Toxicity of Olea africana in Artemia Salina and Mice

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Abstract

The current study investigated the toxicity of the ethanol extract of*Olea africana* in mice and brine shrimp *(Artemia salina)*. Brine shrimp cytotoxicity and acute toxicity of the extract in mice were observed over 24 hours. Subacute toxicity of the extract was studied in mice for 28 days using animal weight, organ-to-body weight ratio, and hematological, biochemical and histological parameters as indicators. Data analysis was done using Probit regression and Two Way ANOVA *($p<0.05$)*. The lethal concentration of the extract responsible for 50% mortality in brine shrimp (LC_{50}) was 2257.84 µg/mL (702.97-7367.95), while the lethal dose of the extract responsible for 50% mortality in mice (LD_{50}) was 4297.30 mg/kg. There were no significant differences in the mean weight or organ-to-body weight ratio of the control and treatment group mice. Some hematological and biochemical parameters in extract-treated mice were significantly different from control group mice. Adverse histopathological changes including fibrosis around the hepatic artery, degeneration of hepatocytes, congestion, and mild fibrosis around the portal vein and artery were observed in the livers of extract-treated mice. Perivascular infiltration, fibrosis, mild glomerular degeneration, mild tubular degeneration, cell infiltration, and hemorrhage were observed in the kidneys of extract-treated mice. Given these findings, prolonged administration of *Olea africana* is associated with significant toxic concern. As a result, caution should be exercised when using the extract.

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1. Background

For the most part, herbal medicines have been regarded as safe and effective alternatives to conventional medicine[1][2]. The perceived lack of side effects has been the strongest selling point of herbal medicine [3][4][5]. This misconception, however, has led to the indiscriminate use of herbal medicine formulations [6][7][8]. Furthermore, long-term use of these formulations, as well as a lack of proper dosage guidelines, highlight the need to investigate the oral toxicity of such formulations.

Olea africana is an ornamental tree with green, glossy leaves, a large trunk, and inconspicuous but fragrant flowers[9]. It is found in Asia (South East) and Africa (East and South). In folk medicine, various communities use the plant to treat urinary tract and eye infections, kidney problems, sore throat, headaches, and backaches. It is also used as a styptic, emollient, hypotensive, antimalarial and febrifuge [10][11][12]. The scientific literature is replete with pharmacological reports on *Olea africana's* anthelmintic, antibacterial, antihypertensive, and anti-diarrheal activities [10][11][13][14]. Moreover, triterpenoids, coumarins, secoiridoid glucosides, phenolic glucosides, and lignans are among the phytochemical compounds found in *Olea africana* [11][15][16][17][18]. Others include saponins, alkaloids, tannins, flavonoids, anthraquinones, and glycosides [13]. However, studies on the safety of *Olea africana* are scarce [19]. As a result, the current study investigated the toxicity of the ethanol extract of *Olea africana* in two animal models; mice and brine shrimp.

2. Methods

2.1. Ethical statement

Written permission was sought from the institutional ethics committee (University of Nairobi; **FVM BAUEC/2019/199)**

before the study began.

2.2. Collection of the plant

Olea africana was collected in Samburu, Kenya and authenticated at the National Museums of Kenya Herbarium. A voucher specimen was deposited.

2.3. Sample preparation and extraction

20 Kgs of air-dried *Olea africana* leaves were weighed, transferred to a mechanical mill (Christy, Hunt Engineering Ltd, Atlas Works, Earls Colne, Essex, England) and pulverized into a fine powder. About 0.2 grams of this powder was weighed, and 1 liter of ethanol (Loba Chemie, India) was added, shaken for 3 days, and filtered. The filtrate was rotary evaporated at 40^{o}C and the percentage yield was calculated[20].

2.4. Animals used in experiments

Brine shrimp eggs (Artemio® mix) were purchased commercially and subsequently hatched. For the brine shrimp cytotoxicity test, *Artemia salina* (brine shrimp) larvae were used. Mice were obtained from the University of Nairobi. They were fed commercial mice pencils (Unga feeds) with free access to water. The mice were housed in polypropylene cages and given time to acclimatize to conditions in the laboratory. An alternate light and dark cycle was maintained.

2.5. Brine shrimp cytotoxicity assay

Two hundred and fifty brine shrimp larvae were transferred to 25 plastic tubes of 5 mL. Ten larvae were transferred to each tube with group 1 as the negative control group, group 2 as the positive control group (vincristine sulphate only), while groups 3, 4, and 5 contained 10 µg/mL, 100 µg/mL, and 100 µg/mL of the extract [21]. Observations were made after 24 hours and the larvae which survived were used to calculate the LC_{50} of the extract using probit analysis[22].

