Acute dysfunctional status of hepatorenal tissues of rats administered with leaf extracts of Ocimum gratissimum L. (Lamiaceae)

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ABSTRACT

Background: Given the vast medicinal properties of Ocimum gratissimum, the present study evaluated, in comparative terms, the acute dysfunctional status of hepatorenal tissues of Wistar rats administered with petroleum ether (PE) and ethyl acetate (EA) leaf extracts of O. gratissimum. Methods: Grouping of the experimental rats was assigned according to the treatments given, in which graded doses (200, 400, 600 and 800 mg/kg body weight (b.w.)) of PE and EA fractions of O. gratissimum leaf extract were administered to the rats by oral gavage on a daily basis for a period of 21 days. Serum levels of hepatorenal tissues biomarkers were measured using standard spectrophotometric methods. The organ-to-body weight ratio of the rats was measured on the 21st day of the experiment. Results: Serum aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio (i.e. AST/ALT) of the experimental rat groups was found to be within the range of 0.919 – 1.022 unit. The experimental rat groups administered with PE and EA fractions of O. gratissimum leaf extract showed dose-dependent increasing levels of serum alkaline phosphatase (ALP) activity. Likewise, rat groups administered with the herbal extracts exhibited increasing serum total bilirubin, urea and creatinine concentrations, in a dose-dependent manner. At the end of the 21-day treatment period, all the experimental rat groups showed increase in body weight, ranging from 0.79 – 1.98% increase. The liver weight and kidney weight to body weight ratios were within the range of (0.0468 ± 0.02 – 0.0981 ± 0.04) unit and (0.00245 ± 0.002 – 0.01968 ± 0.0007) unit, respectively. Conclusion: The results showed that doses of PE and EA fractions of O. gratissimum leaf extract greater than 400 mg/kg b.w. induced dose-dependent hepatorenal toxicity, with the EA fraction provoking greater toxicity than the PE fraction of O. gratissimum leaf extract.

Key words: Body weight, ethylacetate, hepatorenal, Ocimum gratissimum, petroleum ether

INTRODUCTION

In general terms, metabolic events within the liver and kidney are essential to ensure constancy in the internal environment of vertebrates1-4. The control mechanisms of metabolic events in the hepatocytes are regulated at the molecular, organelle, cellular and organ levels5,6. Endogenous metabolic control mechanisms of hepatocytes involve the actions of regulatory enzymes, organelles responsible for protein and lipid biosynthesis, as well as interactions of the hepatocytes with sinusoidal and Kupffer cells. Meanwhile, exogenous control mechanisms are accomplished by biochemical interactions between the liver and the musculature, as well as interactions among the renal, enteric and endocrine systems7. The metabolic heterogeneity of hepatocytes in health and disease is summarized elsewhere8-10. Routine clinical evaluation of the functional status of hepatocytes, the so-called liver function test (LFT)/biliary integrity test (BIT), is established by evaluating activities of non-functional plasma enzyme indicators, namely aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and the inductive hepatocyte smooth endoplasmic reticulum (SER) specific enzyme, γ-glutamyl transferase (γ-GT). As well, albumin, total bilirubin and total protein concentrations in blood samples are examined11-12. The nephron is the functional unit of the kidneys. The renal tissues are primarily involved in the removal of low plasma threshold substances, such as urea, creatinine and uric acid. The renal tissues also regulate blood electrolyte concentrations and, by extension, osmolality, extracellular fluid volume and acid-base balance of the vascular system. Furthermore, the kidneys are sites for the biosynthesis of steroid and polypeptide hormones, such as 1, 25 dihydroxyvitamin D, erythropoietin, and renin13,14. Elevations of plasma low threshold substances in the blood are indicative of compromised renal function. The kidney
function of the kidney function test (KFT) measures plasma creatinine and blood urea nitrogen (BUN) levels, among other blood indicators, such that their raised levels in the blood are diagnostic of presentation and progression of renal disease. The renal/kidney function test indicators are applied in monitoring the efficacy of therapeutic intervention against compromised renal function.

