2) and third (group 3) groups received the extract at 500 mg/kg and 1000 mg/kg body weight, respectively.

The extract was administered daily for 28 days simultaneously, and the animals were observed at least twice daily for morbidity and mortality. All groups received 1 ml/100 bodyweight of the solution. Body weights were recorded daily. Rats were observed for aggression, mobility, diarrhea, appetite, and response to sound stimuli. On day 29, after 12 hours of fasting, rats were first anesthetized with ether. Blood samples were collected from the retro-orbital sinus in dry tubes for biochemical analyses and EDTA tubes for hematological analyses. Samples for biochemical analyses were centrifuged at 2500 rpm for 15 min and serum were collected. Biochemical parameters such as serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), creatinine, urea, creatine phosphokinase (CPK), total cholesterol, triglycerides, C-reactive protein, and glucose were performed. Sodium (Na^+), potassium (K^+), chloride (Cl^-) and calcium (Ca^{++}) ions were measured in serum. Hematological parameters obtained were white blood cell count (WBC), red blood cell count (RBC), hematocrit (HCT), hemoglobin (HB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), platelet count (PLT) and mean corpuscular volume (MCV). Then, the animals were euthanized with ether, and necroscopy of all rats was performed and the weight of selected organs (kidney, liver, spleen, heart, and abdominal fat) was recorded.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by analysis of variance (ANOVA) with Tukey's test to assess the difference between the two groups. Values of $p < 0.05$ were considered significant. The statistical package Instant (GraphPad Prism 6.01) was used to perform all statistical analyses.

RESULTS

Phytochemical screening and extraction of plant materials

Evaporation of the plant extract resulted in a yield of 11.75%. The results of phytochemical screening showed the presence of alkaloids, total phenols, flavonoids, condensed tannins, sterol, terpenes, cardiac heterosides, and coumarin. (Table 1)

Quantitative phytochemical screening of cardiotonic glycosides gave us a concentration of 8.36 ± 0.15 µg Digoxin/mg *O. gratissimum*.

Table 1. Qualitative phytochemical screening of chemical groups in *O. gratissimum* leaf extract.

Researched groups	Extract
	O. gratissimum
Alkaloids *(Bouchardât)*	**+**
Alkaloids *(Mayer)*	**-**
Alkaloids *(Dragendorff)*	**+**
Total Phenols	**+**
Flavonoids	**+**
Condensed Tannins	**+**
Hydrolyzable Tannins	**-**
Sterol	**+**
Terpenes	**+**
Anthracenes	**-**
Saponins	**-**
Cardiotonic Heterosides	**+**
Coumarin	**+**

+: presence; -: absence.

Brine shrimp toxicity screening

From Figure 1, the LC_{50} values for *O. gratissimum* were 0.60 ± 0.19 mg/ml using the equation $y = 2.7738\ln(x) + 9.5795$.

Acute toxicity

For a short (48 hours) or long (2 weeks) observation period, the limit dose of 5000 mg/kg *O. gratissimum* did not cause mortality or acute toxic effects in the three exposed rats. The LD_{50} was greater than 5000 mg/kg.

Sub-chronic toxicity

In the clinical evaluation, no behavioral changes and no deaths were observed at the end of treatment. Similarly, no significant difference in body weight was observed between the control and treated groups during this period (Table 2).

O. gratissimum at 1000 mg/kg significantly ($p < 0.01$) increased the relative weight of abdominal fat compared with the control group (Table 3).

