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Evaluation of Acute and Subacute Oral Toxicity of *Erlangea tomentosa* (Oliv. & Hiern) S. Moore (Asteraceae) Methanol Leaf Extract in Experimental Wistar Albino Rats

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Abstract

Erlangea tomentosa (Oliv. & Hiern) S. Moore's leaves, which are in the Asteraceae family, were tested for acute and subacute toxicity in experimental Wistar rats. Lorke's (bi-phasic) method was used to evaluate the acute toxicity profile of the plant extract. In phase 2, twelve rats of both sexes were administered a maximum dosage of 5000 mg/kg of extract. Observations of toxicity signs were made and recorded for 2 hours consecutively, for 24 hours intermittently, as well as for the next 14 days. For the subacute study, rats were orally administered water and plant extract daily for 28 days. Toxicological effects were recorded daily, and body weights were documented weekly. Hematological, biochemical, and histopathological tests as well as relative organ weights were evaluated at the conclusion of the study. In phase 2 of the acute toxicity study, it was found that the plant extract was toxic at doses of 1600, 3100, and 5000 mg/kg, with only one death at the highest dose. In the subacute toxicity study, animals that were administered 800 mg/kg of extract for 28 days showed symptoms of toxicity. The weight of the kidneys increased; urea and ALT levels increased; as well as total protein and albumin levels decreased compared to the control group. At the same dose, histopathology examination revealed alterations of the liver and kidneys, while hematopoietic cells were significantly disrupted compared to the control animals. Based on the findings, the methanol-extracted leaves of *E. tomentosa* showed a moderate level of acute toxicity. The administration of the extract at a dose of 400 mg/kg was safe, but 800 mg/kg was toxic. This caused damage to their livers and kidneys. This led to an increase in ALT and ALP, changes in blood parameters like Hb, RBC, and PLT, as well as the development of inflammatory cells.

Keywords: *Erlangea tomentosa*, methanolic extract, acute toxicity, subacute toxicity

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1 Introduction

The use of medicinal plants is a part of African culture and tradition [1]. They are also a source of biological diversity and an ethnopharmacological basis for drug production [2]. Herbal medicine continues to be the greatest portion of health care for approximately 75-80% of the international population, mostly in low-developed countries [3]. This is due to the common belief that herbal remedies are safe, affordable, and readily available locally [4]. However, the absence of sufficient consistency, safety precautions, quality control, and adulteration with conservative drugs is the main barrier to the use of phyto-remedies [5].

Toxicology testing is a critical step in determining whether industrial products, drugs, or other compounds constitute a danger to human beings, creatures, or the environment [6]. Acute toxicity is used to describe the negative effects that can happen in the first 24 hours or sometimes 14 days after taking a single dose of a substance [7]. According to regulations, the main goals of this study are to find a dose that has significant side effects and to figure out the minimum lethal dose. Among the many indicators to look out for, sleep, convulsions, salivation, diarrhea, lethargy, coma, tremors, and death should all be taken into consideration [8]. Repeated oral exposure to a substance can have risks and hazards, so these studies may help figure out the dosing regimen for long-term use that is safe for international guidelines [9].

As a result, all phytochemical substances should have their effectiveness as well as safety scientifically confirmed [10]. *E. tomentosa* (Oliv. & Hiern) S.Moore (Asteraceae) is a woody erect herb that can reach a height of 50 cm. In Runyankore, it is known as *Ekyoganyanja*. According to Muhwana et al. [11], people who use traditional herbal medicine often use the leaves of this plant to make herbal remedies for many different types of sickness. According to research done by Asiimwe and his colleagues in Western Uganda, the herb has been used in the past to treat colic, stomachaches, syphilis, fever, and even miscarriage [12].

