

Acute and sub-acute toxicity studies of the methanol extract of *Oecophylla longinoda* by oral administration in rats

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Abstract: *Oecophylla longinoda* has some reported medicinal uses, including antimicrobial, analgesic, and anti-inflammatory activities. This study was designed to investigate the *Oecophylla longinoda* methanol extract to evaluate the toxicity profiles of its acute and sub-acute effects. The experiments were conducted to determine the oral median lethal dose (LD₅₀) and other gross toxicological manifestations on an acute basis. In the first phase of the acute toxicity study, three per group were administered *Oecophylla longinoda* at single oral doses of 10, 100, and 1000 mg/kg body weight, and 1600, 2900, and 5000 mg/kg of *Oecophylla longinoda* in the second phase. In the sub-acute studies, the mice were orally administered 2.5 g/kg *Oecophylla longinoda* per day for 14 consecutive days. Rat body weight and fluid intake were recorded during the 14 days. The rat kidney, heart, liver, and blood sera were obtained for weight, histological, and biochemical markers including glucose, cholesterol, proteins, triglycerides, enzymes including ALT, AST, and ALP, as well as electrolytes (sodium and potassium) examinations. Results show that the methanol extract of *Oecophylla longinoda* is safe, and no significant changes in the biochemical markers that indicate harmful effects on the kidney and liver were observed in mice. However, the liver showed slight changes while the kidneys and heart remained healthy in mice exposed to *Oecophylla longinoda*. The no-observed-adverse-effect limit was 5000 mg/Kg body weight/day.

Introduction

Natural products, such as those from plant, animal, or marine sources, are increasingly being used as disease-preventive agents, health supplements, and revitalizers. The idea of using food as medicine has developed and has been in use for a very long time. According to historical literature, natural products were also the most widely used and trustworthy approach for traditional people to prevent and treat illness [1-5]. Medicinal insects contain many secondary metabolites like terpenoids, phenolics, and alkaloids, some of which are very complex. Nevertheless, insects used to treat certain diseases cough, rheumatism, and skin infections are commonly used without any scientific knowledge or evidence of their toxicological effects [6]. The acute and sub-acute toxicity test is a technique that is founded on the investigation of the mode of action of chemicals as well as an assessment of their safety and innocuousness. In the context of chemical manufacture, handling, and usage, acute and sub-acute systemic toxicity studies are employed for risk management and hazard disclosure [7]. A single-dose test called an acute toxicity test is used to determine symptoms and the degree of

harm in animals [8]. After gathering preliminary data from acute toxicity testing, which does not include or reveal details about the animal's target tissue or organ, sub-acute toxicity studies at repeated dosages can be conducted to get definitive information on the targeted tissue and organs [9]. Traditional Chinese and Indian treatments for arthritis and other abnormal health disorders are derived from extracts of *Oecophylla longinoda* (OL) [10]. Bacteria such as *Proteus penneri*, *Serratia*, and *Citrobacter freundii*, as well as fungi such as *Aspergillus aculeatinus*, *Penicillium chrysogenum*, *Cloridium chlamydosporis*, and *Phytophthora palmivora*, were prevented from infecting cocoa pods with a high population of OL [11]. In the meantime, it has been discovered that Australian Formicine ants also possess bactericidal qualities. Important vitamins, including vitamin B-12, folate, and vitamin C, are typically found in OL, usually called Tailor ants [12]. Tailor ants have also been found to include chemical components such as triterpenoids, alkaloids, and steroids [13].

The methanol extract of OL has been shown to have inhibitory activity against some pathogens. For the antimicrobial activity, OL inhibits *Pseudomonas aeruginosa* (27 mm) as OL's concentration decreased to 25 mg/mL. In *Staphylococcus aureus*, an irregular pattern of inhibition occurred, and in *Escherichia coli*, an upward trend was observed for the result from 20 mm to 25 mm (25 mg/mL, 100 mg/mL, respectively) as the concentration decreased. For the anti-fungal activity, inhibition against *Aspergillus niger*, *Proteus notatum*, and *Mucormycetes* increased as the concentration decreased [5]. OL was seen to be active against pain and inflammation [14, 15]. The analgesic activity was studied using acetic acid to induce writhing, the hot-plate test, and the formalin-induced pain in mice [16], and the anti-inflammatory activity was studied using carrageenan, dextran, and formaldehyde to induce edema in mice [17]. The public's acceptance of natural product remedies as a way to prevent and treat disease is growing. Because natural medications are generally thought to be safe and do not have the serious side effects associated with synthetic treatments, people use them. The safety and toxicological profile of many of the natural product formulations available on the market, however, is not well supported by scientific research. This is particularly important because natural products are increasingly being used for self-medication without a doctor's supervision. Accurate scientific knowledge regarding the toxicity and safe dosage range of natural therapies is, therefore, crucial. Therefore, as part of an ongoing scientific evaluation of the extract, this study aimed to assess its acute and sub-acute toxicity profiles in mice. Experiments were conducted to determine the oral median lethal dose (LD₅₀) and other gross toxicological manifestations on an acute basis and sub-acute basis.

