Trop J Nat Prod Res, July 2025; 9(7): 3280 - 3290 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Ninety Days Repeated Sub-Chronic Toxicity Study of Ethanol Leaf Extract of *Laggera Aurita* Linn (Asteracea) In Wistar Rats

Sa'adatu M. Julde*, Safiya B. Borodo, Abubakar S. Wada, Sani Malami, Lawal A. Bichi

Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University Kano, Nigeria.

ARTICLE INFO

Article history:
Received 11 April 2025
Revised 16 May 2025
Accepted 14 June 2025
Published online 01 August 2025

Copyright: © 2025 Julde *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Laggera aurita Linn (Asteraceae) is widely used in Nigeria, Cameroon, Ghana, Senegal, and India for treating malaria, cancer, epilepsy, tuberculosis, and rheumatoid arthritis. This study seeks to establish the safety profile of the ethanol extract of Laggera aurita (EELA). An acute toxicity limit test at 5000 mg/kg was conducted as per OECD 425 and determined to be > 5000 mg/kg. A 90-day oral chronic toxicity study followed, according to OECD 408, involving five groups of ten rats administered the extract at 75, 150, 300, and 600 mg/kg daily, while the control group received 1 ml/kg distilled water. Daily measurements included food and water intake, and body weights were measured weekly. The animals were humanely killed on day 91, and blood samples and vital organs were collected for assays and histopathology. Results indicated no changes in food intake, water intake, body weights, relative organ weights, haematological indices, or reproductive hormones. No elevations of liver transaminase enzymes, bilirubin, or total protein were observed, nor changes in serum creatinine, blood urea nitrogen, or electrolytes. However, a significant ($p<0.05$) reduction in aspartate transaminase (AST) and urea was observed in the liver and kidney at doses of 150-600 mg/kg compared to control. Additionally, there was a significant ($p<0.05$) reduction in thyroid hormones and an elevation of cholesterol and low-density lipoproteins at doses of 150-600 mg/kg. The study suggests EELA is generally safe at low doses (75 mg/kg), with the no observed adverse effect level (NOAEL) determined to be 75 mg/kg.

Keywords: Safety, Toxicity, *Laggera aurita,* Safety Profile, Acute Toxicity.

Introduction

The use of plants for ethnomedicinal purposes to treat various illnesses is increasingly seen as an alternative to conventional medicines in recent times.[1,2] Furthermore, plants are a source of new drugs and lead compounds with so much potential that they can be explored for their medicinal benefits.[3] Given the significant reliance on medicinal plants for treating diseases and the potential for drug discovery, it is important to seek out potent, effective, and relatively safe plant medicines.[4] Nearly 85 % of the population of developing countries rely on the use of herbal medicinal products for the treatment of diseases and in Nigeria there has been a substantial increase in public awareness and the use of herbal medicinal products for disease treatment and/or prevention.[5] *Laggera aurita* (LA) is a species of *Laggera* from the family Asteraceae; some of its local names in Nigeria include *taba-taba* in Hausa and *eru-taba* in Yoruba. LA grows annually in Nigeria along houses, farmlands and riverbanks, and spreads throughout sub-Saharan Africa.[6] The ethnomedicinal use of LA has been documented in China, Senegal, Ghana, Pakistan, India and Nigeria. The whole plant and or its leaves have been used to treat ailments such as pediatric malaria fever, pain, asthma, cancer, bronchitis, constipation and epilepsy.[7,8,9]

*Corresponding author. Email: smjulde.cpt@buk.edu.ng
Tel: +2347067274295

Citation: Julde SM, Borodo SB, Wada AS, Malami S, Bichi LA. Ninety Days Repeated Sub-Chronic Toxicity Study of Ethanol Leaf Extract of *Laggera Aurita* Linn (Asteracea) In Wistar Rats. Trop J Nat Prod Res. 2025; 9(7):3280 – 3290 https://doi.org/10.26538/tjnpr/v9i7.56

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Some of the folkloric medical uses have been scientifically evaluated, and several scientific studies have reported various bioactive properties of the plant, such as alkaloids, saponins, phenols, flavonoids and cardiac glycosides.[10] This makes it important to obtain more information from toxicity studies to assess the risks associated with its use and improve safety in its use in folk medicine. This research aims to evaluate the sub-chronic toxicity and safety profile and to estimate the no observed adverse effect level (NOAEL) of LA.