Other ailments treated with the plant include diarrhea, skin infections, anemia, appetite stimulation, conjunctivitis, children's convulsions, analgesics, and inflammation, as well as mental confusion [11]. Despite adequate evidence for its efficacy, little scientific evidence supports its safety as an herbal medicine. So, we can only be sure that the use of a certain species is safe after careful investigation [7]. Consequently, the purpose of this research was to investigate the acute and subacute oral toxicity profiles of the methanol leaf extract of *E. tomentosa* in Wistar albino rats.

2 Materials and Methods

2.1 Study design

This was an in vivo experimental study. The evaluation of the toxicity effects of a methanol leaf extract of *E. tomentosa* was conducted in a laboratory setting.

2.2 Collection and identification of plant material

The leaves of *E. tomentosa* were hand-picked from Bwegiragye village, in Bushenyi District, Western Uganda. The plant was identified by a plant taxonomist from Mbarara University of Science and Technology (MUST). A voucher specimen with the number B50891 was deposited in the herbarium of the Department of Botany for future reference.

2.3 Extraction of the plant material

The plant extracts were prepared according to Begashaw and Porwal's method [13] [14]. To summarize, newly collected plant leaves were washed 2-3 times with tap water to eliminate adherent particles before being dried in the shade until a consistent mass was achieved. With the aid of a mortar and pestle, the dried leaves were ground into a fine powder. The powdered materials were packed in closed, dry khaki paper bags at room temperature, out of direct sunlight. To obtain the hydroalcoholic crude extract, we macerated this coarsely powdered plant (300g) in 70% methanol (3000ml) for 3 days at room temperature in an Erlenmeyer flask. Filter paper was used to remove the filtrate from the marc after 72 hours (Whatman No.1). The solvent was then enabled to vaporize from the filtrate using a rotary evaporator under reduced pressure and more concentrated in a water bath at 55 ◦ C. The condensed extract (57.31g) was maintained at 4 °C till the experiment was over [14]. The extraction produced a yield of 19.10 % calculated with respect to the dry weight.

2.4 Experimental animals

The Wistar albino rats (*Rattus norvegicus*) of 8-10weeks used for the experiment were obtained from the animal house facility of the Department of Pharmacology and toxicology, Kampala International University Western Campus (KIU-WC). Rats of the same gender were randomly put into the control and treatment groups. They could be housed separately if it was technically necessary. The rats were kept in their havens for five days prior to the start of the treatment study to permit acclimatization to the laboratory environments. The rats were fed a normal rodent pellet diet and had access to unlimited water [15]. Additionally, the Guidelines for the Uganda animal regulations, and the National Academies Press's Guide for the Concern and Usage of Laboratory-Animals, published in Washington, DC, were followed to ensure animal health and rights [16] [17]. All of the protocols were approved by the Animal Research Ethics Committee at KIU-WC, which gave the reference number of KIU-2022-86 before the start of the experiments.

2.5 Inclusion and exclusion criteria

Normal and mature rats of both sexes were used for both the acute and subacute toxicity studies. Rats that were pregnant or nursing were not used, and rats with external wounds or a rough coat were not used either.

2.6 Test chemicals

Methanol of analytical grade (99.5%) used in this study was purchased at a local Chemical shop supplier, Ltd. (Bushenyi District).

2.7 Acute oral toxicity evaluation

The method described by Lorke [18] and modified by Chinedu et al. [19] was used to determine the oral median lethal dose (LD_{50}) of the plant extract in rats. To summarize, this method is made up of 2 phases. In the first phase, the administration of the extract was applied at doses of 100, 500 and 1000 mg/kg to three groups of animals, involving three animals each. The observation of signs of toxicity like excessive perspiration, tremors, convulsions, salivation, diarrhea, mortality, and other toxicity signs was observed consecutively for 2 hours then intermittently for 24 hours. The 2nd phase consisted of 3 groups of animals randomly chosen, each consisting of a single animal. Based on the results of the first step, no deaths occurred at any of the dose levels in phase I after 24 hours. Then animals received graded doses (1600, 3100, and 5000mg/kg) of the extract and signs of toxicity, including deaths, were observed and documented for 2hours consecutively and 24hours intermittently as well as for the next 14days.