Ocimum gratissimum L. belongs to the family Lamiaceae. The plant is a perennial herb widely distributed in warm and temperate regions of the world. The phytochemical compositions of diethyl ether, ethyl acetate, ethanol and aqueous leaf extracts of Ocimum gratissimum have been exhaustively reported elsewhere, in which it was noted that Ocimum gratissimum contained relatively high quantities of alkaloids, flavonoids, saponins, methyl cinnamate, camphor, thymol, eugenol, linalool, citral, terpenes and lactones. Traditional medicine practitioners administer Ocimum gratissimum extracts for the treatment and management of fever, rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke, influenza, gonorrhrea and mental illness. The use of Ocimum gratissimum extracts by folklore medicine practitioners for the treatment of microbial infections has been validated by empirical investigations. There are empirical evidence that edible vegetables and medicinal plants contain deleterious phytochemicals which are usually eliminated by traditional and conventional processing methods prior to consumption of the plant materials. Some of these edible and medicinal plants have been reported to provoke organ dysfunction and systemic toxicity, especially when ingested in large quantities and unprocessed forms. However, the susceptibility of animal models to chemical-induced hepatic or systemic toxicity is regulated by genetic, environmental, dietary and pathophysiological factors. The vast medicinal properties of Ocimum gratissimum notwithstanding, the present study ascertained, in comparative terms, the acute dysfunctional status of hepatorenal tissues using blood levels of enzyme activities and metabolite profiles of hepatorenal origin, as well as organ/body weight indicator in Wistar rats administered with petroleum ether (PE) and ethyl acetate (EA) leaf extracts of Ocimum gratissimum.

METHODS

Collection and identification of leaf samples

Fresh and healthy leaves of Ocimum gratissimum were collected between the period of August 9th and September 2nd, 2019- from a private botanical garden located within Imo State University, Owerri, Nigeria (Latitude 5° 30.2237’N; Longitude 7° 2.6277’E). The leaves were identified and authenticated by a botanist. A voucher number (IMSUH: 021) was assigned to the leaf samples and, thereafter, deposited in the herbarium for reference purposes.

Preparation of leaf samples

The collected leaves of Ocimum gratissimum were washed using tap water and then transferred into an oven (WTC BINDER-7200 Oven, Tuttingen, Germany). The leaves were dried to constant weight at 50°C for 10-12 h. The dried leaf samples were pulverized and subsequently stored for use as previously described.

Extraction and fractionation of leaf extracts

A 500 g part of the pulverized leaf sample of Ocimum gratissimum was subjected to repeated hydro-ethanol (ratio: 2:3 v/v) extraction for 24 h using Soxhlet apparatus. The hydro-ethanolic leaf extract was fractionated according the methods previously described, but with minor modifications. Fractionation of the hydro-ethanolic leaf extract was carried out by successive partitioning using equal volumes of solvents in the order of increasing polarities, viz. PE > EA. The PE and EA fractions of leaf extract of Ocimum gratissimum were subsequently concentrated under reduced pressure for 12 h at 50°C in a rotary evaporator (Büch Rotavapor R-200, USA). The separate residues of PE and EA fractions of Ocimum gratissimum leaf extract were dried in a vacuum desiccator. The yield of the fractionated leaf extract of Ocimum gratissimum was calculated as the quotient of dried weight of the fractionated leaf extract to 100 g of the dried pulverized sample subjected to extraction protocol.

The dried PE and EA fractions of Ocimum gratissimum leaf extract were weighed and suspended in measured volumes of phosphate-buffered saline (PBS: pH=7.4), osmotically equivalent to 9.0 g/L NaCl [9.0 g NaCl, 1.71 g Na_{2}HPO_{4}.2H_{2}O and 2.43 g Na_{2}PO_{4}.2H_{2}O per liter] to give standard solutions. Graded doses (200, 400, 600 and 800 mg/kg body weight (b.w.)) of PE and EA fractions of Ocimum gratissimum leaf extract were formulated and administered to the rats.