Materials and Methods

Plant Collection and Extraction

The leaves of *Laggera aurita* were collected from Zaria Local Government Area, Kaduna State, in April 2023. The plant sample was identified by Baha'uddeen Said Adam from the Department of Plant Biology at Bayero University, Kano, with the herbarium accession number BUKHAN 0138 and reference number DPB/BUK/HIF/01377. Fresh leaves of Laggera aurita were washed, air-dried, and reduced in size using a pestle and mortar. The powdered leaves were weighed, and 1.8 kg of the powdered plant was extracted using 6 L of 70 % w/v ethanol and 30 % w/v distilled water via cold maceration for 72 hours. The solvent from the extract was evaporated to dryness in a thermostat oven set at 50℃. The dried extract was weighed, labelled as EELA (ethanol extract of Laggera aurita), and stored in a desiccator.

Animals

Wistar rats weighing between 100 and 150 g were purchased from the Animal House of the Department of Pharmacology and Therapeutics, Bayero University Kano, and kept under standard laboratory conditions with access to food and water ad libitum. The animals were housed in their cages and maintained at a temperature of 28±2°C, while the relative humidity ranged from 45 to 55% under a 12-hour natural dark/light cycle. The rats were acclimatized for five days prior to the commencement of the experiment.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Chemicals and Equipment
Ethanol (Sigma-Aldrich), distilled water, chloroform (sigma-Aldrich), formalin (10 %) (sigma-aldrich), mouth cannular, syringes (1ml, 5ml and 10ml), pestle and mortar, spatula, crucible, measuring cylinders, oven, desiccator, electronic weight scales, cotton wool, razor blades, EDTA bottles, plain bottles, plastic containers, centrifuge, ELISA kits (Wuhan Fine Biotech Co.), microplate reader (RT-2100c from RAYTO).

Ethical Approval
Ethical clearance was sought from Bayero University Animal Care and Use Research Ethics (ACUREC), which was approved with animal use protocol (AUP) number BUK/ACUREC/CAP/PG33.

Acute Toxicity
This was carried out using the up-and-down method according to the Organization for Economic Co-operation and Development guidelines.[10] Three female Wistar rats weighing between 120-150g were used for the study. The animals were kept in the laboratory for five days to acclimate to the laboratory conditions. The animals were fasted from food (but with access to water) for 3-4 hours before dosing. The fasting body weight was measured and used to calculate the dose for each animal. The limit test was carried out at 5000 mg/kg to determine the acute toxicity. In the first stage, one animal was orally administered the EELA at a dose of 5000 mg/kg. After administration, food was withheld for a further 3-4 hours in rats and observed for 48 hours, along with careful observation for the first 30 minutes post-treatment and occasionally during the first 24 hours. The animal survived the first stage; therefore, two new fresh animals were further administered EELA at 5000 mg/kg and observed for 48 hours. They were monitored continuously for 14 days for mortality or morbidity. The LD_{50} was subsequently estimated. Animals were observed, including changes in behaviour patterns, eyes, mucous membranes, skin, and fur.