Finally, the calculation of the LD_{50} value was estimated by considering the geometric mean of the smallest dose that has caused death

as well as the large dose at which the animals survived.[19] $LD_{50} = \sqrt{D_0 X D_{100}}$, D_0 = the highest dose without death, D_{100} = the lowest dose that causes death.

2.8 Subacute oral toxicity study experimental setup

A total of twenty-four rats of both sexes were arbitrarily allocated into four classes of six rats each. After the acute toxicity profile was done, 200, 400, and 800 mg/kg were chosen as low, intermediate, and high doses to test on animals.

The changes in physiological and behavioral parameters were recorded, as was the case for the assessment of acute toxicity, including weekly recording of animal body weights. On day 29, the rats were fasted overnight and killed under 4% halothane vapor in a closed chamber. This was done so that histopathology tests could be done on the liver and kidneys, as well as hematological and biochemical tests [3].

2.9 Hematological and biochemical estimations

Using common laboratory procedures, all of the hematological tests, such as red blood cell (RBC) and hemoglobin (Hb), hematocrit (HCT), packed cell volume (PCV), thrombocytes, neutrophils, lymphocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) counts, etc., were measured in blood EDTA-containing tubes. The biochemical analysis was carried out on the plasma/serum and homogenized samples. The serum was used to determine glucose, urea, creatinine, albumin, total bilirubin, direct bilirubin, total proteins, and liver marker enzymes like AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), and ALP (Alkaline Phosphatase) [20].

2.10 Histopathological analysis

Representative rat's liver and kidney per group were separated and immersed in formaldehyde solution 10%. The tissues would then be prepared, fragmented, and paraffin-embedded. The sections (5µm) were stained with Haematoxylin and eosin stain and examined under a light microscope (20X magnification) to look for lacerations and abnormalities [14].

2.11 Relative organ weight estimations

At the conclusion of the study, the rats were euthanized, then the heart, kidneys, liver, brain, lungs, spleen, and thymus were carefully separated. Immediately after dissection, the rat organs were weighed to estimate the comparative organ masses and observed for every gross lesion. The formula in equation 1 was used to determine the relative organ weight [9] [20].

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of organ(gms)}}{\text{Bodyweight (gms)}} \times 100 \quad \text{(Equation 1)}$$

2.12 Statistical analysis and presentation of data

The data were presented using the means as well as the standardized error of the mean (SEM) using Microsoft Excel version 13 and Statistical Package for the Social Sciences (SPSS) Version 22 software. The one-way analysis of variance (ANOVA) followed by Turkey HSD contrast test was used to analyze the data statistical significance. When $p < 0.05$, the differences in measures were deemed important, as well as when $p < 0.01$, the differences in measures were regarded as extremely important [21].

3 Results and Discussion

3.1 Acute toxicity test

In the phase 1 of the acute toxicity study, no death recorded in all the administered doses (100, 500, ad 1000mg/kg) within 24hours of the experiment**.** In the phase 2, toxicity signs such as fatigue, mild to moderate diarrhea, drowsiness, sleepiness, hypnosis, and rough hairs were observed immediately after administration of the extracts up to four hours. After the 4hours, the animals recovered from these effects and

regained their activeness. Meanwhile, the highest dose (5000mg/kg) animal started to lose weight and showed signs of anemia on day 8. Its ears, eyes, noses, and tails turned white while the death of the rat happened on day 10**.** So, it was possible to figure out the mean lethal dose (LD_{50}), which was 3,937 mg/kg when the Lorke method's formula was considered.

3.2 Subacute toxicity study

3.2.1 Toxicity signs and deaths

There were no signs of toxicity or death in the lower doses during the 28 days. Meanwhile, signs of toxicity such as sleepiness, decreased movement, and roughness in the animals' coats were observed at the highest dose (800 mg/kg), but no deaths were recorded as well.