Materials and methods

Ant material: The ants were collected from the base of an almond tree (*Terminalia catappa*) in Edo State, Nigeria, near the Ikpoba settlement of Ikpoba Okha Local Government. The ants were identified as *Oecophylla longinoda* (African Weaver Ants) by a biologist at the Department of Animal and Environmental Biology at the University of Benin, Nigeria.

Ant preparation: After collection, the ants were immersed in distilled water. Following the sorting process, the ants were crushed in a mortar. Over eight hours in a Soxhlet extractor, 64.9 g of the crushed ants were fully extracted using 600 mL of methanol. The crude extract was concentrated at 50°C using a rotary evaporator (Model RE, 200, USA) to produce a semi-solid extract.

Experimental animals: The Department of Pharmacology and Toxicology at the University of Benin's Faculty of Pharmacy's Pharmacology animal house provided the 65-75 g for mature Wistar rats of both sexes of roughly seven weeks old that were used. In addition to being housed in plastic cages with sawdust bedding, animals were kept in a room with a controlled room temperature at 23°C-25°C, a 12: 12 light/dark cycle, and unlimited access to food and drink. The animals were given two weeks to acclimate before being used. The Ethical Committee of the Faculty of Pharmacy examined and approved both acute and sub-acute tests on the 20th of November 2022 with reference number EC/FP/022/23.

Acute toxicity studies: The oral acute toxicity test of the methanol extract of *OL* was evaluated as a previously reported method [18], and the result was obtained at doses 10, 100, and 1000 mg/kg for the first phase with three mice each, were recorded. Body weight was observed for signs of toxicity and death for the first 24 hrs during the first phase. For the second phase, doses at 1600, 2900 and 5000 mg/kg were administered with one mouse in each group, and the mice were observed for 24 hrs for the first day for signs of toxicological symptoms (alertness, touch response, tremors, pinna reflex, writing reflex, etc.) and death (mortality) and 14 more days. This helped to rule out any possible toxic effects of the extract.

Sub-acute toxicity studies: Mice were allotted to two groups of control and treated, such that the number of each sex was the same in both groups, but mice of the opposite sex were not placed in the same cage, and pregnant mice were excluded from the study. The treated group was given daily doses of 2.5 g/kg of extract in 0.4 ml of distilled water by orogastric tube for 14 days. In the sub-acute experiments, the mice were administered orally 2.5 g/kg per day of the extract for 14 consecutive days. Mice weight and fluid intake were recorded during the 14 days. Terminally, kidneys, liver, hearts, and blood/sera were obtained for weight, haematological, and biochemical markers of toxicity. The dose of extract was taken as a quarter of the maximum dose (10.0 mg/kg) used for the acute toxicological experiments. Rats in the control group were sham-treated with 0.4 ml of distilled water (p.o) daily for 14 days. At the end of the treatment period of 14 days, rats were anaesthetized with diethyl ether vapor in a chamber and blood samples were obtained via the abdominal aorta for biochemical and haematological assays. The heart, liver, and kidney were isolated, cleaned of the adherent tissues, and kept on absorbent papers for 10.0 min before they were weighed.

Measurement of body weight and daily fluid intake: Fluid intake was recorded daily over the 14 days. Mice drank from graduated water bottles, and at the same time each day, the decrement in the amount of fluid consumed over 24 hrs was measured by subtracting the day's reading from that of the previous day. End-of-treatment weights were used for weight analysis.

Biochemical assay: After the sub-acute toxicity test, rats are humanely euthanized using chloroform to induce anesthesia and death. Blood is collected via cardiac puncture and allowed to coagulate and clot in specimen vials to extract serum, and organs are quickly removed and washed with cold phosphate-buffered saline. A Blood sample was after being centrifuged at 3000 rpm, the serum samples were divided into clean vials using Pasteur pipettes. Before examination using the Chemwell Chemistry autoanalyzer, the serum samples were kept at -20°C. Using the procedure outlined [19], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. The serum creatinine was measured using Larsen's kinetic technique [20]. Serum albumin was measured as the previous reported method [21], whereas total serum protein was measured by using the Biuret method [22].

Histopathological examination: Comparing the tissue sections of the *OL*-treated rat to those of their respective control groups under a microscope revealed no lesions or aberrant histological alterations. From a physical examination, the organs looked and felt normal. When compared to the corresponding control groups, the relative organ weight index of the rats treated with *OL* extract did not significantly change.

Statistical analysis: Data shown as mean \pm SEM (standard error of the mean). The Student's *t*-test was used to compare the treatment and control groups. $P < 0.05$ indicates a statistically significant difference.

