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Acute and sub-chronic toxicity studies of methanol extract of *Trema orientalis* (Linn) blume in albino wistar rats

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ABSTRACT

Background: The safety potential of the methanol extract of *Trema orientalis* (TOM) leaf was evaluated in albino Wistar rats using biochemical, haematological, and histological indices in both acute and sub-chronic toxicity studies.

Methods: The animals were managed following the National Institute of Health (NIH) stipulated protocols for handling laboratory animals. The weight of each animal was recorded upon arrival and monitored throughout the study. The animals were allowed to acclimatize for 14 days, after which they were reweighed and randomly distributed into four groups of three female rats (n=12) for acute toxicity studies. Group A was given distilled water, and Groups B, C, and D were given a single dose of 2000, 4000, and 5000 mg/kg bw TOM extract, respectively. On day 15, each animal was anaesthetized and then euthanized. For the sub-chronic toxicity study, animals were randomly distributed into five groups of eight female rats (n=40). They were dosed daily for 28 days. Group A (Negative control group) was given distilled water. Groups B, C, and D had 200, 400, and 800 mg/kg bw TOM extract and Group E (Vehicle control group) were given 0.25 % of sodium carboxyl methyl cellulose (CMC). Five animals were anaesthetized and then euthanized on day 29, while three animals were kept for recovery evaluation for another two weeks without further administration of the extract. Ten organs were excised from each animal and weighed. The liver and kidney were processed for histopathological studies, while the blood samples were collected for biochemical and haematological assays.

Results: From acute toxicity studies, the LD_{50} value of TOM extract was estimated to exceed 5000 mg/kg bw via oral passage. From Sub-chronic toxicity studies, biochemical results showed a significant ($p < 0.05$) reduction in total protein, albumin, globulin, AST, ALP, and ALT in a dose-dependent manner. Histology of the liver and kidney tissues of all the animals except the kidney of the 800 mg/kg group had no visible lesions; this sets the safety dose for TOM at above 400 mg/kg but below 800 mg/kg. Recovery animals had significantly ($p < 0.05$) increased total protein, total bilirubin, and ALP and decreased albumin and direct bilirubin levels.

Conclusion: This study reports the safety dose of TOM, a reputable medicinal plant extract. This is the first study reporting that the LD_{50} value of TOM extract exceeds 5000 mg/kg bw via oral passage.

1. Introduction

The use of plants in traditional medicine has been of great value in preventing, managing, and treating diverse diseases and illnesses [1]. Medicinal plants are often considered a natural and safe alternative to pharmaceutical drugs because they are rich in therapeutic secondary metabolites [2], serving as an antioxidant (e.g. phenolic compounds), anti-inflammatory (e.g. flavonoids), anti-fungal (e.g. tannins), anti-biotic (alkaloids and saponins), anti-obesity (polyphenols, flavonoids, and saponins) and many more [3,4]. *Trema orientalis*, a member of the Ulmaceae [5], grows up to 8–12 cm high with a straight, slender trunk of 90 cm girth; its wood is white or faintly tinged in pink. Its English name is Charcoal tree, while its local names in Nigeria are Ajennana by the Hausas, Agbolo (Agboloso) by the Igbos, and Afoforo,

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Afefe or Afe by the Yorubas [6]. The charcoal tree grows in both warm temperate and tropical regions, as it is available in Africa, Asia, Australia, China, and India [6,7]. *T. orientalis* is used to produce gunpowder, fireworks, poles, pulp, and paper. The bark of the plant is used for waterproofing fishing lines and for making rope [7–10]. It is eaten by the Zulus in South Africa and taken as spinach in Congo [11–13]. Folkloric use of the leaves and stem bark of *T. orientalis* includes treatment of diabetes mellitus, asthma, dysentery, hypertension, respiratory diseases, oliguria, and malaria. The root is a remedy for bronchitis, trauma, blood stasis, hematuria, and bleeding of the intestine and stomach. At the same time, the stem bark and the leaves are used in treating poisoning, sore throats, cough, yellow fever, gonorrhoea, and toothache [6,11,13–16]. The pharmacological effects of *T. orientalis* that have been reported are analgesic, anti-bacterial, anti-malarial, antioxidant, anti-helminthic, anti-convulsant, anti-diabetic, anti-inflammatory, anti-plasmodial, laxativity, anti-sickling and diuretic activities [11–13, 17,18]. Though *T. orientalis* leaf is eaten in South Africa and Congo, the toxicity evaluation on *T. orientalis* has not been extensively studied nor adequately situated. The only toxicity report was by [12], which estimated the LD_{50} of *T. orientalis* to exceed 2000 mg/kg of TOM, but higher concentrations were not considered. Also, the sub-acute toxicity study report given by [12] was not explicit, as they used 250, 500, and 1000 mg/kg b. wt. of TOM but reported the safety dose to be 400 mg/kg. The study aimed to evaluate the toxicity of the methanol extract of *T. orientalis* (TOM) using acute and sub-chronic toxicity studies in rat model.

2. Materials and methods

2.1. Plant material

The leaves of *T. orientalis* were collected in October 2019 from Agboye, Oyo-East Local Government Area (LGA) of Oyo town in Oyo State, Nigeria. Identification and authentication of the leaf was carried out by Dr. S. A. Odewo, a botanist at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Herbarium specimens were deposited at FRIN herbarium with voucher number FHI.112967.

2.2. Sample Pre-treatment and Extraction

Fresh leaves were carefully hand-picked and properly cleaned to ensure they were free from dirt and foreign matters. The leaves were air-dried at room temperature (25 ± 2.00°C) and then oven-dried at 45°C before pulverization. The sample was milled and stored in an airtight container. A dried pulverized leaf sample (3.2 kg) was successively extracted using the cold maceration technique on a polarity scale of hexane, ethyl acetate, and methanol to yield hexane (TOH), ethyl acetate (TOE), and methanol (TOM) soluble fractions respectively. The filtrates were after that concentrated using a rotary evaporator (Buchi R-300) at 45°C to afford 66.860, 53.130, and 192.210 g extracts of TOH, TOE, and TOM, respectively. The extracts were stored in the desiccator containing anhydrous calcium chloride for further use [20].

2.3. Experimental animals

For the acute toxicity study, twelve healthy ten weeks old female albino rats of Wistar strain (**n = 2**), weighing between 120 and 150 g, and for the sub-chronic toxicity study, forty healthy ten weeks of female albino rats of the same strain (**n = 40**) but weighing 80–120 g, purchased from the University Teaching Hospital Animal house at the University of Lagos, Akoka, Nigeria, were used for the toxicity study. Ethical approval (Protocol Assigned Number: CHREC /087/2021) was obtained from the Covenant University Health Research Ethics Committee (CHREC). The animals were managed following the National Institute of Health (NIH) stipulated protocols for handling laboratory animals (NIH Office of Animal Care and Use, 2016). They were given access to food, water, *ad libitum* with 12 hours of light and darkness. All the animals were allowed to acclimatize for 14 days, during which they were given the standard starter feed, without induction or treatment.