2.6. Acute toxicity assay in mice

The methods of Muhammad et al[23] and Olaniyan et al [24] were used with modifications. Briefly, 35 female mice were fasted overnight, weighed, and randomly assigned to one of seven groups as follows: Group I animals received distilled water only; Group II animals received 2000 mg/kg, Group III animals received 2048 mg/kg, Group IV animals received 2560 mg/kg, Group V animals received 3200 mg/kg, Group VI animals received 4000 mg/kg, and Group VII animals received 5000 mg/kg. All the animals were observed for 24 hours [23][24].

2.7. Sub-acute toxicity in mice

The method of Olaniyan et al[24] was used. Briefly, 28 female mice were randomly assigned to four polypropylene cages (1, 2, 3, and 4), each housing four mice. Cage 1 served as the control and mice in this cage were given distilled water

orally once daily for 28 days. Mice in cages 2, 3, and 4 were given 100 mg/kg (low), 300 mg/kg (intermediate) and 600 mg/kg (high) body-weight doses of the extract once daily for 28 days [24]. The mice were monitored for toxicity. All surviving mice were fasted for 24 hours after the last treatment was administered, and blood was collected via cardiac puncture into appropriate heparinized or non-heparinized bottles [24]. The mice were anaesthetized humanely with intraperitoneal ketamine injections before the liver and kidneys were surgically removed, cleaned with physiological buffer saline, and weighed [24]. The mean organ-to-body weight ratio and the percentage mean weight gain of treatment and control group mice were calculated [24].

2.8. Hematological parameters

On the 29th day of the experiment, and following an overnight fast, blood from the animals was collected in heparinized bottles and used in the analysis of hematological parameters such as hemoglobin (Hb), hematocrit (HCT) red blood cell (RBC), MCV (mean corpuscular volume), mean corpuscular hemoglobin (MCH), and total white blood cells (WBCs) at the hematology department of the Kenyatta National Hospital.

2.9. Biochemical parameters

On the 29th day of the experiment, and following an overnight fast, blood from the animals was collected in non-heparinized tubes and centrifuged at 3000 r/min for 10 minutes to prepare serum. The levels of sodium, alanine aminotransferase (ALT), direct bilirubin, aspartate aminotransferase (AST), potassium, alkaline phosphatase (ALP), total protein, albumin, gamma glutaryl transferase (GGT), and total bilirubin (TB) in control and treatment group mice were evaluated at the Biochemistry department of the Kenyatta National Hospital.

2.10. Histopathology

The liver and kidneys were harvested from the sacrificed experimental mice and fixed in 10% buffered formaldehyde in labeled bottles before being processed for histological examination. The tissues were embedded in paraffin wax after which sectioning was done to 5 um thickness. Staining of the sectioned tissues was done with hematoxylin and eosin. The stained tissues were mounted on glass slides and examined under a standard light microscope [23].

2.11. Data analysis

Data from treatment and control groups were summarized using mean ± SEM (standard error of the mean) and analyzed using two-way Analysis of Variance (ANOVA) and Dunnet's post hoc test ($p < 0.05$) on GraphPad Prism statistical software (USA) version 8.0.

3. Results

The toxicity of the extract in *Artemia salina* and mice is summarized in (Table 1).

Table 1: Toxicity of *Olea africana* in *Artemia salina* and mice

Sample	*Artemia salina* [a]	Mice [b]
Ethanol leaf extract of *O. africana*	2275.84 (702.97-7367.95)	4297.30

[a] *Presented as median lethal concentration in µg/mL, with 95% confidence intervals in parentheses*

[b] *Presented as median lethal dose in mg/kg*

The toxicity of the extract in *Artemia salina* was 2275.84 (702.97-7367.95) µg/mL while the toxicity of the extract in mice was 4297.30 mg/kg. (Table 1).

A comparison of the mean weight gain (%) between treatment and control group mice is shown in (Figure 1.)