Results

Cage side observations: Mice in all treatment groups demonstrated normal neuronal, motor, and behavioral responses to all doses of *OL* extract given, and no mortality was noted. When *OL* was administered to treated mice, their skin and fur, eyes, behavior patterns like locomotion and posture, and autonomic and central nervous system activity did not vary from those of the control group. Throughout the 14-day observation

period, no behavioral problem was noted, and the extract treatment did not cause weight loss. This demonstrated that the methanol extract of *OL* had an oral LD₅₀ of more than 5000 mg/kg body weight.

Body weight: The body weight of the treatment group rats in the sub-acute study did not show any significant changes when compared with the control, throughout the study period. The feed and water consumption pattern of the mice was regular and consistent throughout the experimental period.

Table 1: Acute effect on the body weight of BALB/c mice

Body weight (g)				
Treatments	Dosage (mg/kg)	Initial Weight (1 st day)	Final Weight (14 th day)	Difference
Control group	2000	67.98±1.45	66.94±1.14	+1.04
Methanolic extract	2000	70.04±2.09	71.96±2.05	+1.92

Table 2: Sub-acute on the body weight of BALB/c mice

Body weight (g)				
Treatments	Dosage (mg/kg)	Initial Weight (1 st day)	Final Weight (14 th day)	Difference
Control group	2000	72.62±1.37	73.07±0.28	+0.45
Methanolic extract	2000	70.16±2.48	67.52±2.16	+2.64

Table 3: Acute toxicity observations of BALB/c mice

Group	Dose mg/kg	Sign of toxicity	Mortality (%)
Control	0.2ml	Nil	0
Extract	10	Nil	0
Extract	100	Nil	0
Extract	1000	Nil	0
Extract	1600	Nil	0
Extract	2900	Nil	0
Extract	5000	Nil	0

The signs of toxicity include; alertness, touch response, tremor, pinna reflex, writing reflex

Table 3: Effects of sub-acute administration of 2500 mg/kg/day of *OL* on biochemical parameters in rats

	Parameters	Control	Methanol extract
Lipid profile	Total cholesterol (mg/dl)	83.75±9.32	78.75±1.70
	Triglycerides(mg/dl)	79.5±21.97	101.0±23.36*
	High density lipoprotein (mg/dl)	41.25±4.03	38.0±1.82
	Low density lipoprotein (mg/dl)	26.5±6.24	21.0±3.82
Electrolytes	Sodium (Mmol/L)	140.5±2.08	142.0±2.44
	Potassium (Mmol/L)	4.08±0.55	4.14±0.85
	Bicarbonate (Mmol/L)	21.5±1.29	22.5±1.29
	Chloride (Mmol/L)	103.75±1.70	102.0±2.94
Renal function	Urea (mg/dl)	26.5±9.14	28.0±2.94
	Creatinine (mg/dl)	0.85±0.15	0.65±0.12
Liver function	ALP (U/L)	432.0±123.45	493.75±158.28
	AST (U/L)	27.5±4.35	18.25±6.81*
	ALT (U/L)	90.25±5.85	94.0±7.52
	TB (mg/dl)	0.25±0.05	0.30±0.08
	CB (mg/dl)	0.10±0.00	0.125±0.05
	TP (mg/dl)	7.15±0.23	6.87±0.17
	ALB (mg/dl)	3.67±0.17	3.62±0.17
	GLO (mg/dl)	3.45±0.12	3.25±0.17

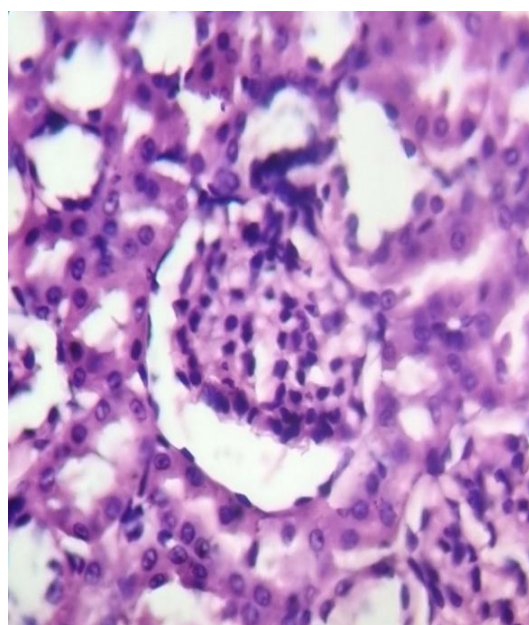
Data are expressed as mean±SD (n=8). *Significantly different from the control group by p<0.05

Table 3 displays the biochemical parameter data. As regards the lipids profile test, when rats in all groups received *OL* extracts orally once a day, their levels of cholesterol and low-density lipoprotein cholesterol remained the same, and at acceptable levels to the rats in the control group. Cholesterol levels for rats are relatively low, except when hyperlipidemia is induced. The cholesterol levels were low; however, it was noticed in the histopathology that there were pockets of fat, which the biochemical analysis didn't pick this. The triglyceride values were seen to be similar to the control groups, and a few rats had normal levels of high-density lipoprotein cholesterol in comparison to the control group. For the high-density lipoprotein levels, the higher the better; for the low-density lipoprotein levels, the lower the better.