Animal handling and experimental design

The male Wistar rats, within the ages of 7 – 9 weeks old and of average weight of 109.74 ± 2.81 g, were obtained from the Animal House of Imo State University, Owerri, Nigeria. Handling of the animals was
performed according to the methods previously described. The Ethical Committee on the use of animals for research, Department of Biochemistry, Imo State University, Owerri, Nigeria (Ethics Approval Number: ODVC/REN/1232/19) approved the present study. Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

A total of 54 rats were divided into 9 groups of 6 rats each. The rats were deprived of pelletized standard guinea feed (PSGF) (United Africa Company Nigeria Plc., Jos, Nigeria) and water 16 h prior to the commencement of treatment. The rats were deprived of pelletized standard feed for 24 h post-fasted rats were killed by cervical dislocation. At the end of the experimental time of 21 days, the 12 rats from each group were dissected to ascertain their respective weights, were done according to the methods previously described.

**RESULTS**

**Liver and kidney weights to body weight ratios**

The liver and right and left kidneys weights were measured on day 21. The organ weight and body weight were reported in grams. Thus:

\[
\text{Ratio of organ weight to body weight} = \frac{o.w.\text{AT}}{b.w.\text{AT}}
\]

Where:
- \(o.w.\text{AT}\): Organ weight after treatment on day 21
- \(b.w.\text{AT}\): Body weight after treatment on day 21
- \(o.w.\text{BT}\): Organ weight before treatment on day 21
- \(b.w.\text{BT}\): Body weight before treatment on day 0

**Data and statistical analyses**

The data collected were analyzed by the ANOVA procedure while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system package of Version 9.1 of 2006.

**Hepatorenal tissues biomarkers**

Serum levels of hepatorenal tissues biomarkers were measured; serum AST and ALT activities were assessed according to the methods of Henry et al., as described, and by serum ALP activity, serum total bilirubin concentration, serum urea concentration, and serum creatinine concentration.

**Body weight of rats**

The body weight of the rats was measured using electronic weighing balance (Digital Precision Weighing Balance (JCS-QC03) – China), on the 1st and 21st days of the experiment. Thus:

\[
\%\Delta_{b.w.} = \frac{(b.w.\text{AT}) - (b.w.\text{BT})}{b.w.\text{BT}} \times 100
\]

Where
- \(\%\Delta_{b.w.}\): Percentage change in body weight
- \(b.w.\text{AT}\): Body weight after treatment on day 21
- \(b.w.\text{BT}\): Body weight before treatment on day 0

**Collection and preparation of blood, liver and kidneys**

At the end of the experimental time of 21 days, the 12 h post-fasted rats were killed by cervical dislocation. Blood volumes of 0.5 mL were drawn from the orbital sinus of rats and allowed to clot. The serum was measured for hepatorenal tissues biomarkers. The collection and preparation of the liver and kidneys, in order to ascertain their respective weights, were done according to the methods previously described.
b.w. PE and EA fractions of *O. gratissimum* leaf extract (Group 2$_{PE200}$ = 50.97 ± 2.12 U/L + Group 6$_{EA200}$ = 51.97 ± 2.32 U/L) were not significant different ($p > 0.05$) from that of Group 1$_{CONTROL}$ (45.53 ± 1.92 U/L). Likewise, serum AST activities of the experimental rat groups administered with 400 mg/kg b.w. PE fraction of *O. gratissimum* leaf extract of Group 3$_{PE400}$ (49.74 ± 1.99 U/L) versus Group 1$_{CONTROL}$ (45.53 ± 1.92 U/L) showed no significant difference ($p > 0.05$).