2.4. Experimental designs

The experimental design was according to the stipulation of the Organization for Economic Cooperation and Development (OECD) guidelines 423 [19] as described by [21]. The acute toxicity study used four groups of three female rats in a 3 ×4 model. Group A was given distilled water, and groups B, C, and D were given single doses of 2000, 4000, and 5000 mg/kg bw of TOM extracts. Animals in the treatment groups were observed for signs of toxicity, morbidity, and mortality hourly for the first 24 hours and then monitored daily for two weeks after treatment. For sub-chronic toxicity studies, animals were dosed daily for 28 days. Group A, the negative control group, was given distilled water. Groups B, C, and D had 200, 400, and 800 mg/kg bw TOM extract, respectively, and Group E, the vehicle control group, were given 0.25 % sodium carboxyl methyl cellulose (CMC). From the sub-chronic group, five animals were anaesthetized and then euthanized on day 29. In comparison, three animals were kept for recovery evaluation for an additional two weeks (Groups 200-R, 400-R, and 800-R) through administration of extract terminated on day 28, and these were euthanized on day 43. The weights of each animal were monitored weekly; the animals were fasted and then euthanized on day fifteen (acute toxicity animals), day twenty-nine (Sub-chronic toxicity animals), and day forty-three (recovery animals). The animals were anaesthetized using xylazine and ketamine (1:1) and euthanized through cardiac puncture. Blood samples of rats were collected in EDTA and Li-Heparin bottles for biochemical (including liver functions, kidney functions, and lipid profile) and haematological studies. The liver and kidney of the animals were excised, and aliquots were fixed in formal saline (10 %) for histology studies.

2.5. Biochemical and haematological assays

Blood concentrations of total protein [22], albumin, globulin, A/G, total bilirubin, direct bilirubin, urea [23], creatinine [24], total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and activities of Alkaline phosphatase (ALP)[25], Alanine transaminase (ALT) [26] and Aspartate aminotransferase (AST) [27] were determined using commercial test kits purchased from Randox Laboratory, UK according to manufacturer's protocol. The haematological assessment for packed cell volume (PCV), white blood cell count (WBC), haemoglobin (Hb), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), percentage lymphocyte, neutrophil, eosinophil, monocyte and basophil done using standard methods [28,29].

2.6. Histopathological studies

Histology analysis was carried out using the method described [30, 31] with slight modifications. Organs fixed in formal saline (10 %) were sliced to a thickness of 2.1 mm, dehydrated with alcohol of different concentrations, treated with paraffin wax, and cast into blocks. Sections of the tissues were then cut into microtones of 5 μm. They were after that attached to a slide, allowed to dry, and after that stained with haematoxylin-eosin, and examined under a light microscope; the photomicrographs of the samples were recorded.

2.7. Statistical analyses

All analyses were performed in triplicate, and data was analyzed using one-way analysis of variance (ANOVA) and Tukey's test using SPSS version 21.0 (SPSS Inc., Chicago, II, USA). The probability of $p < 0.05$ was considered to be statistically significant. All data was expressed

as mean ± SEM.

3. Results and discussion

3.1. Animal weights and weights of organs

In the acute toxicity study, administering a single TOM extract at different doses to the Wistar rats resulted in a significant weight loss in all the treatment groups at week 3. Loss of 10 %, 12 %, and 8 % were recorded in the groups given 2000, 4000, and 5000 mg/kg bw of TOM, from total loss of 13 g, 15 g, and 11.3 g, respectively, while the negative control group gained an additional 8 g. The absolute organ weights of the experimental animals (Tables 1 and 2) show that the lungs, kidney, brain, pancreas, and spleen were not significantly ($p > 0.05$) different across the groups, while the absolute organ weights of adipose tissue, liver, and ovaries are significantly ($p < 0.05$) different between the treatment groups and the control (Table 1). In the sub-chronic toxicity study, the absolute weight of the organs was not significantly ($p > 0.05$) different, except for the adipose tissue (Table 2).

Acute toxicity is defined as "the unwanted effect (s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours". The lethal dose (LD_{50}) assessment is a critical parameter in determining the acute toxicity of pharmacological agents [32]. Acute toxicity serves as the basis for other toxicological or pharmacological screenings. LD_{50} is the dose that kills 50 % of the test animal population [33], which is usually assessed by the Lorke method [34]. However, mortality is not enough, as the organism may not die but might have been negatively impacted by the test agent to the point of causing functional impairments in organs, which is only evident in tissue lesions or other biochemical biomarkers [35,36]. The OECD guidelines 423 [19] utilized in this study allow toxicity assessment over a more extended period of 14 days after dosage.

In this study, there was no mortality at both short-term (24 hours) and long-term (14 days) periods, so the LD_{50} value of TOM $^{\text{extract was estimated to exceed 5000}}$ mg/kg bw via the oral passage. This result validates the findings of [12], which concluded that the acute lethal dose (LD_{50}) of *T. orientalis* extracts is higher than 2000 mg/kg of TOM. This study concluded and estimated that the LD_{50} value of TOM $^{\text{extract exceeds 5000}}$ mg/kg bw via the oral passage.

Table 1
Weight of organs excised from the albino rats of Wistar strain after acute toxicity test.

S/N	Groups/ Organ weight (g)	Negative Control	2000 (mg/kg bw)	4000 (mg/kg bw)	5000 (mg/kg bw)
1	Lung	1.017 ± 0.03^{a}	0.966 ± 0.07^{a}	1.018 ± 0.02^{a}	1.016± 0.01^{a}
2.	Kidney	0.445 ± 0.02^{a}	0.419 ± 0.01^{a}	0.434 ± 0.02^{a}	0.470 ± 0.01^{a}
3	Liver	4.393 ± 0.37^{a}	3.193 ± 0.07^{b}	3.448 ± 0.10$^{a, b}$	3.617 ± 0.15^{b}
4.	Brain	1.424 ± 0.07^{a}	1.410 ± 0.05^{a}	1.293 ± 0.05^{a}	1.307 ± 0.05^{a}
5.	Spleen	0.342 ± 0.07^{a}	0.351 ± 0.05^{a}	0.358 ± 0.03^{a}	0.465 ± 0.00^{a}
6.	Intestine	5.368 ± 0.26^{a}	2.840 ± 0.23^{b}	2.765 ± 0.07^{b}	3.145 ± 0.18^{b}
7.	Ovaries	1.145 ± 0.06^{a}	0.533 ± 0.03^{c}	0.815 ± 0.05^{b}	0.928 ± 0.01$^{b, c}$
8.	Pancreas	0.778 ± 0.10^{a}	0.495 ± 0.05^{a}	0.764 ± 0.01^{a}	0.885 ± 0.11^{a}
9.	Heart	0.432 ± 0.01^{a}	0.369 ± 0.00^{a}	0.393 ± 0.01^{b}	0.445 ± 0.01^{b}
10.	Adipose tissue	1.654 ± 0.15^{a}	1.969 ± 0.26^{b}	1.937 ± 0.06^{b}	1.961 ± 0.24^{b}

The values of three replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 2000, 4000, and 5000 represent TOM of 2000 mg/kg bw; 4000 mg/kg bw and 5000 mg/kg bw respectively.

No severe other sign of toxicity was observed, except for the short-lived 2-hour drowsiness observed in the group dosed with 5000 mg/kg bw of TOM extract, which was after that normalized. However, the histology of the kidney tissue revealed different degrees of degeneration in the kidneys of all dosed animals. At the same time, there was marked congestion of the portal area and the central vein in the liver only in the group dosed with 5000 mg/kg of TOM extract. Therefore, the absence of mortality does not ascertain non-toxicity. The different degrees of kidney damage by TOM extract and the protective effect on the liver up to 4000 mg/kg of TOM implies that TOM can be ironically nephrotoxic but hepatoprotective simultaneously, especially below 5000 mg/kg dosage. These findings are in tandem with the report given on the toxicity study of TOM by [37], where TOM reversed degeneration in the liver tissues but resulted in mild toxicity in rat tissues after a long time. They concluded that TOM was capable of alleviating Cd toxicity, though dose regulation is critical in the administration of TOM.

3.2. Biochemical and Haematological Results

The kidney function assessors, such as urea, uric acid, and creatinine, are robust biomarkers for evaluating renal dysfunction [38]. A raised level of plasma urea and creatinine indicates the inefficiency of the kidney and may lead to chronic kidney disease. TOM reduced the creatinine concentrations of the experimental animals, while there was no significant difference in the serum urea concentrations. All creatinine and urea values of the experimental animals fell within the normal range of 0.7–1.4 and 6–20 mg/dl for creatinine and urea, respectively (Fig. 1 and Fig. 2).