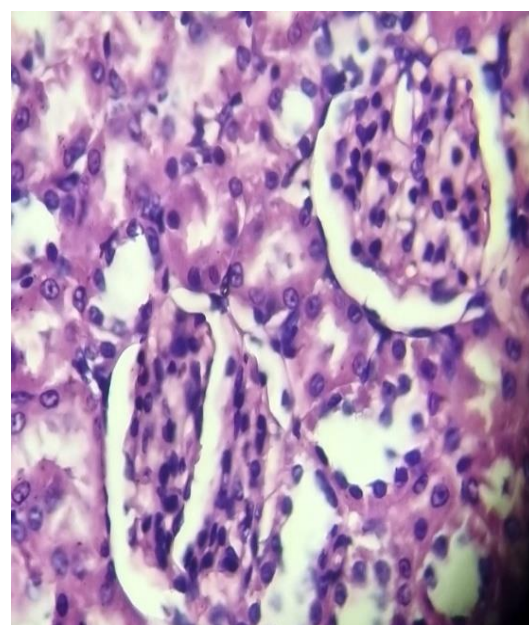
For the renal function, we could see relatively good values for the electrolytes. For the creatinine value, which is the most important renal function, there was a non-significant effect in rats treated with the extract as compared to the control group. Similarly, in urea, bicarbonate, chloride, and potassium values were shown to be all normal as compared to the control group. For sodium levels, there was a non-significant effect in rats treated with the extract, compared to the control group.

For the liver function test, it can be seen that there was a significant increase in rats treated with the extract, which could be a result of bone disease or pregnancy in the rats. AST is not liver-specific; there are other iso-enzymes of AST. For the AST levels, the rats treated with the *OL* extract showed a relatively similar value when compared to the control group. The ALT levels in the group of control rats and the group of rats treated with the extract showed a significant difference. It can be seen, therefore, that the rats treated with methanolic extract had higher levels of ALP and ALT compared to the control group. However, it is important to note that the damage was not so severe; it is mild damage, because the total and conjugated bilirubin is not as low as seen here, compared to rats with damaged livers. The total protein, albumin, and globulin levels were normal, if the damage was severe, the albumin would have been grossly affected, because it is predominantly produced in the liver.

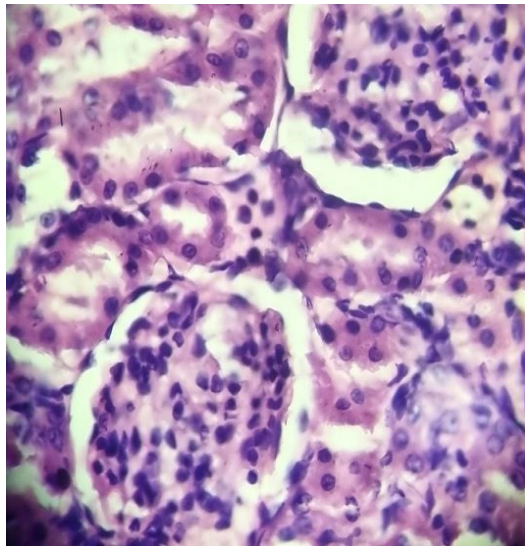
Histopathology: The microscopic evaluation of the tissue sections of the *OL*-treated rats showed mild lesions and abnormal histopathological changes compared to the control groups. This goes to show that the rats may have had liver issues from *OL* administration, although not significant. However, the liver was seen to have experienced changes in the group treated with *OL* extract. The organs (heart and kidney) retained normal texture and appearance on gross examination, with mild changes in the kidney. The relative organ weight index of the rats treated with *OL* extracts did not show significant changes as compared to their respective control groups.



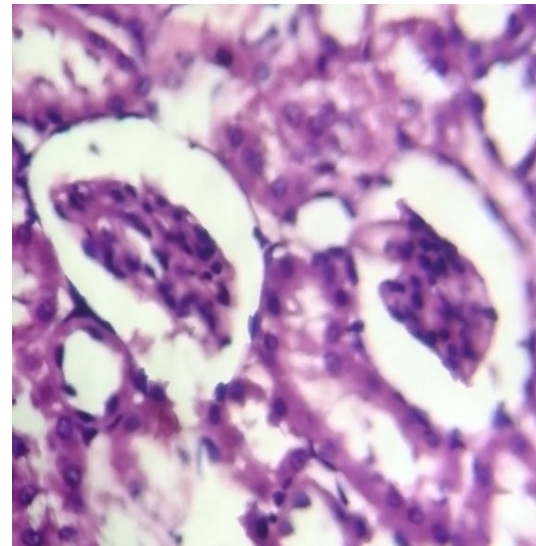
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(b)