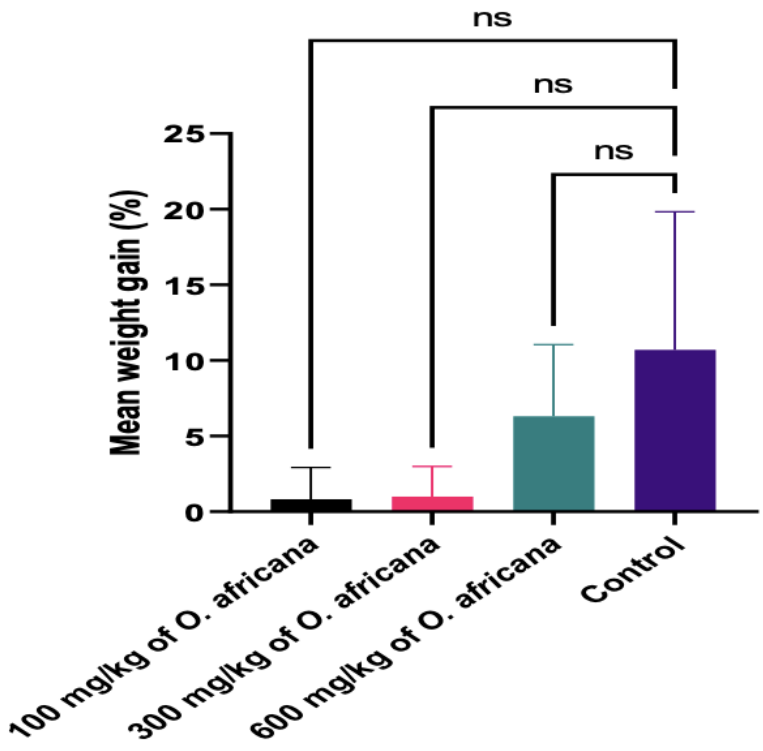

Figure 1. A comparison of the mean weight gain (%) of mice who received graded doses of the ethanol extract of *Olea africana* and mice who received distilled water only.

The mean weight gain (%) in animals given only distilled water did not differ significantly from the mean weight gain (%) in mice given 100 mg/kg *(p=0.0565)*, 300 mg/kg *(p=0.0609)*, or 600 mg/kg *(p=0.5334)* doses of the extract. (See Figure. 1).

The mean organ-to-body weight ratio in mice given the extract over 28 days is summarized in (Figure 1).

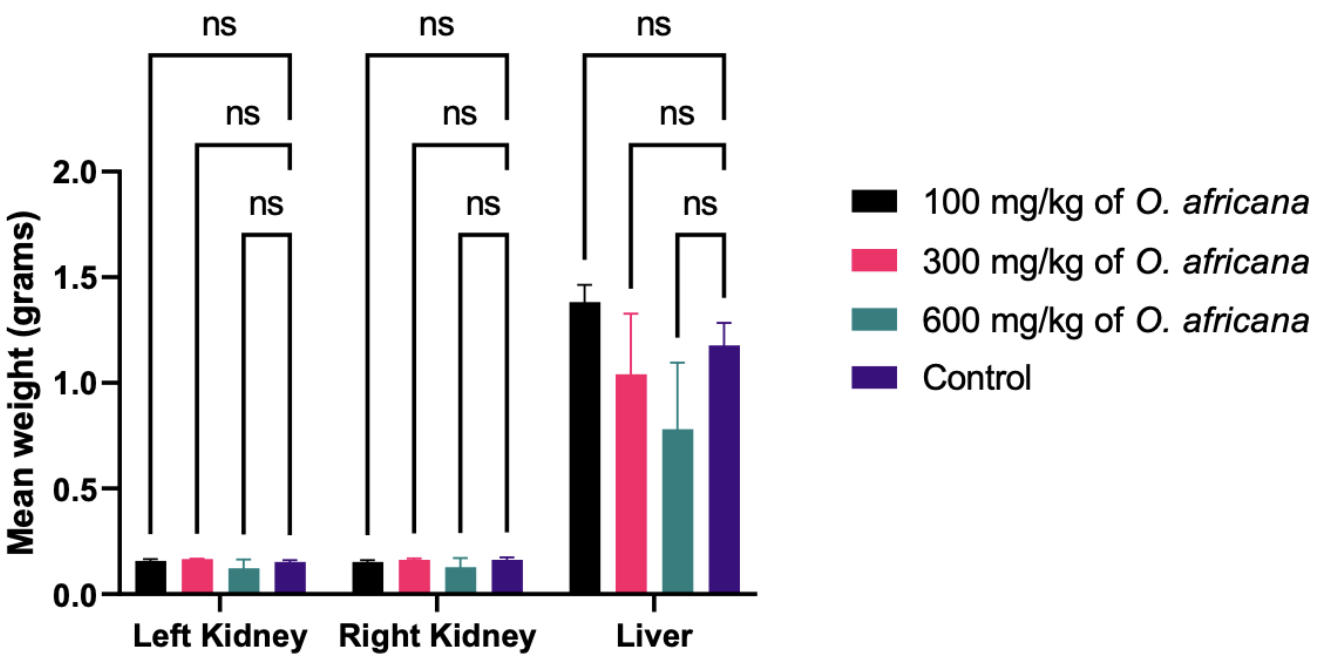

Figure 2: The mean organ-to-body weight ratio of mice given the ethanol leaf extract of *Olea africana* over 28 days

The mean left kidney-to-body weight ratio of mice given only distilled water was not significantly different from the body weight ratio in mice given 100 mg/kg *(p>0.9999)*, 300 mg/kg *(p=0.9997)*, or 600 mg/kg *(p=0.9969)* dose of the extract. (Figure 2).

The mean right kidney-to-body weight ratio in mice given distilled water only was not significantly different from the body weight ratio in mice given 100 mg/kg *(p>0.9999)*, 300 mg/kg *(p>0.9999)*, or 600 /kg *(p=0.9952)* dose of the extract. (Figure 2).