3.2.2 Effects of the extract on bodyweight (BW)

According to the findings, there was no difference between the treatment and control groups when it came to weight gain during the course of the experiment (Table 1). A comparison with the control group revealed no statistically significant differences in the ingestion of water and food.

3.2.3 Effects of the extract on haematological parameters after 28 days

After 28 days of treatment with the plant extract, the results displayed a major reduction in erythrocyte (p<0.05) and hemoglobin (p<0.01) counts, while PLT count (p<0.05) was significantly increased in the highest treated animals (800 mg/kg) compared to the controls. Furthermore, a rise and a diminution in leukocytes and HCT were detected, respectively, but no statistical variation was established when compared to the controls. No changes were detected for the remaining hematological indicators under investigation (Table 2).

Table1. Effects of the extract on bodyweight (BW)

Days	BW(gm)/group			
	Control (DW) (10mL/kg)	Extract (200mg/kg)	Extract (400mg/kg)	Extract (800mg/kg)
1	117.50 ± 5.39	114.80 ± 9.29	118.71 ± 8.01	121.63 ± 11.74
7	132.48 ± 4.59	130.81 ± 9.35	131.92 ± 5.26	135.07 ± 11.70
14	147.52 ± 6.34	138.67 ± 8.08	146.89 ± 6.92	149.63 ± 14.12
21	162.40 ± 7.19	155.46 ± 8.39	159.11 ± 8.14	162.54 ± 15.36
28	173.47 ± 6.74	170.38 ± 8.30	174.02 ± 8.26	174.61 ± 16.53
Weight gain (Day28 – day1)	55.97 ± 5.06	55.58 ± 2.34	55.31 ± 1.23	52.98 ± 8.48

The data represents the mean (n=6) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. All groups compared with the control group, was **not significant**. Key: DW: distilled water.

Table 2. Effects of the extract on haematological parameters after 28 days

Tests	Values/group			
	Control (DW) (10mL/kg)	Extract (200mg/kg)	Extract (400mg/kg)	Extract (800mg/kg)
WBC ($\times 10^9$/L)	9.47 ± 1.19	8.30 ± 0.97	9.30 ± 0.94	12.33 ± 1.84
RBC ($\times 10^{12}$/L)	8.93 ± 0.27	7.64 ± 0.41	7.87 ± 0.47	7.28 ± 0.22*
Hb (g/dL)	17.68 ± 0.53	15.43 ± 0.80	15.67 ± 0.56	13.82 ± 0.59**
HCT (%)	46.40 ± 4.63	45.77 ± 2.37	45.77 ± 1.79	39.65 ± 1.89
MCV (fL)	57.03 ± 0.63	60.05 ± 0.86	58.60 ± 1.48	55.60 ± 1.25
MCH (pg)	19.70 ± 0.18	20.17 ± 0.36	20.02 ± 0.52	19.32 ± 0.39
MCHC (g/dL)	34.70 ± 0.21	33.70 ± 0.37	34.23 ± 0.20	34.80 ± 0.28
RDV (%)	13.58 ± 0.30	13.95 ± 0.42	14.10 ± 0.25	13.71 ± 0.43
RDW (fL)	25.90 ± 0.61	28.50 ± 1.18	27.42 ± 1.03	23.95 ± 1.69
PLT ($\times 10^9$/L)	751.50 ± 19.87	701.00 ± 12.90	719.33 ± 35.58	954.50 ± 78.40*
MPV (fL)	6.75 ± 0.14	6.83 ± 0.12	7.12 ± 0.19	6.42 ± 0.16
PCT (%)	0.51 ± 0.01	0.48 ± 0.01	0.51 ± 0.03	0.57 ± 0.02

The data represents the mean (n=6) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: *-p < 0.05, **-p < 0.01.WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, RDV: Red cell distribution width, RDW: Platelet distribution width, PLT: Platelet, MPV: Mean platelet volume, PCT: Plateletcrit.