The effect of TOM extract on the kidney functions of the experimental animals was assessed using creatinine and urea. Kidney function evaluation of the toxicity animals revealed that TOM extract resulted in a significant ($p < 0.05$) reduction in the creatinine concentrations in the treatment group in a dose-dependent manner (Fig. 1A). Still, there was a notable significant ($p < 0.05$) elevation in the creatinine level of the recovery groups compared with the treatment groups (Fig. 2A). There was no significant difference ($p > 0.05$) in the urea level of the control group and treatment groups in the acute toxicity animals except for the 5000 mg/kg group, where there was significant ($p < 0.05$) reduction in urea level (Fig. 1B). There was no significant ($p > 0.05$) difference in the urea levels of the negative control group and the experimental groups for sub-chronic toxicity animals. However, a significant ($p < 0.05$) difference was observed between the negative control and the vehicle control groups (Fig. 2B).

This is at variance with the histology result because histology assessment shows that the kidney tissues had different degrees of degeneration. However, the liver function results corroborate the histology results of the liver tissue.

Total protein, albumin, globulin and bilirubin values of the treatment groups were reduced, implying TOM's hepatoprotective effect as observed in histology [39,40]. Total protein is a measure of the functionality of the liver, as it is primarily produced by it [41]. Bilirubin, which serves as a blood antioxidant by shielding cells from free radical scavenging, such as lipid peroxidation, showed a significant ($p < 0.05$) reduction in the bilirubin level of the treatment groups compared to the negative control group, indicating that the TOM extract lowered the bilirubin values and invariably capable of protecting the liver. The liver panel tests (ALP, AST and ALT) measure the integrity of the liver. ALP helps in breaking down proteins [42]. ALT converts protein to energy for the liver cells [43], while AST helps in the metabolism of amino acids. All these enzymes are generally present in the blood at low levels, so a raised value of each can be used to diagnose disease conditions. A raised level of ALP means the bile duct is blocked, a raised level of ALT in the bloodstream means liver damage and an increased level of AST in the blood indicates muscle or liver damage [44]. The usual range of ALP, AST and ALT are 40–129, 8–48, and 7–55 U/L respectively [45]. From

Table 2
Weight of organs excised from the albino rats of Wistar strain after sub-chronic toxicity test.

Groups/ Organs weight (g)	Negative Control	200 (mg/kg bw)	400 (mg/kg bw)	800 (mg/kg bw)	200-R (mg/kg bw)	400-R (mg/kg bw)	800-R (mg/kg bw)	Vehicle Control
Lung	0.871 ± 0.212^{a}	1.019 ± 0.116^{a}	1.286 ± 0.028^{a}	1.286 ± 0.143^{a}	1.301 ± 0.086^{a}	0.772 ± 0.275^{a}	1.194 ± 0.022^{a}	1.019 ± 0.031^{a}
Kidney	$0.492 \pm 0.018^{a, b}$	$0.510 \pm 0.027^{a, b}$	$0.519 \pm 0.027^{a, b}$	0.567 ± 0.032^{b}	0.454 ± 0.018^{a}	$0.495 \pm 0.019^{a, b}$	$0.504 \pm 0.012^{a, b}$	$0.510 \pm 0.016^{a, b}$
Liver	4.188 ± 0.167^{a}	4.336 ± 0.319^{a}	4.253 ± 0.313^{a}	4.741 ± 0.354^{a}	4.451 ± 0.293^{a}	4.434 ± 0.179^{a}	4.503 ± 0.161^{a}	4.206 ± 0.173^{a}
Brain	1.371 ± 0.024^{b}	1.116 ± 0.319^{a}	$1.384 \pm 0.063^{a, b}$	$1.245 \pm 0.053^{a, b}$	1.381 ± 0.062^{b}	$1.291 \pm 0.103^{a, b}$	$1.349 \pm 0.072^{a, b}$	$1.307 \pm 0.063^{a, b}$
Spleen	0.487 ± 0.013^{a}	0.452 ± 0.071^{a}	0.567 ± 0.194^{a}	0.449 ± 0.026^{a}	0.456 ± 0.017^{a}	0.441 ± 0.070^{a}	0.457 ± 0.025^{a}	0.462 ± 0.041^{a}
Intestine	5.677 ± 0.273^{a}	5.019 ± 0.030^{a}	5.624 ± 0.338^{a}	5.103 ± 0.728^{a}	6.171 ± 0.017^{a}	4.745 ± 0.587^{a}	5.787 ± 0.457^{a}	5.029 ± 0.341^{a}
Ovaries	0.479 ± 0.186^{a}	0.455 ± 0.590^{a}	0.467 ± 0.132^{a}	0.518 ± 0.250^{a}	0.451 ± 0.249^{a}	0.521 ± 0.038^{a}	0.505 ± 0.057^{a}	0.474 ± 0.140^{a}
Pancreas	1.345 ± 0.140^{a}	0.946 ± 0.057^{a}	0.975 ± 0.109^{a}	0.993 ± 0.253^{a}	0.806 ± 0.151^{a}	1.214 ± 0.179^{a}	1.207 ± 0.162^{a}	0.799 ± 0.101^{a}
Heart	$0.479 \pm 0.007^{a, b}$	0.455 ± 0.020^{a}	$0.467 \pm 0.014^{a, b}$	$0.518 \pm 0.025^{a, b}$	0.451 ± 0.017^{a}	$0.521 \pm 0.012^{a, b}$	$0.505 \pm 0.008^{a, b}$	$0.474 \pm 0.028^{a, b}$
Adipose tissue	$2.066 \pm 0.106^{a, b}$	$2.203 \pm 0.151^{a, b}$	$2.605 \pm 0.075^{b, c}$	$2.407 \pm 0.212^{b, c}$	1.848 ± 0.012^{a}	3.032 ± 0.320^{c}	$2.548 \pm 0.322^{b, c}$	$2.502 \pm 0.323^{b, c}$

The values of five replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 200, 400, and 800 represent TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively. Groups of 200-R, 400-R, and 800-R represent the recovery animals from each of the groups treated with TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively.