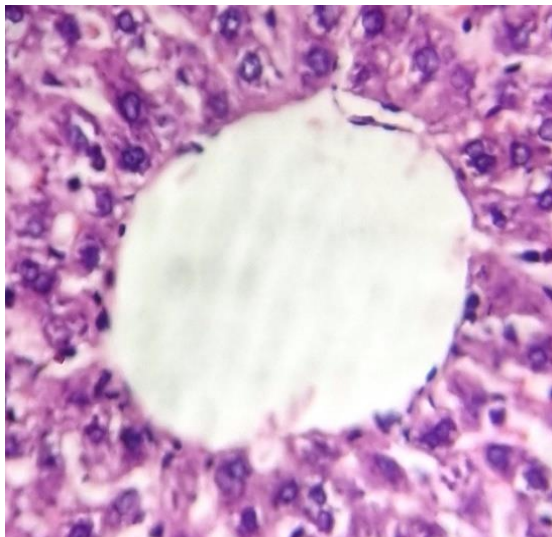
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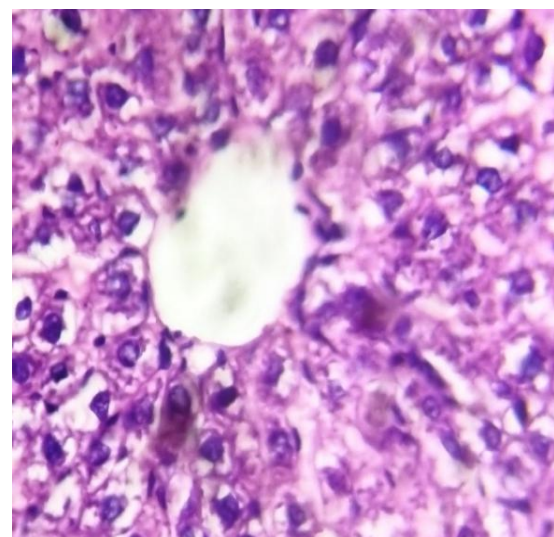
(d)

Figure 1: Effect of *OL* extract on renal histology in rats

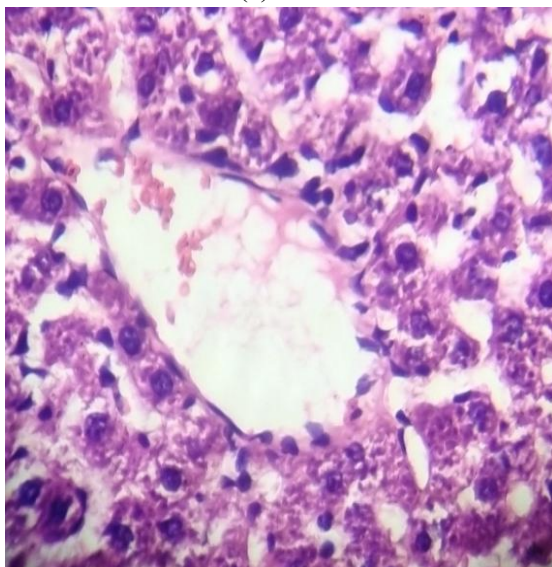
Histological sections were visualized by hematoxylin and eosin staining and observed under a light microscope (a) and (b) control rat, (c) and (d) rat treated with methanol extract of *OL* (200 mg/kg)



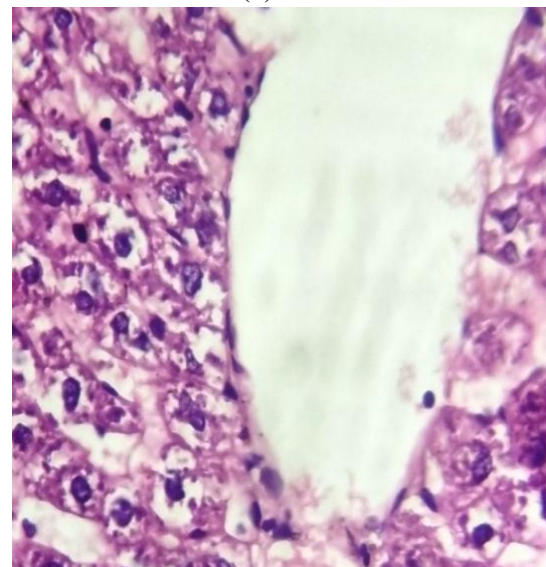
(a)



(b)



(c)



(d)

Figure 2: Histopathology of the liver

Histological sections were visualized by hematoxylin and eosin staining and observed under a light microscope (a) and (b) control rats, (c) and (d) rats treated with methanol extract of *OL* (200 mg/kg)