The experimental rat group administered with 400 mg/kg b.w. EA fraction of *O. gratissimum* leaf extract (Group 7$_{EA400}$ = 59.62 ± 2.12 U/L) showed serum AST activity that was significantly higher ($p < 0.05$) than the group administered with PE fraction of *O. gratissimum* leaf extract (Group 3$_{PE400}$ = 49.74 ± 2.02 U/L).

Figure 1 showed that the serum AST activities of the experimental rat groups administered with 600 mg/kg b.w. and 800 mg/kg b.w. PE and EA fractions of *O. gratissimum* leaf extract (Group 4$_{PE600}$ = 63.51 ± 2.33 U/L + Group 8$_{EA600}$ = 81.8 ± 3.32 U/L and Group 5$_{PE800}$ = 76.84 ± 3.05 U/L + Group 9$_{EA800}$ = 89.57 ± 3.81 U/L) were significantly higher ($p < 0.05$) than that of Group 1$_{CONTROL}$ (45.53 ± 1.92 U/L). An overview of Figure 1 showed increasing levels of serum AST activities of the herbal-treated groups, which occurred in a dose-dependent manner when compared with Group 1$_{CONTROL}$.

Serum ALT activities of rats administered with fractions of *O. gratissimum* leaf extract

Figure 2 showed that serum ALT activity of Group 1$_{CONTROL}$ (49.49 ± 1.52 U/L) was not significantly different ($p > 0.05$) from those of the experimental rat groups administered with 200 mg/kg b.w. PE and EA fractions of *O. gratissimum* leaf extract (Group 2$_{PE200}$ = 52.06 ± 1.82 U/L + Group 6$_{EA200}$ = 52.09 ± 1.71 U/L), as well as the 400 mg/kg b.w. PE fraction of *O. gratissimum* leaf extract (Group 3$_{PE400}$ = 50.04 ± 1.60 U/L).

Specifically, serum ALT activity of the rat groups administered with herbal extract was such that Group 8$_{EA600}$ = 80.03 ± 3.38 U/L > Group 4$_{PE600}$ = 64.08 ± 2.32 U/L, and Group 9$_{EA800}$ = 87.6 ± 3.85 U/L > Group 5$_{PE800}$ = 76.41 ± 2.02 U/L; $p < 0.05$. Furthermore, Figure 2 showed dose-dependent increasing levels of serum ALT activities of the rat groups administered with herbal extract.

Table 1 showed that the serum AST/ALT ratio of the experimental rat groups was within the range of 0.919 – 1.022 unit. Furthermore, an overview of Table 1 showed that serum AST/ALT ratios of Group 1$_{CONTROL}$, as well as Group 2$_{PE200}$ — Group 4$_{PE600}$ were less than 1.0 unit, whereas those of Group 5$_{PE800}$ — Group 9$_{EA800}$ were greater than 1.0 unit.

Serum ALP activities of rats administered with fractions of *O. gratissimum* leaf extract

Figure 3 showed that serum ALP activities of the experimental rat groups administered with 200 mg/kg b.w. and 400 mg/kg b.w. PE and EA fractions of *O. gratissimum* leaf extract (Group 2$_{PE200}$ = 132.56 ± 5.12 U/L + Group 6$_{EA200}$ = 133.8 ± 5.09 U/L and Group 3$_{PE400}$ = 136.78 ± 5.21 U/L + Group 7$_{EA400}$ = 139.28 ± 5.74 U/L) were not significantly different ($p > 0.05$) from that of Group 1$_{CONTROL}$ (124.56 ± 4.34 U/L).

Serum ALP activity of Group 4$_{PE600}$ (153.19 ± 6.74 U/L) was not significantly different ($p < 0.05$) from that of Group 8$_{EA600}$ (167.48 ± 6.88 U/L). Likewise, serum ALP activity of Group 5$_{PE800}$ (158.98 ± 5.28 U/L) and Group 9$_{EA800}$ (171.68 ± 6.93 U/L) showed no significant difference ($p > 0.05$). Figure 3 showed dose-dependent increasing levels of serum ALP activities of experimental rat groups administered with PE and EA fractions of *O. gratissimum* leaf extract.