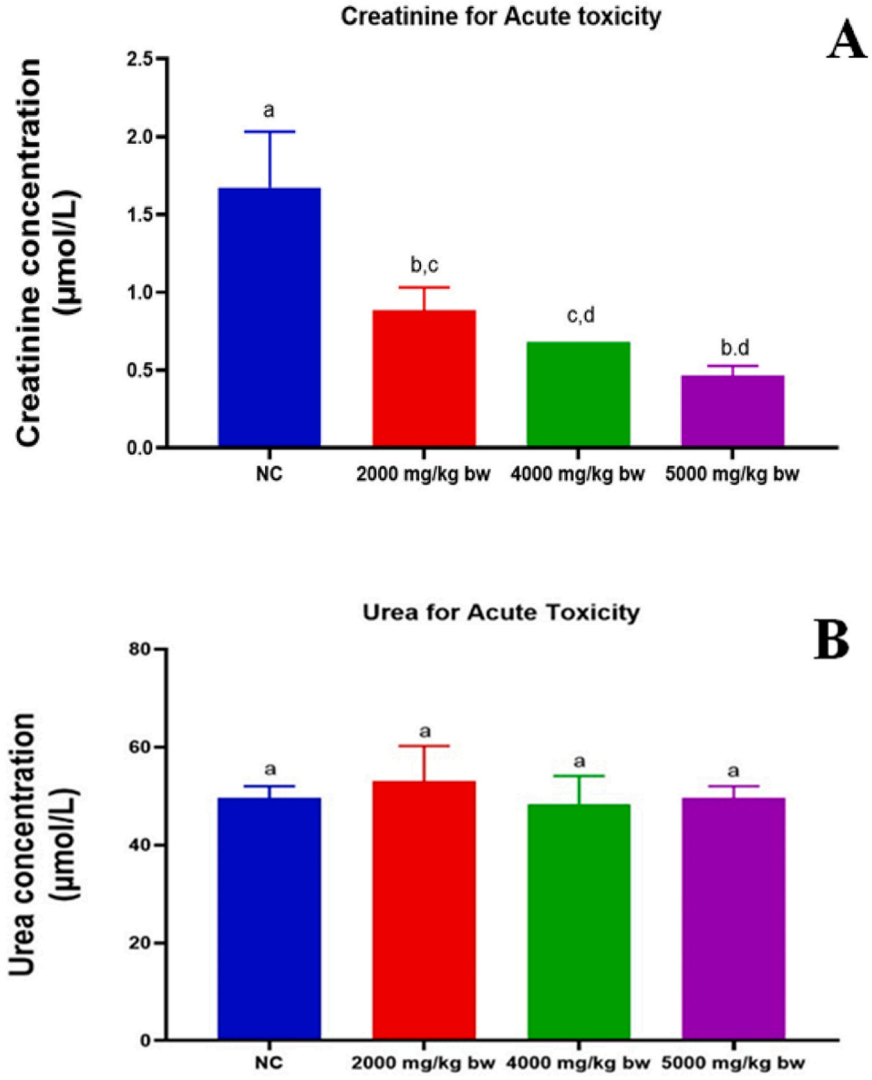

Fig. 1. Effect of TOM extract on the Kidney functions of the acute toxicity animal. **A** - Effect of TOM extract on the creatinine level of acute toxicity animals **B -** Effect of TOM extract on the urea level of acute toxicity animals NC- Negative Control.

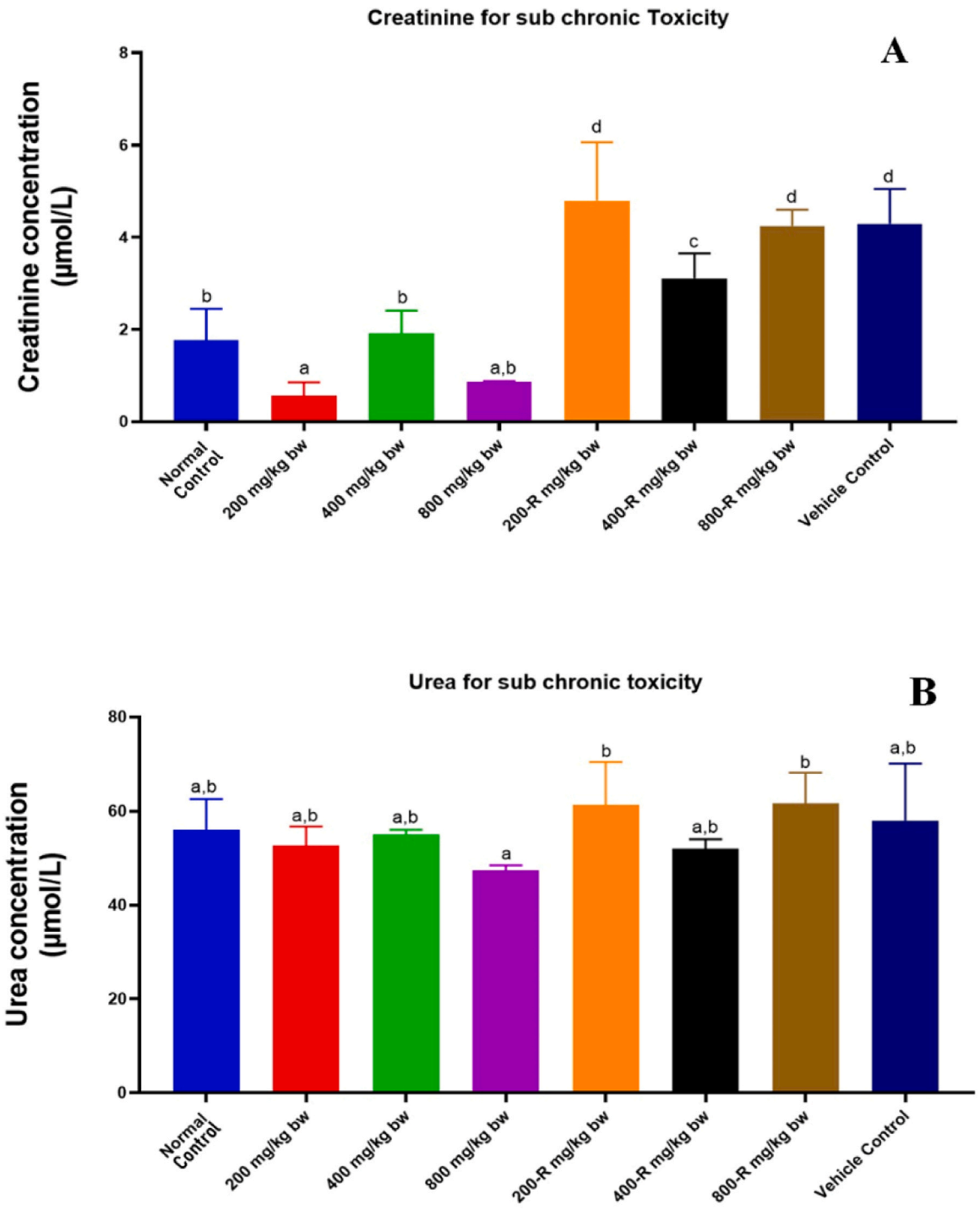

Fig. 2. Effect of TOM extract on the Kidney functions of the Sub-chronic animal. **A** - Effect of TOM extract on the creatinine level of sub-chronic toxicity animals **B** - Effect of TOM extract on the urea level of sub-chronic toxicity animals.

this study, though the ALP, AST and ALT values fell within the acceptable ranges, there was a significant ($p < 0.05$) reduction in the liver panel enzymes of treatment groups compared to the negative control. All liver function markers, including total protein, albumin, globulin, bilirubin, AST, ALP and AST, are in agreement with the findings of [38], who reported that TOM reduced serum total protein, bilirubin, albumin and ALT significantly ($P < 0.05$).

Except for the AST-enzyme level, there was no significant ($p > 0.05$) difference between the control and all the treatment groups of acute toxicity animals. All other liver function biomarkers of the treatment groups were significantly ($p < 0.05$) different from the control group (Table 3). There was a significant ($p < 0.05$) reduction in the total protein, albumin, globulin, total bilirubin, direct bilirubin and serum ALT-enzyme activities in a dose-dependent manner in the treatment groups. Treatment with TOM significantly ($p < 0.05$) increased the serum ALP- enzyme activity in all treatment groups of the acute toxicity animals. In the sub-chronic toxicity group, there was a significant ($p < 0.05$) reduction in the total protein value of the primary treatment groups (200 mg, 400 mg and 800 mg). However, the recovery groups (200-R mg, 400-R mg and 800-R mg) had significantly higher total protein values than the main treatment groups (Table 4). The albumin and total bilirubin levels of the treatment groups increased significantly

Table 3
Effect of TOM extract on liver functions of the rats of Wistar strain used for acute toxicity study.