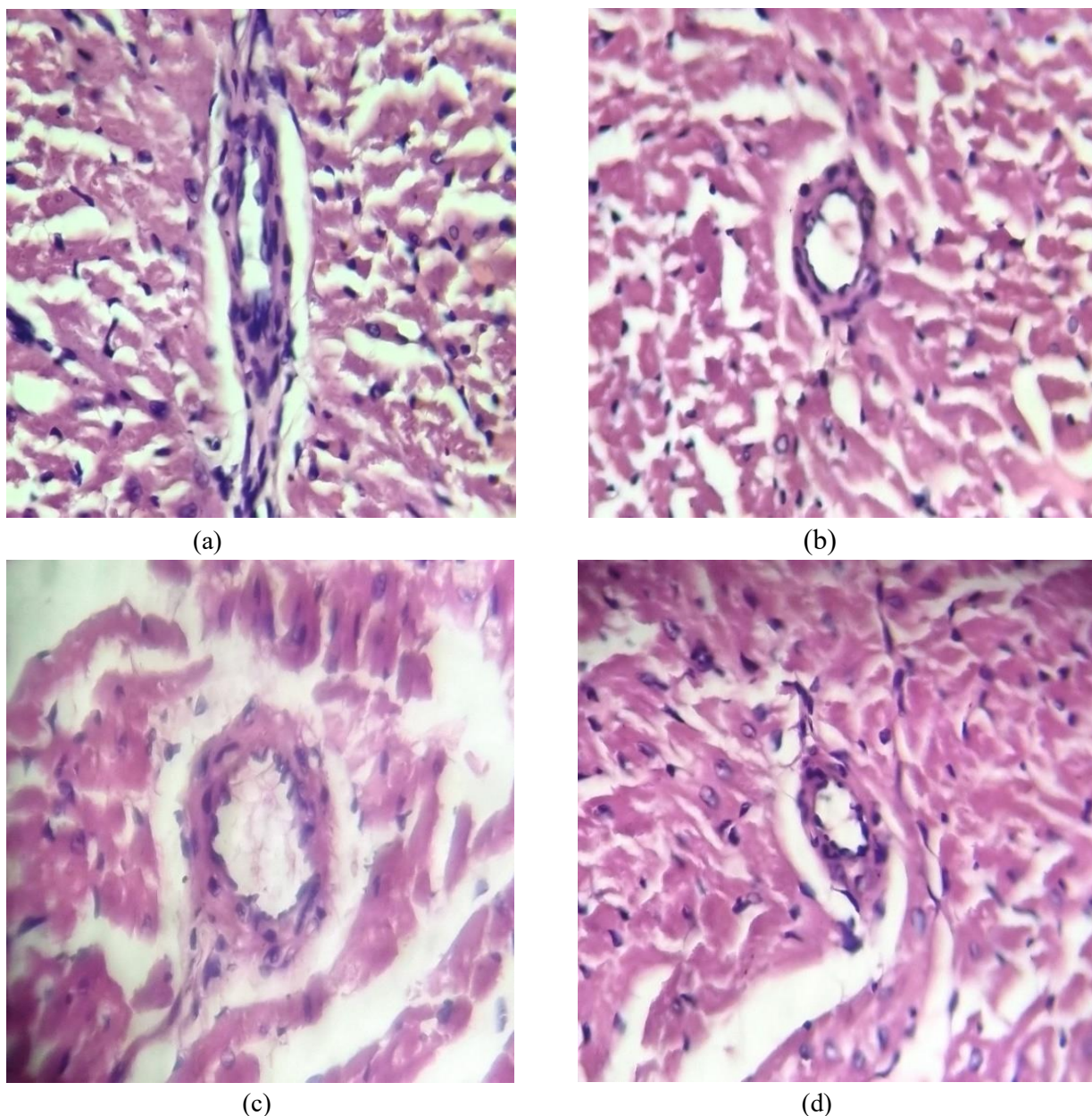


Figure 3: Histopathology of the heart

Histological sections were visualized by hematoxylin and eosin staining and observed under a light microscope (a) and (b) control rats, (c) and (d) rats treated with methanol extract of OL (200 mg/kg)

Histopathology of kidney/renal: Normal histology of rat kidneys (glomeruli, tubules, and interstitium) was found in the control group. The *OL*-treated group (c) revealed a visible renal corpuscle with a glomerulus that appears mildly enlarged, with tubules and interstitial. The clearing between the basement membrane and the glomerulus, which is a spongy tissue necessary for ultrafiltration, reveals how mildly enlarged the glomerulus is in the *OL*-treated group as compared to the control. The other slide (d) revealed a prominently atrophied renal corpuscle with glomerulus, with unremarkable tubules and interstitial space. Histology reveals that there is very little effect on the group treated with *OL*, this was not, however, seen in the serum biochemistry assay as the creatinine, urea, and other electrolyte levels were normal.

Histopathology of the heart: It is seen that *OL* has no such effect on the rats treated. The coronary artery is the heart's lifeline, supplying it with oxygen-rich blood. It helps to give us a clear idea of what is going on around the heart. The coronary artery of both control and *OL*-treated groups looks clear, with no constriction; it is clear that there is no occlusion of the arteries. The heart, therefore, reveals a prominent coronary artery surrounded by bundles of myocardial fibers surrounding it. The heart of the group treated with *OL* showed no visible negative effect.