The mean liver-to-body weight ratio in mice given distilled water only was not significantly different from the body weight ratio in mice given 100 mg/kg *(p=5543)*, 300 mg/kg *(p=0.7986)*, or 600 mg/kg *(p=0.0961)* dose of the extract. (Figure 2).The effect of the ethanol extract of *Olea africana* on biochemical parameters in mice over a 28-day period is shown in Table 2.

Table 2. Effect of the ethanol extract of *Olea africana* on biochemical parameters in mice over a 28-day period

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Albumin (g/dL)	Total protein (g/dL)	Direct bilirubin (g/dL)	Total bilirubin (g/dL)	Sodium (g/dL)	Potassium (g/dL)
Control	45.25 (0.96)^{a}	151.0 (9.06)^{a}	45.50 (6.95)^{a}	12.00 (2.16)^{a}	35.25 (0.96)^{a}	65.25 (0.96)^{b}	24.16 (4.27)^{a}	33.77 (5.61)^{b}	148.50 (0.58)^{b}	7.13 (0.17)^{b}
100 mg/kg	75.00 (2.16)^{b}	246.5 (1.29)^{c}	61.00 (0.82)^{b}	9.25 (0.96)^{a}	34.50 (1.29)^{a}	66.75 (1.71)^{b}	24.68 (0.13)^{a}	34.40 (0.18)^{b}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
300 mg/kg	67.25 (9.91)^{b}	230.5 (19.02)^{c}	41.25 (3.86)^{a}	38.25 (18.41)^{b}	35.77 (2.50)^{a}	65.70 (1.78)^{b}	25.52 (4.76)^{a}	25.43 (1.71)^{a}	145.50 (1.29)^{b}	8.98 (0.28)^{c}
600 mg/kg	55.25 (1.26)^{a}	185.8 (2.22)^{b}	59.50 (1.29)^{b}	2.50 (1.73)^{a}	35.05 (0.21)^{a}	61.12 (0.51)^{a}	61.50 (1.29)^{b}	94.25 (2.50)^{c}	36.25 (72.50)^{a}	10.35 (0.24)^{d}

Values are expressed as mean ± standard deviation. Means with different superscripts along the columns are significantly different ($p<0.05$).

The mean ALT levels in mice given distilled water only were significantly lower than those in mice given 100 mg/kg (*$p<0.0001$)* or 300 mg/kg *($p=0.0007$)* dose of the extract. (Table 2). There was no significant difference in the mean ALT levels between mice that received only distilled water and mice given 600 mg/kg of the extract *($p=0.1813$)*. (Table 2).

The mean AST levels in mice given only distilled water were significantly lower than the mean AST levels in mice given 100 mg/kg (*$p<0.0001$)*, 300 mg/kg *($p<0.0007$)* or 600 mg/kg *($p<0.0001$)* dose of the extract. (Table 2).

The mean albumin levels in mice who received distilled water were not significantly different from the mean albumin levels in mice treated with 100 mg/kg *($p=0.9190$)*, 300 mg/kg *($p=0.9694$)*, or 600 mg/kg *($p=0.9982$)* dose of the extract. (Table 2).

The mean levels of total protein in mice given only distilled water were not significantly different from the mean levels of total protein in mice given 100 mg/kg *($p=0.6070$)* or 300 mg/kg *($p=0.9802$)* of the extract. (Table 2). However, the mean levels of total protein in mice given 600 mg/kg of the extract were significantly lower *($p=0.0167$)* than the mean levels of total protein in mice given distilled water (Table 2).

The mean levels of direct bilirubin in mice given distilled water only were not significantly different from the mean levels of direct bilirubin in mice given 100 mg/kg *($p=0.2703$)*, or 300 mg/kg *($p=0.6726$)* dose of the extract. (Table 2). The mean levels of direct bilirubin in mice treated with 600 mg/kg of the extract were significantly higher *($p<0.0001$)* than the mean levels of direct bilirubin in mice who were given distilled water. (Table 2).

The mean total bilirubin levels in mice given only distilled water were not significantly different from the mean total bilirubin levels in mice treated with 100 mg/kg *(p=0.9503)* of the extract. (Table 2). However, the mean levels of total bilirubin in mice given a 300 mg/kg dose of the extract were significantly lower *(p<0.0001)* than the mean levels of total bilirubin in mice given distilled water only. (Table 2). Furthermore, the mean total bilirubin levels in mice given a 600 mg/kg dose of the extract were significantly higher *(p<0.0001)* than the mean total bilirubin levels in mice given distilled water. (Table 2).