S/N	Groups/ Biochemical Parameters	Negative Control	2000 (mg/kg bw)	4000 (mg/kg bw)	5000 (mg/kg bw)
1.	Total protein (g/dl)	7.145 ± 0.225[a]	3.820 ± 0.503[b]	3.570 ± 0.335[b]	2.750 ± 0.942[b]
2.	Albumin (g/dl)	2.300 ± 0.399[a]	2.100 ± 0.087[a]	1.76 ± 0.058[b, c]	1.38 ± 0.185[c]
3.	Globulin (g/dl)	4.590 ± 0.659[a]	2.220 ± 0.139[b]	1.090 ± 0.107[b, c]	1.180 ± 0.572[c]
4.	A:G	0.450 ± 0.173[a]	0.950 ±0.121[b]	1.300 ± 0.006[b, c]	0.82 ± 0.318[c]
5.	Total bilirubin (mg/dl)	3.490 ± 0.051[a]	0.45 ± 0.104[b]	1.860 ± 0.231[b]	0.83 ± 0.310[b]
6.	Direct bilirubin (mg/dl)	9.910 ± 0.364[a]	6.550 ± 0.021[c]	7.720 ± 0.157[b]	6.52 ± 0.497[c]
7.	ALP (U/L)	31.74 ± 0.006[a]	182.160 ± 1.774[b]	149.73 ± 5.075[b]	121.04 ± 4.566[b]
8.	AST (U/L)	16.000 ± 2.428[a]	18.000 ± 1.000[a]	14.000 ± 1.000[a]	12.000 ± 2.000[a]
9.	ALT (U/L)	29.250 ± 0.023[a]	19.000 ± 1.63[b]	12.730 ± 0.023[c]	11.000 ± 0.462[c]

The values of three replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 2000, 4000, and 5000 represent TOM of 2000 mg/kg bw; 4000 mg/kg bw and 5000 mg/kg bw respectively. ALP - Alkaline phosphatase, ALT-Alanine transaminase, AST- Aspartate aminotransferase A: G- Albumin: Globulin.

($p < 0.05$). Conversely, there was a significant ($p < 0.05$) reduction in the albumin, direct bilirubin and total bilirubin levels of the recovery groups, evidently in a dose-dependent manner (Table 4). There was a significant ($p < 0.05$) increase in serum activities of the ALP enzyme of the treatment group and a notable and significant ($p < 0.05$) increase in the recovery groups except for group 800-R. The serum activities of the ALT-enzymes in the treatment groups and the recovery groups were not significant ($p > 0.05$) different from the negative control group, except for the distinct and significant ($p < 0.05$) increase in the group given 200 mg/kg bw of TOM. There was no significant difference in the serum activities of the AST enzymes in all treatment and recovery groups, except for the group given 800 mg/kg bw of TOM extract. However, unexpectedly, the AST and ALT levels of the vehicle control group were significantly ($p < 0.05$) lower than the negative control group (Table 4).

A lipid profile result with high TC, TG, LDL, and low HDL is a risk for cardiovascular diseases and obesity [48]. While a reverse result will help improve heart, healthy weight and obesity-related health challenges. The optimal range for the lipid profile requires that the value of TC should be under 200 mg/dl, LDL should be under 100 mg/dl, TG should be under 150 mg/dl, and the HDL is meant to be over 60 mg/dl [49].

The lipid panel analysis of the TOM effect in this study shows a significant ($p < 0.05$) increase in HDL values, while the TG and LDL values of the treatment group significantly ($p < 0.05$) reduced compared to the control group. This effect was not observed in TC values because TC is a total of HDL and LDL, and a high HDL will increase the value of total cholesterol when added to LDL concentration. This improvement in HDL and reduction in TG and LDL can indicate the weight loss capability of TOM extract. This may elucidate the 8–12 % loss in body weight of the groups dosed with TOM, compared to the steady weight gain in the control group. This would have been a sign of toxicity if the weight of organs excised from the experimental animals were altered. However, the weight of organs excised from the dosed group was not significantly ($p > 0.05$) different from that of the negative control group, except for the adipose tissue, liver and ovaries. As for the recovery groups, the liver and kidney histology results show no recovery from the effect of TOM extract. This means that any toxicity effect or impairment caused by such an agent might be permanent or require another form of treatment before recovery.

The lipid profile of acute and sub-chronic toxicity animals shows that there was a significant ($p < 0.05$) reduction in the triglyceride level of the treatment groups in comparison to the control (Tables 5 and 6). There was a significant ($p < 0.05$) increment in the total cholesterol levels in both acute and sub-chronic animals (Tables 5 and 6). There was also a significant ($p < 0.05$) increment in the low-density lipoprotein levels in sub-chronic animals, but a reduction in the low-density lipoprotein levels in the acute toxicity animals (Tables 5 and 6). In sub-chronic toxicity animals, high-density lipoprotein was significantly ($p < 0.05$) reduced, while in acute toxicity animals, high-density

Table 5
Effect of TOM extract on the lipid profile of rats of Wistar strain used for acute toxicity study.

S/N	Groups/ Lipid profile parameters	Negative Control	2000 (mg/kg bw)	4000 (mg/kg bw)	5000 (mg/kg bw)
1.	Total Cholesterol (mg/dl)	117±9.77[a, b]	143.5 ±4.94[b, c]	182 ±3.26[c]	121.46 ±4.16[a]
2.	Triglycerides (mg/dl)	111.33± 0.18[a]	45.33 ±0.52[b]	36.66 ±0.27[b]	30.75 ±1.06[b]
3.	High Density Lipoprotein (mg/dl)	173 ± 0.00[a]	87.01± 1.68[b]	44.24 ±0.53[c]	51.15 ±0.80[c]
4.	Low-Density Lipoprotein (mg/dl)	114.24± 1.64[a]	27.32 ±0.06[b, c]	14.03 ±1.05[c]	24.04 ±3.42[b]

The values of three replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 2000, 4000, and 5000 represent TOM of 2000 mg/kg bw; 4000 mg/kg bw and 5000 mg/kg bw respectively

Table 4
Effect of TOM extract on liver functions of the rats of Wistar strain used for sub-chronic toxicity study.

Groups/ Biochemical Parameters	Negative Control	200 (mg/kg bw)	400 (mg/kg bw)	800 (mg/kg bw)	200-R (mg/kg bw)	400-R (mg/kg bw)	800-R (mg/kg bw)	Vehicle Control
Total protein (g/dl)	7.33±0.07 [f]	1.65±0.07[a, b]	3.8±0.27[c]	0.59±0.00[a]	5.1± 0.15[d]	6.52±0.20[e]	5.23±0.15[d]	2.86±0.47[b]
Albumin (g/dl)	2.48±0.18[a]	3.41±0.07[b]	4.16±0.13[c]	4.57±0.15[c]	3.28±0.16[b]	2.31±0.20[a]	2.27 ± 0.12[a]	1.33 ± 0.22[d]
Globulin (g/dl)	4.94±0.20[c]	1.75±0.10[a]	1.61±0.24[a]	2.14±0.43[a, b]	1.94±0.01[a]	2.94± 0.25[b]	2.07±0.02[a, b]	1.40±0.17[a]
A/G	0.49±0.06[a]	1.96 ±0.08[d]	1.49±0.27[c]	1.51±0.10[c]	1.68±0.20[d]	0.75±0.01[a, b]	1.1±0.07[b]	0.51±0.03[a]
Total bilirubin (mg/dl)	3.94±0.34[a, b]	4.52±0.15[b]	5.18±0.33[b]	6.98±0.57[c]	4.39 ±0.57[b]	4.53 ±0.16[b]	4.77 ± 0.16[b]	2.51 ± 0.11[a]
Direct bilirubin (mg/dl)	5.14±0.46[a, b]	5.8±0.00[b]	4.35±0.45[a, b]	9.24±0.53[c]	4.91±0.79[a, b]	3.06±0.33[a]	4.32±0.21[a, b]	2.15±0.33[a]
ALP(U/L)	25.23±0.30[a,	49.97±2.67[b]	75.44±0.58[c]	44.16±0.87[b]	78.66± 0.00[c]	90.99±0.64[d]	35.53±0.65[a]	32.38±3.00[a]
AST (U/L)	27.00±0.00[b, c]	30.5±2.93[c]	25±1.03[b, c]	36±2.84[c]	27±2.52[c, d]	25.67±1.03[b, c]	21± 1.26[b]	12.95±2.06[a]
ALT (U/L)	36.50±1.28[b, c]	52.33±0.165[c, d]	48.0±0.00[b, c, d]	34.90±4.48[b, c]	41.00±4.42[b, c, d]	37.33±1.29[b, c]	34.00±4.02[b]	20.80±0.65[a]

The values of five replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 200, 400, and 800 represent TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively. Groups of 200-R, 400-R, and 800-R represent the recovery animals from each of the groups treated with TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively. ALP - Alkaline phosphatase, ALT-Alanine transaminase, AST- Aspartate aminotransferase A: G- Albumin: Globulin.