Table 3. Effects of the extract on biochemical parameters after 28 days

Tests	Values/group			
	Control (DW) (10mL/kg)	Extract (200mg/kg)	Extract (400mg/kg)	Extract (800mg/kg)
FBG (mmol/L)	5.38 ± 0.44	4.23 ± 0.29	3.75 ± 0.18	3.32 ± 0.31*
UREA(mg//dL)	12.93 ± 1.33	11.75 ± 1.50	16.38 ± 2.03	25.47 ± 3.88**
CRT (mg/dL)	0.98 ± 0.846	0.91 ± 0.041	0.67 ± 0.11	1.18 ± 0.12
T.protein (g/dL)	6.62 ± 0.41	5.77 ± 0.18	5.27 ± 0.09	4.41 ± 0.33*
Albumin (mg/dL)	5.44 ± 0.012	5.31 ± 0.07	5.07 ± 0.12	3.79 ± 0.17***
T. bil (mg/dL)	0.15 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.20 ± 0.02
D. bil (mg/dL)	0.12 ± 0.01	0.14 ± 0.02	0.13 ± 0.01	1.28 ± 1.14
ALT (U/L)	27.97 ± 2.18	24.20 ± 1.54	29.03 ± 3.20	42.01 ± 4.84*
ALP (U/L)	31.57 ± 2.22	36.38 ± 1.94	39.38 ± 1.88	42.22 ± 3.59*
AST (U/L)	28.60 ± 1.32	26.10 ± 1.05	27.43 ± 2.93	31.66 ± 1.98

The data represents the mean (n=6) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: *-$p < 0.05$, **-$p < 0.01$, ***-$p < 0.001$.FBG: Fasting blood glucose, CRT: Creatinine, T.protein: Total protein, T.bil: Total bilirubin, D.bil: Direct bilirubin, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase.

Table 4. Effects of the extract on body organs after 28 days

Body Organs	Relative organ weight /group			
	Control (DW) (10mL/kg)	Extract (200mg/kg)	Extract (400mg/kg)	Extract (800mg/kg)
Heart	0.44 ± 0.01	0.45 ± 0.01	0.45 ± 0.02	0.48 ± 0.02
Liver	3.28 ± 0.15	3.28 ± 0.07	3.35 ± 0.15	3.80 ± 0.16
Kidneys	0.70 ± 0.01	0.67 ± 0.02	0.68 ± 0.01	0.81 ± 0.03**
Brain	0.96 ± 0.02	1.22 ± 0.11	1.09 ± 0.06	1.16 ± 0.12
Lungs	0.55 ± 0.03	0.78 ± 0.07	0.80 ± 0.07	0.76 ± 0.09
Spleen	0.29 ± 0.02	0.66 ± 0.08	0.62 ± 0.14	0.60 ± 0.11
Thymus	0.18 ± 0.02	0.21 ± 0.03	0.20 ± 0.15	0.19 ± 0.13

The data represents the mean (n=6) and standard error of the mean (SEM). They were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. Key: **-p < 0.01