90-Day Repeated Dose Sub-Chronic Toxicity Studies
The 90-day repeated dose sub-chronic toxicity study was conducted according to the Organization for Economic Co-operation and Development guidelines.[11] Fifty rats, 8-9 weeks old, weighing between 120- 150 g, were randomized into five groups of ten animals each (five males and five females) and allowed to acclimatize for five days before starting the experiment. The animals were kept under standard laboratory conditions and permitted access to laboratory diet and water *ad libitum*. Group I was the control, and they were orally administered distilled water at a dose of 1 ml/kg body weight once daily. Groups II, III, IV and V were the treatment groups and were orally administered 75, 150, 300 and 600 mg/kg bodyweight of EELA once daily. Administration was done continuously at the same time once daily for 90 days, during which animals were observed for any signs of toxicity, such as changes in fur, skin, eyes, mucus, posture, gait, and bizarre behaviour. These observations were done daily, and the weight of animals was recorded weekly. After 90 days, the animals were starved overnight but allowed water access and weighed the next day. The animals were humanely euthanized by cervical dislocation after mild anaesthesia using chloroform. Four milliliters of blood sample were collected from each rat. Whole blood was collected in EDTA bottles and plain bottles for each rat.

Assessment of Haematological Parameters
The blood samples collected in the EDTA-coated bottles were assessed for haematological parameters using the Biobase automated haematological analyzer. The haematological parameters analysed include white blood cell (WBC), Lymphocytes (LYMP), Mid Cells (MID), Granulocytes (GRAN), Red blood cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean cell haemoglobin concentration (MCHC), and Platelets (PLT).

Assessment of Biochemical Parameters
The blood samples collected in the plain bottles were centrifuged at 2500 rpm for 10 minutes with a Centurion centrifuge machine, model 4040 series. The serum was then separated from the cells for the analysis. Liver function tests include alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), bilirubin and albumin. The kidney function (urea, creatine, sodium, potassium, bicarbonate and chloride), while lipid profile include cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride, in addition, oxidative stress markers assayed were glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT). They were all assayed using the biobased chemistry analyzer.

Hormonal Assay
The serum obtained from whole blood was used to assay the thyroid hormones (T3, T4, and TSH) and reproductive hormones (estrogen, testosterone, and progesterone) for both the male and female rats. The assay was carried out using the sandwich ELISA protocol as described by the manufacturer (ELISA kits obtained from Wuhan Fine Biotech Co.), after which the absorbances were measured using a microplate reader (RT-2100c from RAYTO).

Histopathological Analysis
The liver, kidney, heart, stomach, and brain were harvested from each animal. The organs were weighed to determine the relative organ weight and fixed in 10 % formalin. The fixing was done for 48 hours, after which the tissues were dehydrated through ascending grades of alcohol from 70 % alcohol to 90 % alcohol and finally absolute alcohol (100 %) for 16 hours. The tissues were cleared in toluene for 2 hours and then impregnated with molten paraffin for 4 hours. The tissues were then embedded in paraffin wax, sectioned using a rotary microtome at 5-micron thickness. The sections were stained using the haematoxylin and eosin staining technique, and the stained sections examined using a light microscope and relevant photomicrographs taken using a digital camera of the microscope.

Statistical Analysis
The data obtained from the study were analyzed using repeated measures and one-way ANOVA, where appropriate, and Bonferroni's post hoc test with significance at $p < 0.05$. The statistical package for social sciences (SPSS) software version 26 was utilized for the analysis. The results obtained were presented as Mean ± standard error of mean.

Results and Discussion

Acute Toxicity
LD_{50} was determined to be greater than 5000 mg/kg, as shown in Table 1. There were no changes in behavioural patterns, such as gait, and no changes were observed in the eyes, mucous membranes, skin, and fur. Acute oral toxicity is typically assessed to evaluate the potential toxic effects of a test substance following single-dose exposure and to provide doses for subsequent toxicity testing. [12] An LD_{50} of a test substance exceeding 1000 mg/kg body weight can be considered to indicate low toxicity and, therefore, safety. [13,14] The result of the acute oral toxicity in this study showed that the LD_{50} was above 5000 mg/kg, which agrees with the acute oral toxicity conducted on the same plant by other studies.[16]

Table 1: Acute Toxicity (LD_{50}) of Ethanol Extract of *Laggera aurita* in Rats

Route of administration	Dose	LD_{50}
Oral	5000 mg/kg	> 5000 mg/kg

Effects of Laggera aurita on Body Weight of Rats
Laggera aurita extract at 600 mg/kg significantly ($p<0.05$) reduced the mean body weight of male rats in the fourth week of the experiment compared to the control. Furthermore, in the seventh week, the extract at 150 mg/kg significantly ($p<0.05$) decreased the mean body weight of the male rats when compared to the control. However, on comparison over time, the oral administration of the *Laggera aurita* extract (75, 150, 300, 600 mg/kg) significantly increased the mean body weight compared to week zero (Fig. 1A).