Table 6
Effect of TOM extract on lipid profile of the rats of Wistar strain used for sub-chronic toxicity study.

Groups/ Lipid profile parameter (mg/dl)	Negative Control	200 (mg/kg bw)	400 (mg/kg bw)	800 (mg/kg bw)	200-R (mg/kg bw)	400-R (mg/kg bw)	800-R (mg/kg bw)	Vehicle Control
Total Cholesterol	118.50±7.52^{b}	161.50 ±6.56^{c}	230±15.15^{d}	226±14.28^{d}	180±0.00^{c}	161±10.73^{c}	135±0.63^{b}	80.36±6.25^{a}
Triglycerides	102.33±3.79$^{a, b}$	63 ±2.63^{a}	90 ±5.68$^{a, b}$	88±15.68$^{a, b}$	83.5±2.04$^{a, b}$	58.5±2.86^{a}	82.67±0.05$^{a, b}$	162 ±10.42^{c}
High-Density Lipoprotein	78.22±0.79^{a}	84.29±0.65^{a}	155.61 ±1.66$^{c, d}$	94±3.63^{c}	196.4±0.00^{d}	88.37±1.20^{b}	95.58±0.23^{c}	57.71±0.67^{a}
Low-Density Lipoprotein	91.3±4.30^{a}	132±9.58	121.17 ±0.98^{b}	216.98 ±11.61^{c}	159.15±0.00$^{b, c}$	137.4±8.32^{b}	89.39±10.12^{a}	67.03 ±4.43^{a}

The values of five replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 200, 400, and 800 represent TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively. Groups of 200-R, 400-R, and 800-R represent the recovery animals from each of the groups treated with TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively.

lipoprotein was significantly ($p < 0.05$) increased, compared to the negative control groups.

The effect of drugs, xenobiotics and other substances can be assessed through the blood via their impact on haematological functions; haematological criteria such as packed cell volume, red blood cell count, and white blood cell count are used to determine the level of harmful effects or beneficial functions of plant extracts [31]. The normal range for PCV is (36−51), Hb (11.5–16.5 g/dL), MCHC is (27–32 g/L), WBC (3.6–11.0 cell x 10^9/L), neutrophils (40–60 %), lymphocytes (20–40 %), monocytes (2–8 %), eosinophils (1–4 %), and basophils (0.5–1 %) [46, 47]. There was no significant difference ($p > 0.05$) in all experimental animals' PCV, Hb, MCHC, WBC, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

In acute toxicity studies, the haematology result shows that there was no significant ($p > 0.05$) difference between the packed cell volume (PCV), Haemoglobin (Hb), MCHC, WBC, eosinophil, monocyte count, and basophil count of the control and all treatment groups (Table 7). There was no significant ($p > 0.05$) difference between the Hb levels of the control group and the groups given 2000 and 5000 mg/kg bw of TOM, however, there was a slight reduction in the Hb level of the group given 4000 mg/kg bw of TOM. The lymphocyte counts were significantly ($p < 0.05$) higher in the group given 4000 mg/kg bw of TOM than in the control group; however, there is no significant difference ($p > 0.05$) between the control group and the group given 5000 mg/kg bw of TOM, while the group was given 4000 mg/kg bw of TOM had a significantly ($p < 0.05$) lower lymphocyte count when compared to the control group. For basophil count, there is no significant ($p > 0.05$) difference between the control and other treatment groups, all were 2.00 % except for the group given 4000 mg/kg bw of TOM has a slightly lower value of 1.30 % (Table 7). From the Sub-chronic toxicity studies, there was no significant ($p > 0.05$) difference between the packed cell volume (PCV) of the normal and vehicle control groups as well as the group given 800 mg/kg bw of TOM. And except for the group given 800 mg/kg bw of TOM, the PCV of other recovery groups was lower than their corresponding treatment groups (Table 8). There was no significant ($p > 0.005$) difference between the Haemoglobin (Hb) levels of the control groups and the groups given 200 and 400 mg/kg bw of TOM, however, there was a significant ($p < 0.05$) increase in the Hb level of the group given 800 mg/kg bw of TOM. The Hb levels of all recovery groups were notably lower than those of their primary treatment groups (Table 8). There was no significant ($p > 0.05$) difference in the MCHC, WBC, and certain differentials, including monocyte, eosinophil, and neutrophil of the control group and the treatment groups (Table 8). There was no significant ($p > 0.05$) difference between the lymphocyte counts of the negative control group and the groups given 800 mg/kg bw of TOM extract, while the group was given 400 mg/kg bw of TOM was slightly higher in count than the negative control. There was a notable reduction in the lymphocyte count of the recovery groups compared to the treatment groups (Table 8).

Table 7
Effect of TOM extract on Heamatology parameters of the rats of Wistar strain used for acute toxicity study.

S/N	Groups/ Heamatology Parameters	Negative Control	2000 (mg/kg bw)	4000 (mg/kg bw)	5000 (mg/kg bw)
1.	PCV	34.67 ± 2.19^{a}	33 ± 5.51^{a}	30 ± 5.78^{a}	38 ± 1.16^{a}
2.	Hb (g/L)	11.17 ± 0.17^{a}	10.6 ± 5.51^{a}	9.7 ± 1.85^{a}	12.3 ± 0.38^{a}
3.	MCHC (g/L)	32.20 ± 7.44^{a}	32.19 ± 3.29^{a}	32.36± 3.39^{a}	32.28 ± 0.35^{a}
4.	WBC cell x 10^9/L	5.37 ± 1.90^{a}	3.88 ± 0.35^{a}	2.62 ± 0.08^{a}	3.00 ± 0.01^{a}
5.	Neutrophil (%)	49.00 ± 6.94^{a}	37.00 ± 3.29^{a}	59.00 ± 3.39^{b}	45.00 ± 2.89^{b}
6.	Lymphocyte (%)	35.00 ± 4.92^{a}	47.00 ± 2.91^{b}	26.00 ± 0.88^{a}	34.00 ± 4.64^{a}
7.	Eosinophil (%)	11.00 ± 1.58^{a}	12.00 ± 1.45^{a}	10.00 ± 3.06^{a}	17.00 ± 4.060^{a}
8.	Monocyte (%)	3.00 ± 1.00^{a}	2.00 ± 0.67^{a}	3.00 ± 0.58^{a}	3.00 ± 1.64^{a}
9.	Basophil (%)	2.00 ± 0.82^{a}	2.00 ± 0.82^{a}	1.30 ± 0.33^{a}	2.00 ± 0.58^{a}

The values of three replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 2000, 4000, and 5000 represent TOM of 2000 mg/kg bw; 4000 mg/kg bw and 5000 mg/kg bw respectively. Packed cell volume (PCV), haemoglobin (Hb), mean cell haemoglobin count (MCHC), white blood count (WBC)

3.3. Histopathological studies

Fig. 3 shows the histological architecture of the kidneys of the acute toxicity animals, where A, B, C, and D are the kidneys of the negative control group, the group given 2000, 4000 and 5000 mg/kg bw of TOM extract respectively. Fig. 3A showed no visible lesion, but the kidneys of the dosed groups had various degrees of necrosis ranging from degeneration of renal tubules in 3B, moderate interstitial congestion, as well as moderate congestion of the renal interstitium in 3 C and tubular and glomerular degeneration, debris in the tubules, and cast in the lumen in Fig. 3D.