Histopathology of the liver: The *OL*-treated group Liver histology reveals a visible (clear) central vein and well-fenestrated sinusoids and hepatocytes with prominent hepatic steatosis (fatty liver cells); the hepatocytes have adipocytes surrounding them. Histology reveals that there is little effect on the group treated with *OL*, which corroborates the biochemical findings. The histology of liver sections from the control rats showed a little bit of abnormality in the hepatocellular architecture, as well as not-so-well-preserved liver cells, visible central veins, and a few histological abnormalities. The liver sections from the *OL*-treated group showed some histological changes, such as slight intralobular inflammation around the centrilobular veins (CLVs) and slight cytoplasmic hydropic degeneration of hepatocytes.

Discussion

Natural products are becoming more and more accepted by the general public as a means of preventing and curing illness [1-5]. People use natural medicines because they generally believe that these products are safe and do not have the severe side effects that synthetic drugs have [1, 2]. However, there is a dearth of scientific evidence about the safety and toxicological profile of many of the natural product formulations on the market [5]. This is especially crucial because natural products are more frequently utilized for self-medication without a doctor's supervision [5]. Therefore, it is essential to have an accurate scientific understanding of the toxicity and safe dosage range of natural medications. The investigation, therefore, concentrated on the acute and sub-acute toxicity assessment of the methanol extract of *OL*. Rats in the 14-day acute toxicity trial of the aforementioned extract of *OL* did not die or exhibit aberrant motor-neuronal or behavioral responses [23, 24]. While researching the toxicity and safety of a natural product, it is crucial to keep track of the experimental animals' body weight and feed/water consumption because these data provide insight into their physiological and metabolic status and prevent the researcher from drawing any false conclusions based on the rats' abnormal nutritional status. The weight gain that each rat in the current study displayed was similar and followed a broad pattern. After evaluating the toxicity profile of *OL*, it was discovered that *OL* did not exhibit any toxicological symptoms in rats. The present study's conclusions proved that the methanol extract of *OL* is safe. This toxicity study also includes several significant biochemical markers. The kidneys and liver are the main organs that are vulnerable to the harmful effects of medications. Serum creatinine, urea, and total protein levels were measured to evaluate renal function, while the levels of ALP, AST, and ALT were measured to evaluate liver function. The experiment's findings revealed slight changes in the liver, which were not overly detrimental to the rats and perfectly healthy kidneys. Upon gross inspection, there were no indications of necropsy or unusual morphological abnormalities in the organs. Comparing the tissue sections stained with hematoxylin and eosin under a microscope to the tissues. *OL* was found to be largely harmless in this sub-acute oral toxicity study's results, and the no-observed-adverse-effect limit (NOAEL) of *OL* was found to be 5000 mg/Kg body weight/day. To validate *OL*'s safety for extended use, additional toxicity assessments, such as chronic or genotoxic tests utilizing repeated doses, should be carried out.

Conclusion: The methanol extract of *Oecophylla longinoda* showed a favorable safety profile in toxicity studies in rodents; aside from mild liver changes, there was no mortality or significant behavioral, biochemical, or histological abnormalities. The kidneys and heart remained unaffected, and serum biochemical markers remained within normal ranges. The no-observed-adverse-effect level was established at 5000 mg/kg/day.

References

1. Sofowora A (1982) Medicinal plants and traditional medicine in Africa. 1st Ed., John Wiley and Sons, Chichester, New York, USA. ISBN-10: 0471103675.
2. Sami A, Usama M, Saeed MM, Akram M (2021) Medicinal plants with non-steroidal anti-inflammatory-like activity. Mediterranean Journal of Pharmacy and Pharmaceutical Sciences. 1 (3): 25-32. doi: 10.5281/zenodo.5534605