Serum bilirubin concentrations of rats administered with fractions of *O. gratissimum* leaf extract

Serum total bilirubin concentrations of Group 2$_{PE200}$ (1.23 ± 0.08 mg/dL) and Group 6$_{EA200}$ (1.50 ± 0.07 mg/dL) showed no significant difference ($p > 0.05$) compared with that of Group 1$_{CONTROL}$ (1.00 ± 0.04 mg/dL) (Figure 4). Although serum total bilirubin concentrations of Group 3$_{PE400}$ (1.98 ± 0.09 mg/dL) and Group 7$_{EA400}$ (2.03 ± 0.09 mg/dL) showed no significant difference ($p > 0.05$), their corresponding

| Table 1: Serum AST/ALT ratios of experimental rat groups |
|---------------------------------------------------------------|
| Rat Groups | AST/ALT |
| Group 1$_{CONTROL}$ | 0.919 |
| Group 2$_{PE200}$ | 0.979 |
| Group 3$_{PE400}$ | 0.994 |
| Group 4$_{PE600}$ | 0.991 |
| Group 5$_{PE800}$ | 1.005 |
| Group 6$_{EA200}$ | 0.997 |
| Group 7$_{EA400}$ | 1.002 |
| Group 8$_{EA800}$ | 1.022 |
| Group 9$_{EA800}$ | 1.022 |
values were significantly higher \((p < 0.05)\) than that of Group 1\(_{\text{CONTROL}}\) \((1.00 \pm 0.04 \text{ mg/dL})\).

Figure 4 showed that increase in the administered doses of PE and EA fractions of \(O.\ gratissimum\) leaf extract caused increasing serum bilirubin concentrations of the experimental rat groups in a dose-dependent manner.

**Serum urea concentrations of rats administered with fractions of \(O.\ gratissimum\) leaf extract**

Figure 5 showed that serum urea concentrations of the experimental rat groups were such that Group 2\(_{\text{PE200}}\) \((12.26 \pm 2.04 \text{ mg/dL})\), Group 6\(_{\text{EA200}}\) \((11.33 \pm 1.94 \text{ mg/dL})\) and Group 7\(_{\text{EA400}}\) \((13.83 \pm 1.99 \text{ mg/dL})\) were not significantly different \((p > 0.05)\) from that of Group 1\(_{\text{CONTROL}}\) \((12.78 \pm 1.15 \text{ mg/dL})\). Addition-
ally, serum urea concentration of Group 3<sub>PE<sub>400</sub></sub> (16.6 ± 1.75 mg/dL) was significantly higher (p < 0.05) than that of Group 7<sub>EA<sub>400</sub></sub> (13.83 ± 1.12 mg/dL). The herbal extract- administered rat groups exhibited increasing serum urea concentration in a dose-dependent manner. In comparative terms, serum urea concentrations of Group 4<sub>PE<sub>600</sub></sub> (22.67 ± 2.32 mg/dL) and Group 8<sub>EA<sub>600</sub></sub> (22.00 ± 2.04 mg/dL), as well as Group 5<sub>PE<sub>800</sub></sub> (29.67 ± 2.48 mg/dL) and Group 9<sub>EA<sub>800</sub></sub> (29.67 ± 2.38 mg/dL), showed no significant difference (p > 0.05).

Serum creatinine concentrations of rats administered with fractions of <i>O. gratissimum</i> leaf extract

Serum creatinine concentrations of Group 2<sub>PE<sub>200</sub></sub> (1.07 ± 0.08 mg/dL) and Group 6<sub>EA<sub>200</sub></sub> (1.27 ± 0.13 mg/dL) showed no significant difference (p > 0.05).
Figure 5: Serum urea concentrations of experimental rat groups administered PE and EA fractions of *O. gratissimum* leaf extract. Means of serum urea concentrations of bars with hash tag (#) are not significantly different from that of the CONTROL at p > 0.05 according to LSD.