Figs. 4A, 4B, 4 C and 4D are the livers of the negative control group, and the group was given 2000, 4000 and 5000 mg/kg bw of TOM extract, respectively. Figs. 4A, 4B, and 4C had no visible lesions. However, marked congestion of the portal area and the central vein was observed in Fig. 4D.

Figs. 5A, 5B, 5 C and 5D are the liver and kidney tissues of both negative and vehicle controls; no visible lesion was observed in the four tissues of the control groups.

Figs. 6A and 6B are the kidney and liver of the group given 200 mg/kg bw of TOM extract, which showed no sign of toxicity as there was no visible lesion. Figs. 6C and 6D are the kidney and liver of the recovery

Table 8
Effect of TOM extract on Heamatology results of the rats of Wistar strain used for sub-chronic toxicity study.

Groups/ Heamatology Parameters	Negative Control	200 (mg/kg bw)	400 (mg/kg bw)	800 (mg/kg bw)	200-R (mg/kg bw)	400-R (mg/kg bw)	800-R (mg/kg bw)	Vehicle Control
PCV	34.00±1.02$^{b, c, d}$	29.00±2.73$^{b, c}$	28.00±2.80$^{b, c}$	35.00±2.66$^{b, c, d}$	19.00±0.26^{a}	26.00±1.83$^{a, b}$	42.00±2.87^{d}	36.00±3.28^{d}
Hb (g/L)	11.00±0.34$^{c, d}$	9.90±0.53$^{b, c}$	9.10±0.88$^{b, c}$	34.00±1.02^{f}	6.00±0.07^{a}	8.50±0.72^{b}	13.70±0.93^{e}	12.93±0.62$^{d, e}$
MCHC (g/L)	32.28 ± 0.05^{a}	32.29±0.5^{a}	32.31 ± 0.15^{a}	32.25 ± 0.07^{a}	32.17±0.02^{a}	32.29±0.02^{a}	32.25±0.04^{a}	32.21±0.01^{a}
WBC cell x 10^9/L	3.80±0.36$^{a, b}$	5.30±0.50^{b}	3.40±0.50$^{a, b}$	4.50±0.87$^{a, b}$	4.70±0.54$^{a, b}$	2.10±0.49^{a}	1.90±0.17^{a}	3.30±0.37$^{a, b}$
Neutrophil	39.00±2.92^{a}	36.00±2.80^{a}	48.00±4.34$^{a, b}$	44.00±4.38$^{a, b}$	39.00±1.78^{a}	54.00 ± 4.15^{b}	54.00±1.43^{b}	55.00±3.45^{b}
Lymphocyte	43.00±2.47$^{b, c, d}$	49.00±0.99^{d}	34.00±4.25$^{a, b, c}$	42.00±5.52$^{b, c, d}$	46.00±1.28$^{c, d}$	25.00±2.20^{a}	28.00±3.31^{a}	31.00±3.16$^{a, b}$
Eosinophil	15.00±2.25^{a}	11.00±1.69^{a}	13.00±0.87^{a}	11.00±2.57^{a}	11.00±0.56^{a}	16.00±1.64^{a}	14.00±1.52^{a}	15.00±1.68^{a}
Monocyte	2.00±0.58^{a}	2.00±0.54^{a}	2.50±0.55^{a}	3.00±0.56^{a}	3.00±0.37^{a}	2.00±63^{a}	4.00±0.63^{a}	3.00±0.70^{a}
Basophil	2.00±0.50^{b}	2.00±0.40^{b}	2.50±0.22^{b}	3.00±0.22$^{a, b}$	2.00±0.37^{b}	2.00±0.43^{b}	1.00±0.00^{a}	3.00±0.26^{c}

The values of five replicates are indicated as the Mean ± SEM. Values within the same row having the same superscript are not significantly ($p > 0.05$) different from each other. Groups of 200, 400, and 800 represent TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively. Groups of 200-R, 400-R, and 800-R represent the recovery animals l from each of the groups treated with TOM of 200 mg/kg bw, 400 mg/kg bw and 800 mg/kg bw respectively.
Packed cell volume (PCV), haemoglobin (Hb), mean cell haemoglobin count (MCHC), and white blood count (WBC).

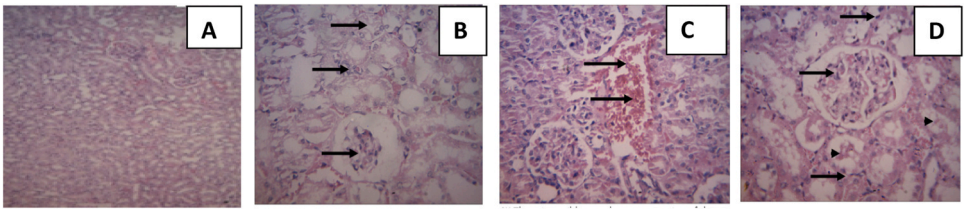

Fig. 3. : Effect of TOM extracts on histology of rat's kidney in acute toxicity studies. A - Negative Control B- 2000 mg/kg bw of TOM C- 4000 mg/kg bw of TOM D - 5000 mg/kg bw of TOM Hematoxylin and Eosin x 100 of tissues for representing each of the treatment clusters.

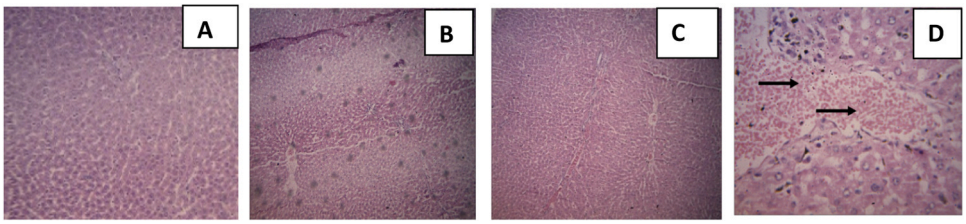

Fig. 4. : Effect of TOM extracts on histology of rat's liver in acute toxicity studies. A - Negative Control B- 2000 mg/kg bw of TOM C- 4000 mg/kg bw of TOM D - 5000 mg/kg bw of TOM Hematoxylin and Eosin x 100 of tissues for representing each of the treatment clusters.

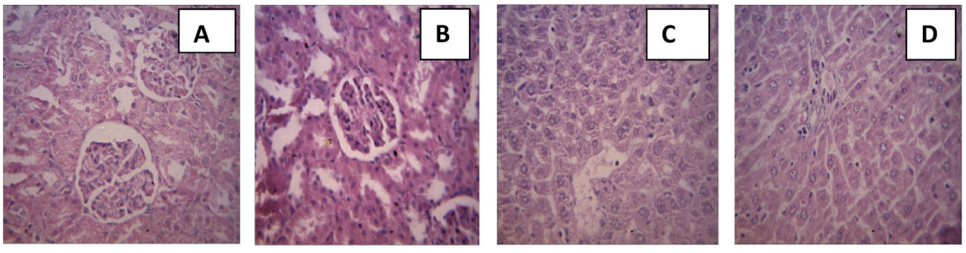

Fig. 5. : Effect of standard and vehicle control on the histology of rat kidney and liver in Sub-chronic toxicity studies. A - Negative control kidney B- Negative control liver C- Vehicle control kidney D - Vehicle control liver Hematoxylin and Eosin x 100 of tissues for representing each of the treatment clusters.

groups.

The group's kidney and liver given 400 mg/kg bw of TOM extract had no visible lesions (Figs. 7A and 7B). The kidney tissue of the 400-R group (Fig. 7C) had diffused glomerular and interstitial congestion (arrow) and congestion at the renal cortex. The liver of the 400-R group also had mild hydropic degeneration (Fig. 7D).