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

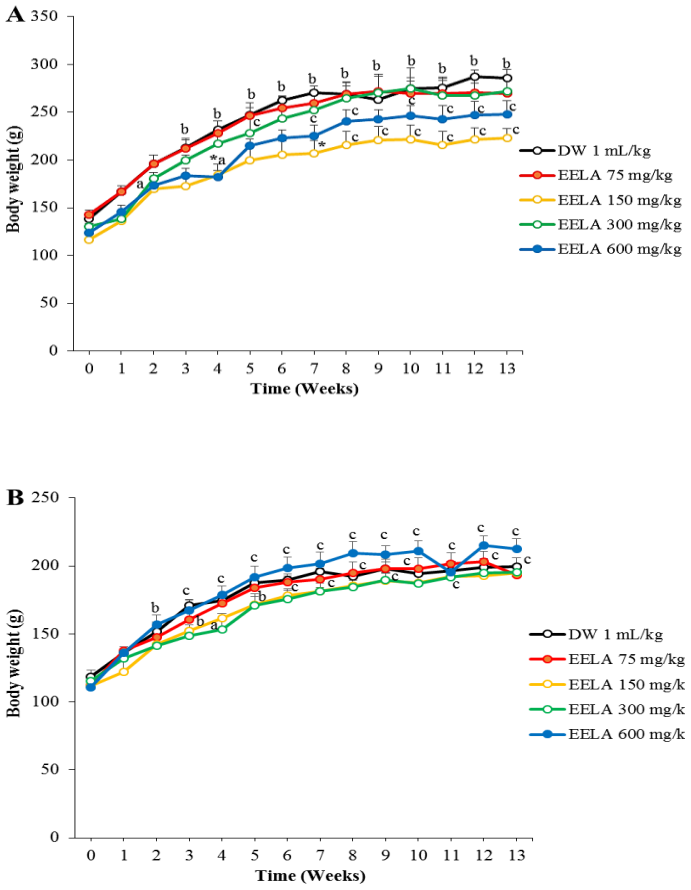

Figure 1: Effect of Ethanol Extract of *Laggera aurita* on Body Weight of Rats, A = Male, B = Female
Values are Mean ± S.E.M. * =p<0.05 as compared to DW group, [a] =p<0.05, [b] =p<0.01, [c] =p<0.001 as compared to Week 0 – Repeated Measure ANOVA followed by Bonferroni's post hoc test, n=5 (Male or Female), DW=Distilled water, EELA = Ethanol Extract of *Laggera aurita*

In the female rats, *Laggera aurita* extract did not show a significant (p<0.05) change between the treated rats compared to the control. However, on comparison over time, the oral administration of the *Laggera aurita* extract (75, 150, 300, 600 mg/kg) significantly increased the mean body weight compared to week zero (Fig. 1B). Reduction in body weight can be an index of toxicity, where a reduction of up to 5 % can be considered a predictor of degeneration within the body.[17] In this study, there was no significant change observed in the body weight of the female animals in all treatment groups compared with the control. Although a significant change was seen in the male animals at weeks 4 and 7 at 600 and 150 mg/kg doses, respectively, this did not affect the final body weights after completing the experiment. No statistically significant difference was found between the weight of the test group compared to the control group over time, at the end of the 90 days. Therefore, a progressive increase in the body weight of all the animals in both control and treatment groups were observed, which suggests a growth response. This suggests that the extract may not have the potential that led to weight loss or stimulate weight gain.[18]