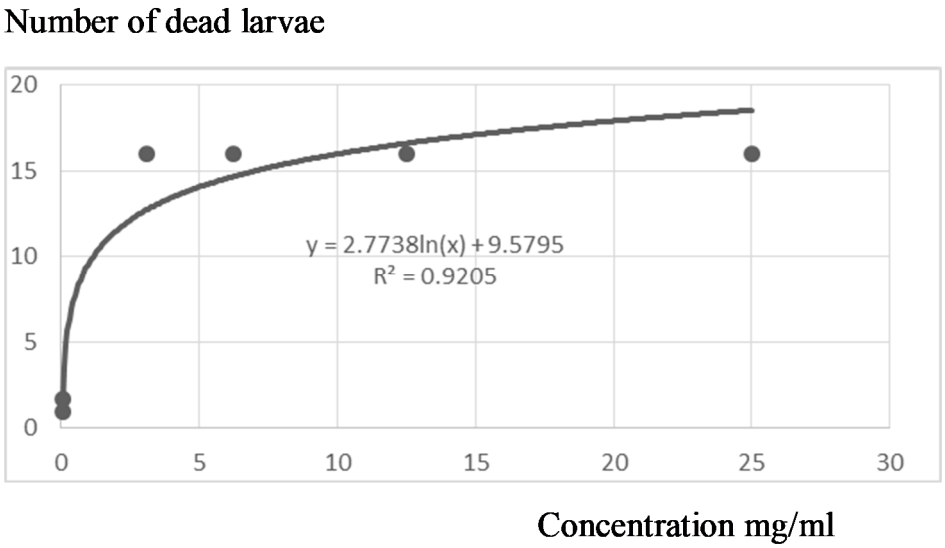

Figure 1. *In vitro* cytotoxicity of *O. gratissimum* leaves on *Artemia salina* larvae. Concentration lethality curve. All values are expressed as mean ± SEM (n = 3).

Table 2. Effect of hydroethanolic extract of *O. gratissimum* on the bodyweight (g) rats after 28 days of the experiment.

Week	Control	*O. gratissimum* extract dose	
		500 mg/kg	1000 mg/kg
0	139.00 ± 5.29	137.66 ± 9.04	135.83 ± 11.80
1	142.50 ± 4.95	145.16 ± 8.06	138.16 ± 10.87
2	147.83 ± 4.92	149.83 ± 9.06	143.50 ± 10.19
3	150.66 ± 5.45	154.83 ± 9.41	147.16 ± 9.27
4	156.66 ± 6.80	164.00 ± 13.42	157.00 ± 10.04

Each value is expressed as mean ± S.E.M.; ANOVA followed by Tukey's test; n = 6.

Table 3. Effect of *O. gratissimum* hydroethanolic extract on relative organ weights of rats after 28 days of the experiment.

Organs	Control (%) (n = 6)	*O. gratissimum* extract (n = 6)	
		500 mg/kg (%)	1000 mg/kg (%)
Kidney	0.65 ± 0.06	0.69 ± 0.03	0.66 ± 0.03
Heart	0.41 ± 0.02	0.43 ± 0.02	0.44 ± 0.02
Spleen	0.44 ± 0.11	0.30 ± 0.04	0.47 ± 0.07
Liver	3.35 ± 0.21	3.33 ± 0.19	3.28 ± 0.22
Abdominal fat	2.83 ± 0.50	2.98 ± 0.57	3.88 ± 0.59*

Each value is expressed as mean ± S.E.M; ANOVA followed by Tukey test; * $p < 0.01$; n = 6.

Tables 4 and 5 show the hematological and biochemical parameters, respectively. No changes in parameters were observed, except for a significant increase in alkaline phosphatase (500 and 1000 mg/kg), creatine phosphokinase (500 and 1000 mg/kg), and a significant decrease in platelet count (500 and 1000 mg/kg) compared with controls. Biochemical parameters such as SGOT, SGPT, and blood glucose were not significantly modified compared to the control.

O. gratissimum extract at a dose of 1000 mg/kg causes a significant ($p < 0.01$) decrease in urea. At 500 and 1000 mg/kg doses, there is a significant increase in alkaline phosphatase (ALP) and a significant decrease in creatine phosphokinase (CPK). (Table 6).

Table 4. Effect of hydroethanolic extract of *O. gratissimum* on hematological parameters after 28 days of the experiment.