The mean sodium levels in mice given 600 mg/kg of the extract were significantly lower*(p<0.0001)* than the mean sodium levels in mice given distilled water (Table 2). There was no significant difference *(p=0.9968)* between the mean sodium levels in mice that received 300 mg/kg of the extract and mice that received distilled water. (Table 2). The mean sodium levels in mice given 100 mg/kg of the extract were significantly lower *(p<0.0001)* than the mean sodium level in mice given distilled water only. (Table 2).

There was no significant difference *(p=0.9604)* between the mean potassium levels in mice given 100 mg/kg of the extract and the mean sodium levels in mice given distilled water. (Table 2). There was no significant difference*(p=0.9992)* between the mean potassium levels in mice given 300 mg/kg of the extract and the mean sodium levels in mice given distilled water. (Table 2). There was no significant difference *(p=0.9960)* between the mean potassium levels in mice given 600 mg/kg of the extract and the mean sodium levels in mice given distilled water. (Table 2).

The effect of the ethanol extract of*Olea africana* on hematological parameters in mice over a 28-day period is shown in Table 3.

Table 3. Effect of the ethanol extract of *Olea africana* on hematological parameters in mice over a 28-day period

Treatment	WBC (10^9/L)	RBC (10^{12}/L)	MCV (f/L)	MCH (pg)	HCT (%)	Hemoglobin (g/dL)	MCHC (g/dL)
Control	2.66 (0.96)^a	9.74 (0.36)^a	52.55 (1.65)^a	15.10 (0.42)^a	50.65 (3.09)^a	14.78 (0.66)^a	28.57 (0.36)^a
100 mg/kg	5.60 (2.16)^b	10.42 (0.74)^a	52.10 (0.79)^a	15.00 (0.29)^a	55.95 (2.79)^b	16.13 (0.80)^b	27.95 (1.10)^a
300 mg/kg	4.51 (9.91)^b	9.56 (0.52)^a	53.58 (0.85)^{ab}	15.28 (0.13)^{ab}	53.20 (0.48)^{ab}	15.25 (0.13)^{ab}	28.52 (0.44)^a
600 mg/kg	4.65 (1.26)^b	9.53 (0.20)^a	55.88 (1.08)^b	15.90 (0.46)^b	54.42 (0.71)^{ab}	15.30 (0.25)^{ab}	28.38 (0.22)^a

Values are presented as mean ± standard deviation (in parenthesis). Means with different superscripts along the columns are significantly different from each other ($p<0.05$).

The mean white blood cell levels in mice given distilled water were significantly lower than those in mice given 100 mg/kg *($p=0.0016$)*, 300 mg/kg *($p=0.0326$)*, or 600 mg/kg *($p=0.0220$)* dose of the extract (Table 3).

There was no significant difference *($p=0.1780$)* between the mean red blood cell levels in mice that received 100 mg/kg of the extract and the mean red blood cell levels in mice that received distilled water only. (Table 3). There was no significant difference *($p=0.9114$)* between the mean red blood cell levels in mice that received 300 mg/kg of the extractand the mean red blood cell levels in mice that received distilled water only. (Table 3). There was no significant difference *($p=0.8749$)* between the mean red blood cell levels in mice that received 600 mg/kg of the extract and the mean red blood cell levels in mice that received distilled water only. (Table 3).

There was no significant difference *($p=0.9026$)* between the mean corpuscular volume levels in mice given 100 mg/kg of the extract and the mean corpuscular volume levels in mice given distilled water only. (Table 3). There was no significant difference *($p=0.4677$)* between the mean corpuscular volume levels in mice given 300 mg/kg of the extractand the mean corpuscular volume levels in mice given distilled water only. (Table 3). The mean corpuscular volume levels in mice given 600 mg/kg of the extract were significantly higher *($p=0.0039$)* than the mean corpuscular volume levels in mice given distilled water. (Table 3).

There was no significant difference *($p=0.9565$)* in the mean corpuscular hemoglobin levels in mice given 100 mg/kg of the extract and mice given distilled water. (Table 3). There was no significant difference *($p=0.8213$)* in the mean corpuscular

hemoglobin levels in mice given 300 mg/kg of the extract and mice given distilled water. (Table 3). The mean corpuscular hemoglobin levels in mice given 600 mg/kg of the extract were significantly higher *(p=0.0185)* than the mean corpuscular hemoglobin levels in mice given distilled water only. (Table 3).

The mean hematocrit levels in mice given 100 mg/kg of the extractwere significantly higher *(p=0.0109)* than the mean hematocrit levels in mice given distilled water only. There was no significant difference *(p=0.2591)* between the mean hematocrit levels in mice given 300 mg/kg of the extract and the mean hematocrit levels in mice given distilled water only. (Table 3). There was no significant difference *(p=0.0675)* between the mean hematocrit levels in mice given 600 mg/kg dose of the extract and the mean hematocrit levels in mice given distilled water only. (Table 3).