Effects of Laggera aurita on Organ Weight of Rats
There was no significant (p<0.05) change between the organ weights of the liver, kidney, heart, stomach, ovaries, testes, and brain of treated male and female rats compared to the control (Figures 2A and B). Organ weight (organ-to-body/brain weight) analysis is an important indicator of endpoint toxicity. Morphological changes may or may not accompany changes in the organ weight between treated and untreated animals.[19] Although histopathology identifies treatment-related effects on any organ, organ weight can also provide some insights to identify a target organ for toxicity.[20] In this study, the relative organ weight measured was the organ-to-body weight ratio, which considers the differences in body weights of the test animals and the control. The result did not significantly change between the treated and the control groups. The relative organ weight results did not indicate the possibility of any target organ toxicity.[21] The body weights of the treated animals were also not affected by the administration of the plant extract, as previously explained, because there was a progressive increase in the body weight of the rats.

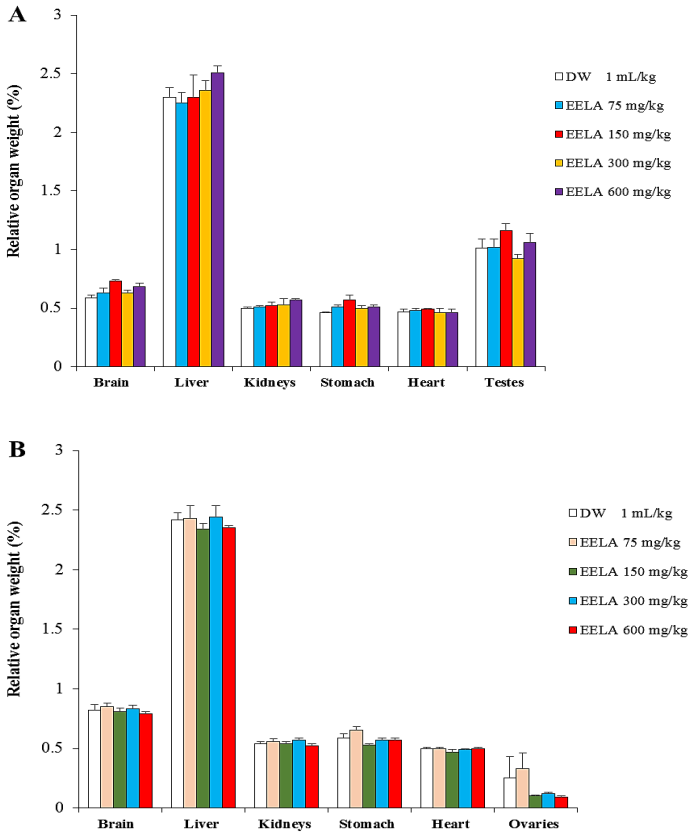

Figure 2: Effect of Ethanol Extract of *Laggera aurita* on Relative Organ Weight of Rats, A = Male, B = Female

Values are Mean ± S.E.M., No significant differences as compared to DW group – One-way ANOVA followed by Bonferroni's post hoc test, n=5 (Male), n=5 (Female), Distilled water, EELA = Ethanol Extract of *Laggera aurita.*

Effect of Laggera aurita on Haematological Parameters of Rats
Haematological parameters such as red blood cells (RBC), white blood cells (WBC), Mean cell haemoglobin concentration (MCHC), Haemoglobin (HG), Haematocrit, Platelets, granulocytes and lymphocytes were normal in the treated male and female rats compared to the control, as presented in Table 2. The Hematopoietic system is one of the main targets for potential toxicants, as the blood cells are the first cells exposed to toxic substances as soon as they reach the systemic circulation.[17] The system is responsible for the delivery of oxygen to tissues throughout the body, maintenance of vascular integrity and provision of immune functions necessary for defence.[22] A reduction in haematological parameters such as RBC, MCHC, and HGB usually indicates the presence of anaemia, which may also be associated with a deficiency in the B vitamins as a result of malnutrition, and other factors;[12] an elevation may be due to dehydration and other health-related factors. White blood cells (WBC), which include granulocytes (neutrophils, eosinophils, basophils), monocytes, and lymphocytes (T cells and B cells), play a crucial role in the body's defence against