Parameters	Control	*O. gratissimum* extract	
		500 mg/kg	1000 mg/kg
WBC	9.08 ± 1.31	8.05 ± 2.79	7.66 ± 1.12
RBC (×10^6/µl)	6.95 ± 0.99	7.11 ± 0.63	6.05 ± 0.78
HB (g/dl)	15.58 ± 1.76	15.10 ± 1.29	15.06 ± 2.19
HCT (%)	42.9 ± 3.99	40.86 ± 3.36	42.70 ± 5.05
MVC (fl)	52.58 ± 3.54	52.78 ± 2.79	55.21 ± 3.71
MCH (pg)	18.68 ± 0.82	19.46 ± 1.06	19.11 ± 0.99
MCHC (g/dl)	35.85 ± 0.96	36.88 ± 0.36	34.96 ± 1.33
PLT (×10^5/µl)	758.33 ± 31.01	490 ± 19.08****	651.66 ± 36.88****

WBCs: White Blood Cells; RBCs: Red Blood Cells; HB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: mean corpuscular hemoglobin; PLT: Platelets
Each value is expressed as mean ± S.E.M; ANOVA followed by Tukey test; **** $p < 0,0001$; n = 6.

Table 5. Effect of hydroethanolic extract of *O. gratissimum* on biochemical parameters after 28 days of the experiment.

Parameters	Control	*O. gratissimum* extract	
		500 mg/kg	1000 mg/kg
Urea (mg/dl)	71.62 ± 3.44	75.30 ± 4.89	49.80 ± 3.16*
Creatinine (mg/dl)	0.55 ± 0.02	0.61 ± 0.03	0.56 ± 0.02
Glycemia (mmol/L)	85.00 ± 1.52	94.66 ± 1.20	92.66 ± 2.33
SGOT (UI/L)	139.00 ± 4.93	122.00 ± 2.16	138.80 ± 2.26
SGPT (UI/L)	43.50 ± 1.50	46.33 ± 2.02	43.00 ± 3.30
ALP (UI/L)	112.50 ± 5.61	136.66 ± 11.89*	207.00 ± 7.54****
CPK (UI/L)	653.00 ± 19.85	382.50 ± 18.85****	549.33 ± 17.45****
TOTAL CHOL (mg/dl)	60.50 ± 1.55	59.00 ± 2.50	56.20 ± 1.39
TG (mg/dl)	111.50 ± 7.64	108.50 ± 3.37	112.00 ± 5.56
CRP (mg/dl)	2.68 ± 0.15	2.41 ± 0.23	2.58 ± 0.08

* : $p < 0,05$; *** : $p < 0,001$; **** : $p < 0,0001$; n = 6 ; SGOT : Serum glutamic oxaloacetic transaminase ; SGPT : Serum glutamic pyruvic transaminase ; ALP : Alkaline Phosphatase ; CPK : Creatine phosphokinase ; TOTAL CHOL : Total Cholesterol ; TG : Triglycerides ; CRP : C-reactive Proteine.
Each value is expressed as mean±S.E.M; ANOVA followed by Tukey test; * $p < 0,01$; *** $p < 0,0001$; **** $p < 0,00001$; n = 6.

Table 6. Effect of hydroethanolic extract of *O. gratissimum* on ionogram parameters after 28 days of the experiment.

Ion	Control (n = 6)	*O. gratissimum* extract n = 6)	
		500 mg/kg	1000 mg/kg
Na^+ (mmol/L)	141.00 ± 1.47	148.75 ± 4.02	148.25 ± 7.14
K^+ (mmol/L)	6.17 ± 0.32	5.90 ± 0.23	5.30 ± 0.36
Cl^- (mmol/L)	102.25 ± 0.85	107.25 ± 2.83	106.75 ± 5.10
Ca^{++} (mg/dl)	9.30 ± 0.34	7.60 ± 0.35	8.01 ± 0.59

Na^+: Natremia; K^+: Kaliemia; Cl^-: Chloraemia; Ca^{++}: Calcemia
Each value is expressed as mean ± S.E.M; ANOVA followed by Tukey test; n = 6.

DISCUSSION

In our current study, the extract revealed the presence of tannins, steroids, alkaloids, terpenoids, flavonoids, phenolics, coumarin, and cardiac glycosides while anthracenes and saponins were absent. Udoha et al.

(2019) identified in the ethanolic extract the presence of flavonoids, cardiac glycosides, saponins, steroids, and phenolics while tannins and alkaloids were absent. Talabi and Makanjuola (2017) reported the presence of alkaloids, saponins, cardiac glycosides, phenolics, tannins, and anthracenes while steroids, terpenes, and flavonoids were absent in the aqueous extract. Jumare et al. (2018) found tannins, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids, and coumarin while phenol was absent in the aqueous leaf as well.