There was no significant difference *(p=0.6749)* between the mean hemoglobin levels in mice treated with 100 mg/kg and the mean hemoglobin levels in mice treated with distilled water only. (Table 3). There was no significant difference *(p=0.9769)* between the mean hemoglobin levels in mice treated with 300 mg/kg and the mean hemoglobin levels in mice treated with distilled water only. (Table 3). There was no significant difference *(p=0.9694)* between the mean hemoglobin levels in mice treated with 600 mg/kg and the mean hemoglobin levels in mice treated with distilled water only. (Table 3).

There was no significant difference *(p=0.9503)* between the mean corpuscular hemoglobin concentration levels in mice treated with 100 mg/kg and the mean corpuscular hemoglobin concentration levels in mice treated with distilled water only. (Table 3). There was no significant difference *(p>0.9999)* between the mean corpuscular hemoglobin concentration levels in mice treated with 300 mg/kg and the mean corpuscular hemoglobin concentration levels in mice treated with distilled water only. (Table 3). There was no significant difference *(p=0.9982)* between the mean corpuscular hemoglobin concentration levels in mice treated with 600 mg/kg and the mean corpuscular hemoglobin concentration levels in mice treated with distilled water only. (Table 3).

The liver sections of mice who received distilled water, 100 mg/kg, 300 mg/kg, and 600 mg/kg of the ethanol extract of *Olea africana* are presented in Figure 3 below.

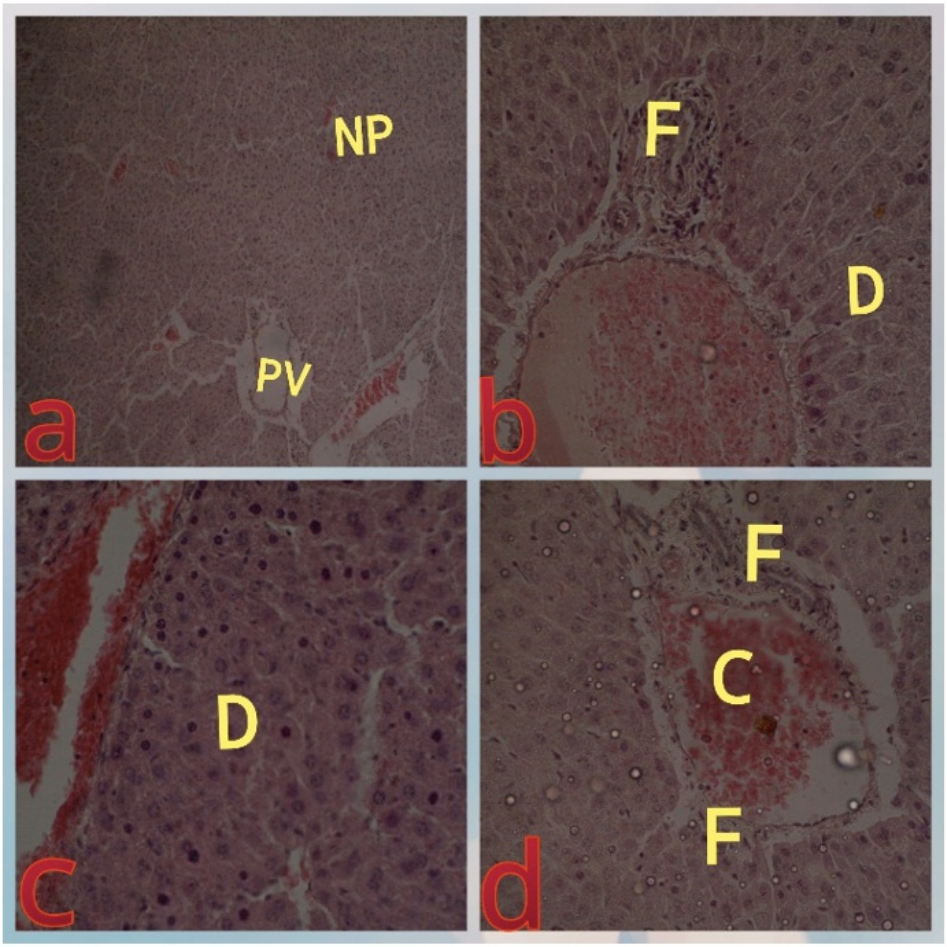

Figure 3. Photomicrograph (X10) of liver sections of mice treated with distilled water (a), 100 mg/kg (b), 300 mg/kg (c), and 600 mg/kg (d) of the ethanol extract of *Olea africana* over 28 days. NP: Normal parenchyma; PV: Portal vein; F: Fibrosis; D: Degeneration of hepatocytes; C: Congestion

The liver sections of mice in the control group (distilled water group) presented normal histology formations of the parenchyma and hepatic veins as illustrated in Figure 3a. There was fibrosis around the hepatic artery and degeneration of hepatocytes in the liver of mice treated with 100 mg/kg of the ethanol extract of *Olea africana* (Figure 3b). The liver sections of mice given 300mg/kg of the ethanol extract of *Olea africana* for 28 days generally presented with degeneration of hepatocytes in tissues surrounding the blood vessels (Figure 3C). The liver sections of mice given 600mg/kg of ethanol extract of *Olea africana* for 28 days generally presented with congestion and mild fibrosis around the portal vein and portal artery (Figure 3d).