3. Akhlaq M, Alum MK, Alam MM (2022) Anti-inflammatory potential of medicinal plants. *Mediterranean Journal of Pharmacy and Pharmaceutical Sciences*. 2 (1): 13-21. doi: 10.5281/zenodo.6399381
4. Imansuri NO, Mhani LA, Elhaddar SE, Shushni MA (2022) Libyan mothers' awareness of natural products among infants. *Mediterranean Journal of Pharmacy and Pharmaceutical Sciences*. 2 (2): 38-43. doi: 10.5281/zenodo.6780482
5. Ahmed R, Khandaker MS (2025) Natural products as of nutraceuticals treatment for neurological disorders: An overview. *Mediterranean Journal of Pharmacy and Pharmaceutical Sciences*. 5 (2): 62-69. doi: 10.5281/zenodo.15226021
6. Koriem KMM, Arbid MS, El-Attar MA (2019) Acute and subacute toxicity of *Ammi visnaga* on rats. *Interdisciplinary Toxicology*. 12 (1): 26-35. doi: 10.2478/intox-2019-0004
7. Li Y, Zhuang Y, Tian W, Sun L (2020) *In vivo* acute and subacute toxicities of phenolic extract from rambutan (*Nephelium lappaceum*) peels by oral administration. *Food Chemistry*. 320: 126618. doi: 10.1016/j.foodchem.2020.126618
8. OECD/OCDE (2001) Ligne Directrice de l'Ocde pour les Essais de Produits Chimiques. OCDE 423: Paris, France. doi: Nil.
9. OECD/OCDE (2008) Lignes Directrices de l'Ocde pour les Essais de Produits Chimiques. OCDE 407: Paris, France. doi: Nil.
10. Iyekowa O, Momo DO, Agbonifoa E, Ngbodina J, Akinyemia K, Samuela VE, Chiomaa CG, Obasuyib EI, Adeyeye DO (2022) Bioactive chemical constituents screening and inhibitory activities of methanol extract of *Oecophylla Longinoda* (Tailor Ant) against some pathogens. *Journal of Science and Technology Research*. 4 (3): 80-86. doi: 10.37933/nipes/4.3.2022.9
11. Oladunmoye M, Akinmaye A, Oladosu T (2018) Antimicrobial activity of abdominal gland extracts of African Weaver Ants (*Oecophylla longinoda*) on isolated microorganisms from kola nut pods. *Acta Scientific Microbiology*. 1 (2): 09-14. doi: 10.31080/ASMI.2018.01.0028
12. Kim TK, Yong HI, Kim YB, Kim HW, Choi YS (2019) Edible insects as a protein source: a review of public perception, processing technology and research trends. *Food Science and Animal Resources*. 39 (4): 521-540. doi: 10.5851/kosfa.2019.e53
13. Igue O, Eza PN (2015) Chemical analyses of volatile compounds from cuticular and non-cuticular abdominal glands of African weaver ants (*Oecophylla longinoda*). *Chemical Science Journal*. 5 (4): 304-312. doi: 10.9734/ACSJ/2015/14255
14. Momo DO, Iyekowa O, Owolabi OJ (2023) Characterizations of the active ingredients of methanol extract of weaver ant and its analgesic activity in mice. *Mediterranean Journal of Pharmaceutical Sciences*. 3 (2): 34-44. doi: 10.5281/zenodo.7905327
15. Iyekowa I, Oderanti AO (2023) Chemical analysis and analgesic activity of methanol extract of Crinum Jagus bulb in BALB/c mice. *Mediterranean Journal of Pharmaceutical Sciences*. 3 (1): 19-25. doi: 10.5281/zenodo.7771339
16. Momo DO, Iyekowa O, Owolabi OJ (2025) GC-MM analysis and evaluation of the anti-inflammatory activity of the oil fraction of methanol extract of *Oecophylla Longinoda* (Weaver ant) in Wistar rats. *Acta Chemica Malaysia*. 9 (2): 45-51. doi: 10.26480/acmy.02.2025.45.51
17. Lorke D (1983) A new approach to practical acute toxicity testing. *Archives of Toxicology*. 54 (4): 275-87. doi: 10.1007/BF01234480
18. Schumann G, Bonora R, Ceriotti F, Féraud G, Ferrero CA, Franck PF, Gella FJ, Hoelzel W, Jørgensen PJ, Kanno T, Kessner A, Klauke R, Kristiansen N, Lessinger JM, Linsinger TP, Misaki H, Panteghini M, Pauwels J, Schiele F, Schimmel HG, Weidemann G, Siekmann L; International Federation of Clinical Chemistry and Laboratory Medicine. (2002) IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. *Clinical Chemistry and Laboratory Medicine*. 40 (7): 725-733. doi: 10.1515/CCLM.2010.137
19. Larsen K (1972) Creatinine assay by a reaction-kinetic principle. *Clinica Chimica Acta*. 41: 209-217. doi: 10.1016/0009-8981(72)90513-x
20. Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*. 31 (1): 87-96. doi: 10.1016/0009-8981(71)90365-2
21. Doumas BT, Bayse D, Bornerk Carter, RJ, Peters TJr, Schaffer R (1981) A candidate reference method for determination of total protein in serum: I Development and validation. *Clinical Chemistry*. 27 (10): 1642-1650. PMID: 6169466.
22. Mohamed EAH, Lim CP, Ebrika OS, Asmawi MZ, Sadikun A, Yam MF (2011) Toxicity evaluation of a standardized 50% ethanol extract of *Orthosiphon stamineus*. *Journal of Ethnopharmacology*. 133 (2): 358-363. doi: 10.1016/j.jep.2010.10.008

23. Abdullah NR, Ismail Z, Ismail Z (2009) Acute toxicity of *Orthosiphon stamineus* benth standardized extract in Sprague Dawley rats. Phytomedicine. 16 (2-3): 222-226. doi: 10.1016/j.phymed.2007.04.013
24. Agarwal S, Sharma G, Verma K, Latha N, Mathur V (2022) Pharmacological potential of ants and their symbionts - a review. Entomologia Experimentalis et Applicat. 170 (12): 1032-1048. doi: 10.1111/eea.13236

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