Figs. 8A and 8B are the kidney and the liver of the group given 800 mg/kg bw of TOM extract. The presence of proteinous casts was observed in the lumen of the kidney, and debris was seen in the tubules of the kidney tissue (Fig. 8A). However, the liver of the group given 800 mg/kg bw of TOM extract had no visible lesion (Fig. 8B). Both the kidney and liver of the recovery groups had lesions.

3.4. The chemical profile of the methanol extract of Trema orientalis leaf

Infrared (IR) spectrum of TOM (Fig. 9) gives an IR υ_{max} at

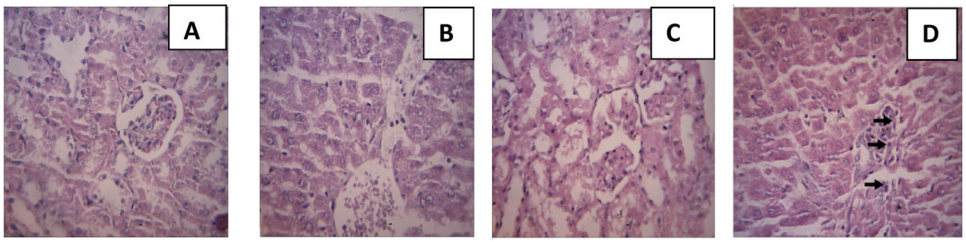

Fig. 6. : Effect of 200 mg/kg bw of TOM extracts on histology of rat's kidney and liver in treatment and recovery groups of sub-chronic toxicity studies. A - 200 mg/kg kidney B- 200 mg/kg liver C- 200-R mg/kg kidney D - 200-R mg/kg liver Hematoxylin and Eosin x 100 of tissues for representing each of the treatment clusters.

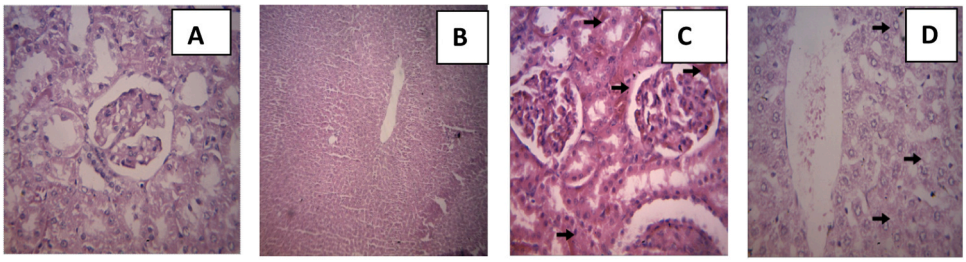

Fig. 7. : Effect of 400 mg/kg bw of TOM extracts on histology of rat's kidney and liver in treatment and recovery groups of sub-chronic toxicity studies. A - 400 mg/kg kidney B- 400 mg/kg liver C- 400-R mg/kg kidney D - 400-R mg/kg liver. Hematoxylin and Eosin x 100 of tissues for representing each of the treatment clusters.

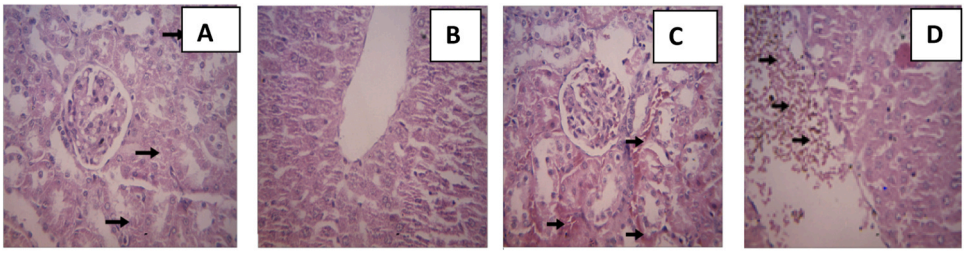

Fig. 8. : Effect of 800 mg/kg bw of TOM extracts on histology of rat's kidney and liver in the treatment and recovery groups of sub-chronic toxicity studies. A - 800 mg/kg kidney B- 800 mg/kg liver C- 800-R mg/kg kidney D −800-R mg/kg liver. Hematoxylin and Eosin x 100 of tissues for representing each of the treatment clusters.

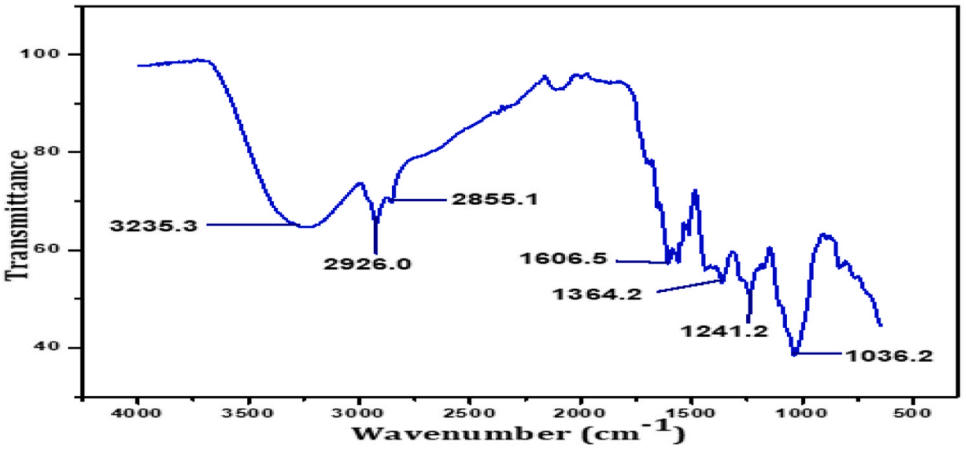

Fig. 9. FT-IR Spectrum of TOM.

3235.3 cm^{-1} showing a broad band of O-H, between 3500 and 3235 (O-H stretching, broad), 2926, 2855 (C-H stretching, CH_3, CH_2), 1703 (C═O), 1606 (C═C, aromatics or alkenes), 1364, 1241 (C-O stretching), 1036 cm^{-1} (possibly O-H deformation). The major peaks in the study are in the same ranges with the infrared spectrophotometric data of methanol leaf extract of TOM previously reported [50].

4. Conclusion

From this study on the acute and sub-chronic toxicity of TOM, the lethality dose of TOM (LD_{50}) is estimated to exceed 5000 mg/kg bw. The study also concludes that the acute toxicity index should not be limited to mortality rate within 24 hours, but other biochemical evaluations like

liver function, kidney function, lipid profile, and haematological evaluation, as well as histology of tissues, especially the liver and the kidney should be used to assess toxicity indices. The safety dose of TOM extract is set at 400 mg/kg bw of TOM, while higher doses, especially 800 mg/kg TOM, can lead to various degrees of lesions in the kidney and liver of animals. The study also concludes that the recovery animals did not show signs of reversed effects. Furthermore, the solution or damage caused by TOM extract can be permanent. Therefore, any pharmacological effect exerted by TOM will most likely be a lasting effect.

Authors' contribution

Omokehinde Taiwo- performed the major wet lab experiments and animal care, analyzed and interpreted the data, drafted the manuscript, and funded the research. Olubanke Ogunlana- Methodology development, supplied some of the reagents used, reviewed and edited the original draft. Abiodun Adebayo- Experimental design, supervision, data validation, and editing of the original draft. Joseph Olugbuyiro- Research supervision, data curation and validation, general reviewing and editing of the original draft, and corresponding author.

CRediT authorship contribution statement

JOSEPH ADEBISI O. OLUGBUYIRO: Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Omokehinde F. Taiwo:** Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Abiodun H. Adebayo:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Olubanke O. Ogunlana:** Writing – review & editing, Validation, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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