Okoduwa et al. (2017) in their investigations revealed the presence of alkaloids, flavonoids, tannins, terpenes, and saponosides while steroids and anthracenes were absent in the n-butanol extract. The variation in phytochemical content in this current investigation of the hydroethanolic extract of the leaf could be due to planting location, seasonal variation, and extraction variables such as temperature, time, and concentration (Aglave et al., 2019).

The various health benefits (Ajila et al., 2011) of medicinal plants are attributed to the presence of secondary metabolites. Yet, the particular presence of alkaloids and cardiotonic glycosides in our extract may be a source of toxicity or benefit to the body depending on the dose ingested. Alkaloids can present toxicity to cells (Igbinosa et al., 2013), organs such as the heart, but also the nervous system. However, cardiac glycosides due to the very narrow margin between the therapeutic dose and the toxic dose can, therefore, at high doses, cause cardiac arrest.

On the other hand, on the quantitative level, the dosage of cardiotonic glycosides gives us a concentration equal to 8.36 ± 0.15 µg of Digoxin/mg of *O. gratissimum*. Considering the use of this plant as a food source, the administration of the hydroethanolic extract of this plant for 28 days allowed, through the evaluation of the effects on various biological parameters, to evaluate the toxicity relative to this concentration.

The LC_{50} values calculated for the brine shrimp lethality test were 0.598 ± 0.19 mg/ml. Therefore, the hydroalcoholic extract of *O. gratissimum* leaves is not toxic according to the table of Mousseux (1995), who classified crude extracts and pure substances into toxic (LC_{50} value < 0.1 mg/ml) and non-toxic (LC_{50} value > 0.1 mg/ml). This study is corroborated by Ajayi et *al*. (2017) who found an LC_{50} of 152.06 ± 0.38 mg/ml. Therefore, *O. gratissimum* would not exhibit cell toxicity.

The acute toxicity study is used to test the adverse effects of an agent on the organism during a single or short-term exposure (Krishnaraju et *al*., 2005). The acute oral toxicity study of OG leaf extracts at 5000 mg/kg showed no obvious mortality or toxic symptoms. This means that the median lethal dose (LD_{50}) of *O. gratissimum* was greater than 5 g/kg body weight. This result was synonymous with that of Ogundipe et al. (2016) and Okoduwa et al. (2017) who reported the non-toxic nature of the leaf extract to either the kidney or liver. Nevertheless, other researchers have reported the opposite such as Udoha et al. (2019) who reported an LD_{50} of OG of 2075 mg/kg, and Onaolapo and Onaolapo (2012) who reported 912.3 mg/kg for an oral ethanol extract. However, these differences in LD50 may be due to plant age, geographic location, season, and time of harvest, which have been reported to affect plant phytochemical compositions and ultimately toxicity (Okoduwa et al., 2017). OG would not show toxicity after acute administration of 5 g/kg body weight or less.

Repeated dose toxicity tests provide information on toxic effects, target organ identification, effects on animal physiology, hematology, biochemical profile, and histopathology (Kharchoufa et al., 2020).

Assessment of body weight during treatment with a defined compound provides information on overall animal health (Njan et al., 2019). A decrease in body weight or a decrease in weight gain may indicate various responses, including treatment-induced systemic toxicity (Rocha et *al*., 2012). Concerning body weight, the hydroethanolic extract of *O. gratissimum* did not induce significant changes in body weight in Wistar rats, indicating an absence of systemic toxicity. There were no clinical signs of toxicity or death during the experimental procedure.

In addition, the relative weight of an organ indicates whether the organ was exposed to injury or not. Injured organs often show abnormal atrophy (Wang et al., 2007). Evaluation of the weight of organs such as the liver, kidney, spleen, testis, heart, pancreas, and brain is very important in toxicological studies (Assih et al., 2021).