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Table 2: Effect of Ethanol Extract of *Laggera aurita* on Haematological Parameters of Rats

Indices	Units	Treatment (mg/kg)				
		DW 1mL/kg	EELA 75	EELA 150	EELA 300	EELA 600
Male						
WBC	($\times10^3$/µL)	4.58±0.27	4.88±0.23	4.10±0.42	4.37±0.58	4.50±0.83
LYMP	(%)	62.72±3.14	63.20±2.24	58.20±3.50	55.67±2.14	59.73±3.72
MID	(%)	3.92±0.27	4.76±0.20	4.83±0.79	4.53±0.33	3.48±0.86
GRAN	(%)	34.04±3.18	32.40±2.15	36.30±5.49	39.93±2.49	37.05±4.11
RBC	($\times10^6$/µL)	6.06±0.16	5.58±0.65	5.03±0.61	5.83±0.27	5.40±0.49
HGB	(g/dL)	13.52±0.60	12.40±0.29	11.70±1.55	14.40±1.09	13.23±0.86
HCT	(%)	41.40±2.01	35.40±1.69	34.67±5.61	42.33±2.03	39.50±2.72
MCHC	(g/dL)	33.36±0.44	34.90±0.43	33.47±0.15	35.03±1.39	32.53±0.98
PLT	($\times10^3$/µL)	212.20±31.27	209.80±19.58	181.33±19.20	218.00±31.21	200.50±18.59
Female						
WBC	($\times10^3$/µL)	4.72±0.14	4.50±0.04	4.12±0.34	5.30±0.21	4.52±0.52
LYMP	(%)	62.54±1.47	61.20±4.13	59.66±2.82	60.58±3.61	57.78±3.58
MID	(%)	5.53±0.30	5.40±1.42	5.26±0.61	5.13±0.66	5.18±0.48
GRAN	(%)	32.18±1.69	33.73±4.33	35.14±3.02	34.65±2.91	36.98±3.40
RBC	($\times10^6$/µL)	5.92±0.12	5.08±0.66	5.56±0.23	5.13±0.40	6.06±0.16
HGB	(g/dL)	13.26±0.57	11.63±0.86	12.78±0.80	11.83±0.30	13.44±0.68
HCT	(%)	40.60±2.32	35.75±2.25	39.20±2.35	35.75±0.75	40.00±3.13
MCHC	(g/dL)	36.38±2.24	33.23±0.24	34.20±0.46	35.48±1.29	33.82±0.55
PLT	($\times10^3$/µL)	228.60±17.87	179.50±30.62	210.80±36.97	185.25±33.19	175.00±23.72

Values are Mean ± S.E.M. No significant differences as compared to DW group – One way ANOVA followed by Bonferroni's post hoc test, n=5 (Male) n=5 (Female), DW=Distilled water, EELA = Ethanol Extract of *Laggera aurita*, WBC=White blood cells, LYMP=Lymphocytes, MID= Mid Cells, GRAN=Granulocytes, RBC=Red blood cells, HGB=Haemoglobin, HCT=Haematocrit, MCHC=Mean cell haemoglobin concentration, PLT = Platelets

infections, tissue injury, and inflammation mechanisms.[23] Neutrophils and lymphocytes build up as a result of bacterial and viral infections; as such toxicity of the WBC would have reduced the body's ability to fight against invading microorganisms.[24] The platelets are part of the hemostatic system, responsible for maintaining homeostasis and therefore help to prevent blood loss in case of vascular injury, as well as maintaining the blood in a fluid state. A disruption of the haemostatic system may manifest as excessive bleeding or thrombosis.[25] From the results obtained, no such changes were seen in the haematological indices.