Figure 6: Serum creatinine concentrations of experimental rat groups administered PE and EA fractions of *O. gratissimum* leaf extract. Means of serum creatinine concentrations of bars with hash tag (#) are not significantly different from that of the CONTROL at p > 0.05 according to LSD.
from that of Group 1CONTROL (0.97 ± 0.02 mg/dL) (Figure 6). Additionally, Figure 6 showed that the experimental rat groups administered with PE and EA fractions of O. gratissimum leaf extract, at doses greater than 400 mg/kg b.w., exhibited serum creatinine concentrations that were significantly higher ($p < 0.05$) than that of Group 1CONTROL (0.97 ± 0.02 mg/dL). Serum creatinine concentration of Group 7EA400 (2.03 ± 0.10 mg/dL) was significantly higher ($p < 0.05$) than that of corresponding Group 5PE400 (1.40 ± 0.07 mg/dL). On the contrary, serum creatinine concentrations of Group 4PE600 (3.13 ± 0.16 mg/dL) and Group 8EA600 (2.80 ± 0.12 mg/dL), as well as Group 5PE800 (4.50 ± 0.20 mg/dL) and Group 9EA800 (4.50 ± 0.18 mg/dL), showed no significant difference ($p > 0.05$).

**Body weights and organ-to-body weight ratios of rats administered with fractions of O. gratissimum leaf extract**

At the end of the 21-day treatment period, all the experimental rat groups exhibited increase in body weight within the range of 109.11 ± 1.03 to 112.26 ± 1.01 g (Table 2). Additionally, Table 2 showed that Group 2PE200 (110.98 ± 0.18 g) exhibited comparatively the highest gain in body weight after treatment, whereas Group 8EA600 (110.37 ± 1.04 g) gave the lowest gain in body weight. Overall, the gain in body weight of the experimental rat groups varied within the range of 0.79 – 1.98%. Specifically, the cumulative gain in body weight of the herbal extract-treated rat groups was such that Group 2PE200 — Group 5PE800 (1.98 – 1.03%) was greater than that of Group 6EA200 — Group 9EA800 (0.95 – 0.79%). The relative gain in body weight of the Group 1CONTROL (112.65 ± 1.01 g) was greater than that of the herbal treated rat groups, except that of Group 2PE200 (110.98 ± 1.08 g).

Table 2 showed that the liver weight to body weight ratios of Group 2PE200 (0.0542 ± 0.02) and Group 3PE400 (0.0582 ± 0.02) were not significantly different ($p > 0.05$) from that of Group 1CONTROL (0.0468 ± 0.02). The cumulative liver weight to body weight ratio of Group 2PE200 — Group 5PE800 was comparatively greater than those of Group 6EA200 — Group 9EA800. Similarly, there was no significant difference ($p > 0.05$) between the kidney weight to body weight ratio of Group 2PE200 (0.00260 ± 0.002) and Group 3PE400 (0.00379 ± 0.003) (Table 2). However, further increase in the experimental dose of the herbal extract caused increased kidney weight to body weight ratio. The liver weight and kidney weight to body weight ratios of Group 2PE200 were significantly different ($p < 0.05$) from the corresponding Group 2EA200.