The kidney sections of mice who received distilled water (a), 100 mg/kg (b), 300 mg/kg (c), and 600 mg/kg (d) of the ethanol extract of *Olea africana* are presented in Figure 4 below.

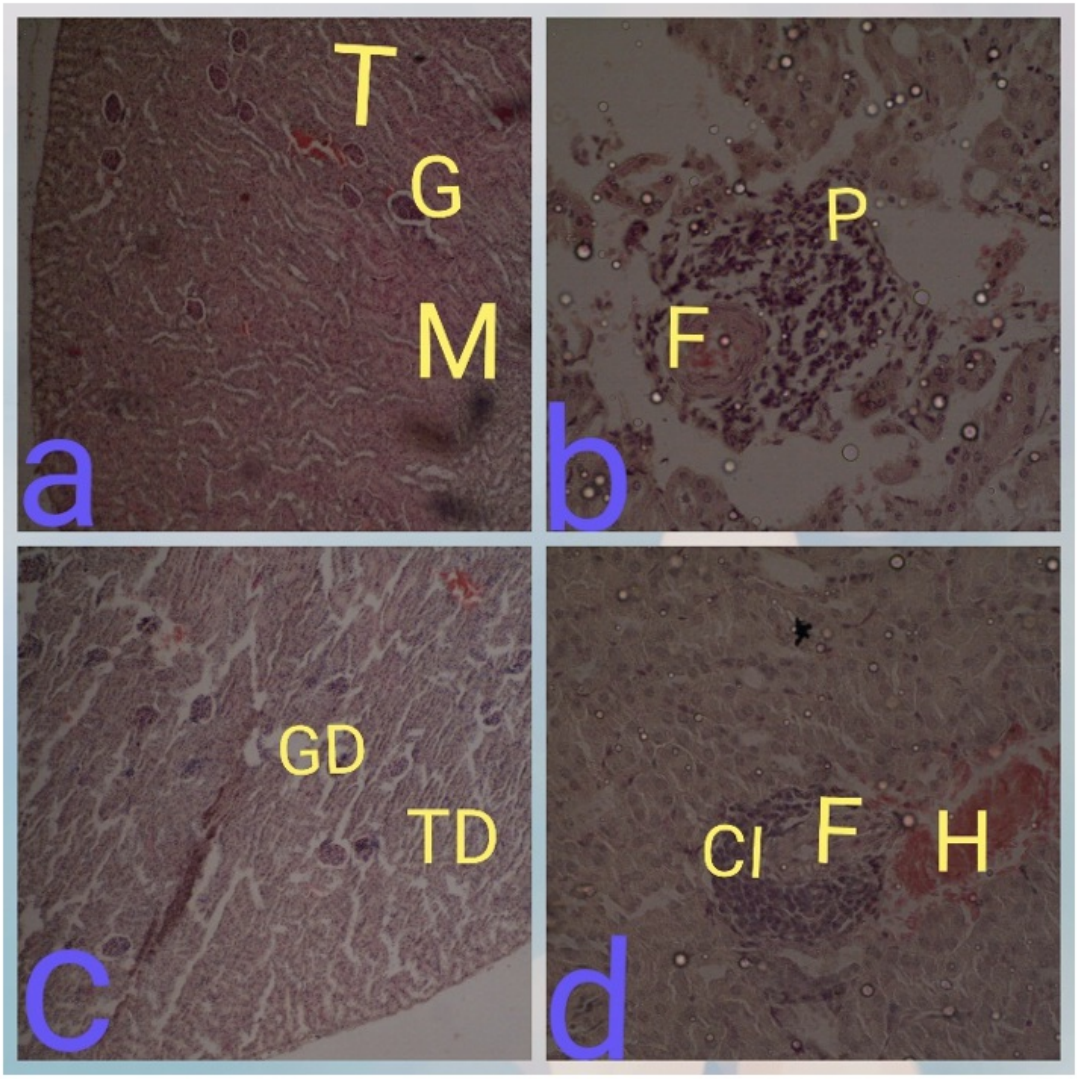

Figure 4: Photomicrograph (X40) of kidney sections of mice treated with distilled water (a), 100 mg/kg (b), 300 mg/kg (c), and 600 mg/kg (d) of *Olea africana* over 28 days. T: Tubules; G: Glomerulus; M: Medulla; F: Fibrosis; P: Perivascular infiltration: GD: Glomerular degeneration; TD: Tubular degeneration CI: Cell infiltration; H: Hemorrhage.