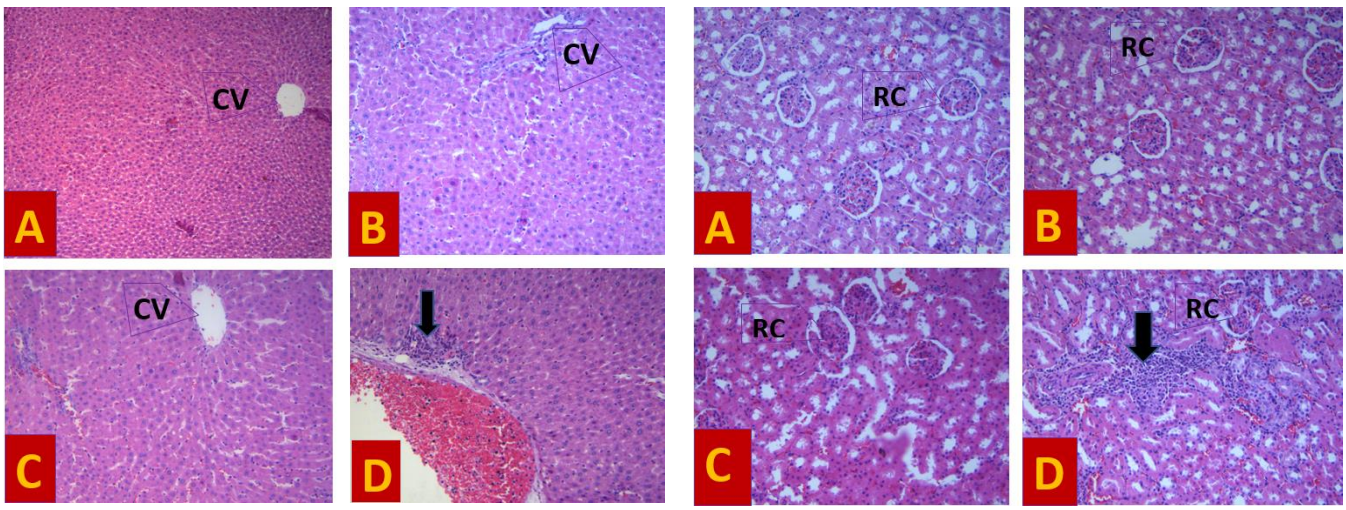

Figure 1: Subacute histopathology investigation

Figure 1A: Liver
A: Group 1 (control group), B: Group 2 (200mg/kg);
C: Group 3 (400mg/kg); and, D: Group 4 (800mg/kg).
CV: central vein; black arrow represents mild lymphocytic inflammation.

Figure 1B: Kidney
A: Group 1 (Control group), B: Group 2 (200mg/kg);
C: Group 3 (400mg/kg), and D: Group 4 (800mg/kg).
RC: renal capsule and black arrow represents lymphocytic chronic inflammation.

3.2.4 Effects of the extract on biochemical parameters after 28 days

The effect of subacute administration of methanol extract of *E. tomentosa* on the biochemical parameters of rats is shown in Table 3. Although albumin varied with a significant decrease in values in all treated groups, most other tests showed significant differences in the highest dose administration

compared to the control group. The observation was made in the cases of fasting blood glucose (FBG) levels (decreased), urea (increased), total protein (decreased), ALT (increased), and ALP (increased). However, there was no difference between the control group and the highest treatment group of 800 mg/kg with respect to creatinine, total bilirubin, and direct bilirubin.

3.2.5 Effects of the extract on body organs after 28 days

The extract did not change the weight of the organs much compared to the control group, except for the kidneys, which got bigger at an 800mg/kg dose (Table 4).

3.2.6 Subacute histopathology effects

Generally, the histological examinations revealed normal liver morphology from control to the intermediate dosage of 400mg/kg of body weight, with clear central veins and no lesions. But at the highest dose of 800mg/kg of body weight, abnormalities were seen, such as mild lymphocytic periportal inflammation (Figure 1A). Similarly, the histopathological examinations revealed normal kidney morphology from control to the intermediate dosage of 400mg/kg of body weight with clear renal capsules and no lesions were present. But the highest dose, 800 mg/kg of body weight, showed abnormalities that have been linked to chronic lymphocytic pertubular inflammation (Figure 1B).

For centuries, plants traditionally used in medicine have provided a foundation for the diagnosis and management of a wide range of illnesses [22]. In an acute toxicology study, a single-dose of a substance is given to an animal, and then the test subject is watched for one to fourteen days to see if there are harmful effects on the it [22]. The main points of this study are death, behavioral modifications, physiology, and additional alterations in the animals' health that happen on their own.