**DISCUSSION**

The combinations of distinctive molecular species present in PE and EA fractions of O. gratissimum leaf extract obviously dictated the toxic outcomes in the experimental rats. Chemical-induced hepatorenal injuries and resultant dysfunction is often initiated by metabolic transformation of molecular species to reactive intermediate species, such as electrophiles, which alter the function and structure of cellular macromolecules. Measurement and evaluation of blood indices are fundamental in establishing the pathological and physiological statuses relevant to the clinician, nutritionist and toxicologist. The liver is primarily rich in aminotransferases, namely AST and ALT, such that their presence in the blood system indicates hepatic necrosis as well as extrahepatic tissue damage or both. The findings of the present study suggest that doses of PE (≥ 600 mg/kg b.w.) and EA (≥ 400 mg/kg b.w.) fractions of O. gratissimum leaf extract administered to the rats provoked hepatic tissue injuries by virtue of the reported serum AST activities of the experimental rat groups. Likewise, the pattern of serum ALT activities of the experimental rat groups administered with PE and EA fractions of O. gratissimum leaf extract exhibited a mutual relationship with serum AST activities in terms of the dose-depended elevation of serum AST activity. Extrahepatic tissues contain appreciable quantities of the aminotransferases, whereby their raised levels in serum are also diagnostic of extrahepatic tissues necrosis. However, the measure of elevated serum AST and ALT activities are non-specific confirmatory tests for hepatic functional status. Accordingly, for the purpose of differential diagnosis, evaluation of serum AST/ALT ratio is applied in order to ascertain the severity and pathologic status of the animal, as well as to identify and confirm the organ of pathologic interest. For instance, serum AST/ALT ratio > 1 unit indicates advanced liver fibrosis and chronic hepatitis, whereas serum AST/ALT ratio of 0.9 is diagnostic of nonalcoholic steatohepatitis. Based on serum aminotransferases indicators, the present study showed that administration of PE and EA fractions of O. gratissimum leaf extract did not substantially cause hepatic dysfunction at relatively low dose of less than 400 mg/kg b.w.; however, the rats exhibited acute hepatic dysfunction following the administration of relatively higher doses of PE and EA fractions of O. gratissimum leaf extract. Elevation of ALP in the blood is linked to pathology of the liver as well as the mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone,
and placenta. Serum ALP activities of experimental rat groups administered with PE and EA fractions of *O. gratissimum* leaf extract at doses greater than 600 mg/kg b.w. were substantially higher than the control rat group, which further confirmed a compromised hepatobiliary function and was in agreement with previous reports. Additionally, previous studies had noted that mild elevation of ALP activity in the blood was indicative of cirrhosis, hepatitis, and congestive cardiac failure. Serum total bilirubin concentrations of the experimental rat groups also confirmed that at relatively low dose, PE and EA fractions of *O. gratissimum* leaf extract did not provoke hepatic dysfunction. Hyperbilirubinemia, which is diagnostic of hepatic dysfunction and hemolytic disorders, is diagnostic when blood serum total bilirubin concentration is greater than 1.0 mg/dL. Accordingly, serum total bilirubin concentrations of the experimental rat groups administered with 200 mg/dL PE and EA fractions of *O. gratissimum* leaf extract did not exhibit hepatic dysfunction and hemolytic disorders. Nevertheless, higher doses of PE and EA fractions of *O. gratissimum* leaf extract elicited hyperbilirubinemia, which was indicative of compromised hepatic dysfunction in the rats. On the contrary, aqueous leaf extract of *O. gratissimum* was reported to enhance hematological parameters following oral administration to experimental rats. It therefore implies that the phytocomponents from aqueous leaf extract of *O. gratissimum*, as compared to PE and EA fractions of *O. gratissimum*, did not provoke hemolytic disorders and hepatic dysfunction; the blood bilirubin concentration was greater than the upper normal limit of the reference range of blood bilirubin concentration. The findings of the present study showed that the pattern of renal tissue dysfunction appeared to correspond to that of hepatic tissues following the administration of PE and EA fractions of *O. gratissimum* leaf extract. Specifically, elevation of serum urea and creatinine concentrations of the experimental rat groups suggest that the severity of compromised renal function was dose-dependent on the administered PE and EA fractions of *O. gratissimum* leaf extract. In a related research finding, Goniothalamus (GTN), which is a phytocompound from several plants of the genus Goniothalamus, engendered dose-dependent renal dysfunction in Sprague-Dawley rats. Contrary to the outcome of the present study, Ogundipe et al. reported that aqueous leaf extract of *O. gratissimum* ameliorated gentamicin-induced renal tissues injury in rats. However, based on empirical evidence of low creatinine clearance after 28 days of treatment, they noted that the risk profile of renal dysfunction is not unlikely following the administration of aqueous leaf extract of *O. gratissimum*. Another report showed that aqueous leaf extract