The kidney sections of mice in the control group (distilled water only) generally presented normal formations of the glomeruli, tubules and medulla as illustrated in Figure 3a. The Kidney sections of mice given 100mg/kg of ethanol extract of *Olea africana* for 28 days generally presented with perivascular infiltration and fibrosis Figure 3b. The Kidney sections of mice given 300mg/kg of the ethanol extract of *Olea africana* for 28 days generally presented normal formations of the cortex for most parts with mild glomerular degeneration (the glomeruli are smaller and spaces around them are larger), mild tubular degeneration (no clear delineation of the tubules), as illustrated in Figure 3c. The Kidney sections of mice given 600mg/kg of the ethanol extract of *Olea africana* for 28 days generally presented cell infiltration, fibrosis, and hemorrhage (Figure 3d).

Discussion

Acute toxicity using small animal models is a preliminary step in establishing the safety profile of medicinal plant extracts [25]. The current study used brine shrimp *(Artemia salina)* and mice to determine the acute toxicity of the ethanol leaf extract of *Olea africana.* The LC_{50} of the extract in brine shrimp was 2275.84 µg/mL (702.97 µg/mL to 7367.95 µg/mL) after 24 hours. A previous study on the aqueous extract of *Olea africana* in *Artemia salina* revealed an LC_{50} of 1269 µg/mL (1016 µg/mL to 1586 µg/mL) [19]. Meyer and Clarkson's criteria suggest that this extract may be considered non-toxic [21][26]. This study reported an LD_{50} (oral) of 4297.30 mg/kg for the ethanol leaf extract of*Olea africana*. A previous study by Amabeoku and Bamuamba reported an LD_{50} of 3475 mg/kg for the methanol extract of *Olea africana* [14]. Considering the recommendations of the Globally Harmonized System of Classification and Labeling of Chemicals, the ethanol leaf extract of *Olea africana* may be classified as non-toxic as far as the acute toxicity protocol in mice is concerned [27]. Because no toxicity was observed during the acute toxicity study, a repeat dose experimental design (28 days) was used to assess the subacute toxicity of the extract. The effect of dose on animal organs and the identification of adverse effects can be evaluated using indicators such as body weight changes, organ-to-body weight ratios, and hematological, biochemical, and histopathological parameters [28][29][30].

There was no significant difference between the mean weight gain of control and treatment group animals suggesting that the extract does not alter metabolism in mice. Previous research has shown that the mean organ-to-body weight ratio in experimental animals is a good predictor of toxicity [29][31]. No significant differences were observed in the mean organ body weight ratios of control and treatment group mice in this study.

Blood provides nutrition, oxygen and is a conduit for waste removal. As a result, it is constantly exposed to foreign substances, which may have negative consequences [32]. Blood analysis can be used to determine the status of health in wildlife and in man [32]. The studied extract induced a non-significant effect on mean RBC, Hb, and MCHC levels in the current study, indicating that the extract has no deleterious impact on osmotic fragility of cells, erythropoiesis, or cell morphology [33]. White blood cells (WBC) elicit an immune response in response to foreign agents in the body[29]. WBC levels in treatment group animals were significantly higher than in control animals, implying that the ethanol leaf extract of *Olea africana* may pose an immune challenge in treatment animals. The significantly higher mean corpuscular volume in mice given the highest extract dose suggests that such a treatment may induce an increase in red blood cell size. MCH reflects MCV i.e., the amount of hemoglobin in a RBC depends on the size of the RBC.

Serum electrolytes (e.g. sodium and potassium) are important indicators of kidney function[34]. Sodium is a major cation of extracellular fluid and plays an important role in the normal distribution of water and osmotic pressure in the different fluid compartments [34]. The mean sodium levels in mice given the low and high doses of the extract were significantly lower than the mean sodium levels in control mice. Insufficient sodium may cause cellular malfunction and death [34]. However, it is not clear why this effect was not dose-dependent.

Albumin, total protein, and bilirubin (direct and total) are good indicators of liver function[34]. The mean total protein, direct and total bilirubin levels in animals given the high dose of the extract were significantly higher than in control animals. The primary indicators of cholestatic liver injury include albumin, bilirubin, and total protein [35]. It could thus be argued that high doses of the extract are associated with cholestatic liver injury. There were significant elevations in the mean ALT,

AST, ALP, and GGT levels in mice who received the extract relative to control group animals suggesting that the sub-acute administration of the extract may have untoward effects on the liver of mice. This was confirmed by histopathology where significant liver and kidney damage was observed.

Conclusions

In light of these findings, prolonged administration of the ethanol leaf extract of *Olea africana* is associated with significant toxic concern. As a result, the extract should be used with caution.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The authors declare that they have no competing interests regarding the publication of this paper.

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