In the oral acute toxicity study, proven symptoms of toxicity were only found at dosages of 1600, 3100, and 5000mg/kg in phase 2, such as decreased movement, rough hairs, napping, diarrhea, and hypnosis; while the highest dose of 5000mg/kg caused the animal's death on day 10. Therefore, based on the findings of the acute toxicity test, it was determined that the lethal dose (LD_{50}) value of the plant extract is approximately 4,000 mg/kg. Based on the general classification criteria, compounds with an LD_{50} level of more than 2000 mg/kg can be said to be moderately nontoxic [23]. In the oral subacute toxicity study for 28 days, the highest dose of 800 mg/kg administered daily, caused rats to be less active and slept more; the rats became hypnotized, and had rough hair, but no mortality was recorded during the experiment.

The kidney is known for its function of removing toxic waste from the body via the hepatic enzyme's biotransformation process. To keep the body in a balanced state, the kidneys reabsorb essential nutrients and get rid of wastes [24]. To evaluate the wellbeing and functions of the kidneys, the concentrations of some biomarkers, such as creatinine and urea in the blood, including histological tissue of the kidney are examined. When the plant extract was given subacutely, there was a significant increase in the concentrations of these kidney function markers in the plasma, and changes in the tissue of the kidney, like pertubular inflammation (lymphocytic). These alterations proved that the extract, at 800 mg/kg dosage, had a detrimental effect on the renal functions of the test rats.

The assessment of blood enzymes like ALP, ALT, and AST, in addition to the total protein concentrations, is the standard approach for determining whether or not the liver has been damaged [7]. In this experiment, the biochemical tests showed that the highest dosed animals had much higher levels of ALT and ALP but lower levels of total protein and albumin than the control animals, and the liver's histology revealed alterations in the tissue, such as periportal inflammation (lymphocytic), which proved that the extract affected the liver function at the dose of 800 mg/kg.

The red blood cell count and hemoglobin level in the blood can be used as indicators of the functional and pathophysiological state of the organism, respectively. If there are major alterations, the given substance is either defensive of the hemopoietic organ or poisonous to it [22]. Animals given 800 mg/kg had a significant decrease in the number of red blood cells and the levels of hemoglobin in the blood, whereas the platelet concentrations

increased. However, this is not important from a toxicology point of view because these values are within the normal range of variation for this species (RBC: 7.27-9.65, Hb: 13.7-17.6, and PLT: 638-1177 for rats 8 to 16weeks old) [25]. Though, this reason can be further deepened and related to the condition of increased biochemical values and kidney conditions because it occurs at the same dose.

The main roles of leukocytes are to boost the immune system and protect the organism from infections or poisons [26]. So, the least variations in the levels of white blood cells between the treated rats and the control rats in groups 2 and 3 showed that the given doses had no major effect on the formation of these types of hemopoietic blood cells. Therefore, all of the findings indicate that when the rats are given the plant extract at a dose of 800 mg kg^{-1} for 28 days, their livers and kidneys were harmed. This study shows that the dose of 400 mg/kg of this plant's extract should not be harmful in subacute toxicity settings.

4 Conclusions

An investigation into the acute toxicity of *E. tomentosa* methanol leaf extract found that a single- dose of 1000 mg/kg of extract did not hurt rats, and the mean mortality dose, or LD_{50}, was found to be approximately 4,000 mg/kg of body weight. The subacute toxicity exploration, on the other hand, found that giving the extract to animals daily at doses of 800 mg/kg over a period of 28 days could hurt their livers and kidneys. So, this study suggests that a low dose of this plant's extract of 400 mg/kg should be safe for the treatment of subacute toxicity settings.

5 Declarations

5.1 Acknowledgments

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5.2 Author Contributions

All authors participated in the study concept and design. The laboratory analysis was performed by Mboneye Anselme under Odoma Saidi's supervision. Statistical data analysis and interpretation were done by Timothy Neeza, while literature and writing were done by Mboneye Anselme with Odoma Saidi and Albert N. Onchweri's supervision.

5.3 Funding Statement

Ministry of National Education and Scientific Research of the government of Burundi.

5.4 Conflicts of Interest

No conflicts of interest have been declared by the authors.

5.5 Ethic

All procedures followed have been approved by the University Animal Research Ethical Committee (AREC/KIU-WC) under KIU-2022-86 reference number.

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