In the present study, the relative organ weights of all treated rats did not differ significantly from those of the control groups. This indicates that the extract did not affect appetite or have adverse effects on the growth of the animals. But *O. gratissimum* significantly increased abdominal fat weight at the dose of 1000 mg/kg. The increase in abdominal fat weight could be due to an effect on lipogenesis.

The results of the hematological evaluation in this study did not show significant changes in hematological parameters. However, the significant decrease in platelet count observed at 500 and 1000 mg/kg may be due to the antiproliferative effect on this cell line. The decrease in platelet count by the extract at all doses implies that the leaf extract affects hemostasis, which is controlled by platelets. It would seem likely that the extract also contains certain compounds such as coumarin and flavonoids that are capable of blocking the release of thrombopoietin (Zaragoza et al., 2016; Zaragozá et al., 2021). No significant changes were observed in total white blood cells and their differentials. As these cells are the main effectors of innate and adaptive immunity (Bidie et al., 2010; Kaur et al., 2014), it can be inferred that the extract does not affect immune responses. All observations indicate the non-hepatotoxic nature of the extract at the doses used in this study. Similar effects on platelets were observed by Ofem et al. (2012).

AST, ALT, ALP, and glucose are serum liver marker enzymes, and serum urea, uric acid, and creatinine are biomarkers of renal function (Corns, 2003). Serum creatinine, uric acid, and urea are common biomarkers for predicting renal dysfunction (Okoduwa et al., 2017).

ALP is present in the liver, bone, heart, skeletal muscle, kidney, brain, pancreas, and blood cells (Timbrell, 1996). The significant increase in ALP activity at 500 and 1000 mg/kg, respectively, compared to the control group may be due to impaired hepatobiliary function or may be due to unknown tissue damage (cytolytic effects) at this dose caused by some compounds such as alkaloids (Ke et al., 2017) present in the extract. The increase in ALP activity is then a hallmark of cholestasis characterized by insufficient biliary excretion (Odou, 2005).

Some biochemical parameters such as CPK, a marker level of cardiac injury (Dossou-Yovo et al., 2020), decreased significantly at 500 and 1000 mg/kg compared to the control group. This result may be due to the presence of compounds such as cardiac glycosides in the extracts. *O. gratissimum* is believed to have myo and cardioprotective properties.

Repeated administration of the aqueous extract of *O. gratissimum* leaves did not affect the lipid profile of the animals.

Renal function was assessed using important predictive biomarkers such as urea and creatinine. Creatinine and urea are non-protein nitrogen end products of protein metabolism that must be continuously eliminated. Therefore, increases in these indices of renal function indicate kidney dysfunction that is primarily caused by injury (Akindele et al., 2014). During deamination, NH_3 is removed from the blood by conversion to urea and an increase may be the result of elevated glomerular filtration (Olaniyan et *al*., 2016). The elevation of urea concentrations with subchronic administration of *O. gratissimum* leaf extract at 1000 mg/kg suggests efficient renal function produced by the extract for NH_3 removal (Njinga et *al*., 2020).

Estimation of electrolyte levels (Na^+, K^+, Cl^-, and Ca^{++}) in serum may be important in the assessment of renal function since the outcome of the mechanism of regulation of osmotic equilibrium and ionic loads can be determined by the electrolyte levels in the blood (Eccles, 1993).

Sodium is the major cation in the extracellular fluid, regulating the acid-base balance and protecting the body from excessive fluid loss. Potassium is the major intracellular cation and plays a similar role to sodium. However, imbalances in these ions may be due to renal failure or renal tubular acidosis, or alkalosis (Eccles, 1993; Holmes, 1993). In this study, serum electrolytes of rats exposed to subchronic doses of the extract were not significant.

It is possible that OG does not significantly influence electrolytes, acid-base balance, and water at these doses. This suggests that renal function is not compromised.

Conclusion

In this study, we evaluated the in vitro cytotoxicity of the hydroethanolic extract of *O. gratissimum* leaves on *Artemia salina* and its acute and subchronic toxicity by oral administration of the extract on female Wistar rats. The results suggest that acute administration of the aqueous extract of *O. gratissimum* is not associated with any signs of toxicity despite some changes mainly in hematological and biochemical parameters.

Therefore, some caution should be taken when administering *O. gratissimum* leaves for long periods.

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