### Table 2: Body weight and organ-to-body weight ratio of experimental rat groups

| Groups | Body weight (g) | %Δb.w. | L/b.w.-R | K/b.w.-R |
|--------|----------------|--------|----------|----------|
|        | b.w.-BT | b.w.-AT |          |          |
| Group 1 | CONTROL | 110.50 ± 1.06 | 112.65 ± 1.01 | 1.95 | 0.0468 ± 0.02 | 0.00245 ± 0.002 |
| Group 2 | PEC200 | 108.83 ± 1.06 | 110.98 ± 1.08 | 1.98 | 0.0542 ± 0.02 | 0.00260 ± 0.002 |
| Group 3 | PEC400 | 109.50 ± 1.00 | 111.33 ± 1.01 | 1.67 | 0.0582 ± 0.02 | 0.00379 ± 0.003 |
| Group 4 | PEC600 | 110.67 ± 1.09 | 112.37 ± 1.08 | 1.54 | 0.0949 ± 0.05 | 0.00836 ± 0.004 |
| Group 5 | PEC800 | 108.00 ± 1.06 | 109.11 ± 1.03 | 1.03 | 0.0981 ± 0.04 | 0.00890 ± 0.005 |
| Group 6 | EA200 | 111.50 ± 1.09 | 112.56 ± 1.08 | 0.95 | 0.0629 ± 0.04 | 0.00456 ± 0.004 |
| Group 7 | EA400 | 109.50 ± 1.01 | 110.47 ± 1.05 | 0.89 | 0.0678 ± 0.04 | 0.00483 ± 0.004 |
| Group 8 | EA600 | 109.50 ± 1.09 | 110.37 ± 1.04 | 0.79 | 0.0996 ± 0.05 | 0.00978 ± 0.005 |
| Group 9 | EA800 | 109.67 ± 1.09 | 110.68 ± 1.04 | 0.92 | 0.0972 ± 0.05 | 0.01968 ± 0.007 |

*b.w.-BT*: Body weight before treatment  
*b.w.-AT*: Body weight after treatment  
%Δ*b.w.*: Percentage change in body weights  
L/b.w.-R: Liver weight to body weight ratio  
K/b.w.-R: Kidney weight to body weight ratio

* Asterisk (*): Not significantly different from Group 1 at *p* > 0.05 according to LSD.  
The mean (X) ± S.D of six (*n = 6*) determinations. Means in the column with the same letter are not significantly different at *p* > 0.05 according to LSD.
CONCLUSION
For the most part, the administration of PE and EA fractions of *O. gratissimum* leaf extract at a dose less than 200 mg/kg b.w. did not cause hepatorenal toxicity in the experimental rats. On the contrary, doses of PE and EA fractions of *O. gratissimum* leaf extract greater than 400 mg/kg b.w. caused dose-dependent hepatorenal toxicity, with the EA fraction provoking greater toxicity than the PE fraction of *O. gratissimum* leaf extract. Further investigations are required in order to identify, quantify, and characterize the molecular species present in the PE and EA fractions of *O. gratissimum* leaf extract that elicited the toxic outcomes in the experimental rats.

ABBREVIATIONS
ALP: Alkaline phosphatase
ALT: Alanine transaminase
AST: Aspartate transaminase
EA: Ethylacetate
PE: Petroleum ether

COMPETING INTERESTS
Authors declare that there are no conflicts of interests.

AUTHORS’ CONTRIBUTIONS
PCC; conceived and designed the research and supervised the laboratory work. PCC prepared the manuscript. PCC/FOO/VNI/VUE; collected the plant samples and carried out the laboratory work. PCC/VNI/VUE; analyzed the data. Authors declare that there are no conflicts of interests.

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