Effect of Laggera aurita on Biochemical Parameters of Rats

The liver function tests assessed include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total Protein (TP), Albumin (ALB), Direct bilirubin (DB), and Total bilirubin (TB). All parameters, as shown in Table 3, were normal compared to the control, except for AST, where a significant reduction was observed at 300 and 600 mg/kg doses in male rats, and at 150, 300, and 600 mg/kg doses in female rats compared to the control. The hepatic system is an important system responsible for the metabolism of xenobiotics and foreign materials through different reactions. The liver is the main organ where exogenous chemicals are metabolized and eventually excreted, liver cells are therefore exposed to significant concentrations of chemicals or drugs, which can lead to liver dysfunction, cell injury, and even organ failure.[26] This makes the liver a target organ for toxicity due to its function in the detoxification processes in the body.[27] The liver is responsible for metabolic activities through different reactions, mainly including oxidase, reductase, and hydrolase mechanisms, transforming the lipophilic chemicals into water-soluble compounds to be excreted from the body. Medicinal plants may cause hepatocellular or cholestatic injury or both.[28] Several medicinal plants are hepatotoxic after consumption, which may be due to inherent toxicity, overdose or contamination with other plants that contain hepatotoxic substances.[29]

A liver function test is usually carried out to give information about the state of the liver and assess the functional efficiency of the organ. These tests include ALT, AST, ALP, bilirubin, albumin, and total protein, among others. The ALT is largely found in the liver, specifically the hepatic cytoplasm, and it is the most sensitive marker for hepatic cellular injury, while the AST is present in the heart, RBC, skeletal muscles, pancreas, kidney, brain and liver. The ALP originates mainly from the bone and hepatic biliary tract.[30] Hepatocellular damage is associated with elevation of serum ALT and AST, while changes in serum ALP are an indication of biliary cirrhosis, hepatitis and disease characterized by inflammation and bile obstruction.[31] In the present study, there were no significant changes seen in ALT and ALP, but a significant reduction was seen in AST at 300 and 600 mg/kg doses in the male rats and 150 and 300 mg/kg doses in the female rats.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Table 3: Effect of Ethanol Extract of *Laggera aurita* on Liver Function Parameters of Rats

Treatment (mg/kg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/dL)	ALB (g/dL)	DB (mmol/L)	TB (mmol/L)
Male							
DW 1 mL/kg	20.25±4.87	48.20±9.66	27.38±6.42	4.12±0.33	1.10±0.33	3.36±0.43	11.32±0.92
EELA 75	15.40±1.86	30.80±8.55	30.82±4.56	3.26±0.16	2.00±0.46	3.74±0.88	13.34±1.69
EELA 150	15.67±4.10	40.00±11.55	13.50±0.90	3.87±0.72	2.70±0.51	6.03±0.73	12.63±1.00
EELA 300	28.00±3.21	9.67±5.24*	20.90±3.66	3.13±0.03	2.20±0.21	6.10±0.87	12.63±0.68
EELA 600	20.25±2.29	8.33±5.84*	36.85±9.46	3.35±0.13	2.68±0.44	5.03±0.70	14.83±1.27
Female							
DW 1 mL/kg	19.60±1.54	86.67±12.02	33.06±3.36	4.38±0.38	1.52±0.31	4.58±1.47	11.02±1.36
EELA 75	11.25±2.56	70.00±0.01	28.55±4.33	4.30±0.72	1.98±0.39	6.48±1.30	15.78±1.17
EELA 150	18.00±2.49	35.00±8.40*	23.70±6.61	3.62±0.24	2.30±0.33	6.20±1.14	13.50±1.26
EELA 300	22.00±1.78	20.00±10.00*	28.33±7.03	3.23±0.47	1.93±0.10	5.23±1.02	14.48±1.16
EELA 600	17.00±2.59	42.50±7.50	33.56±3.54	3.52±0.17	1.70±0.20	3.90±0.26	15.16±1.63

Values are Mean ± S.E.M. * =p<0.05 as compared to DW group – One way ANOVA followed by Bonferroni's post hoc test, n=5 (Male) n=5(female), DW = Distilled water, EELA = Ethanol Extract of *Laggera aurita*, ALT Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, TP = Total Protein, ALB= Albumin, DB = Direct bilirubin, TB = Total bilirubin

Although low biomarker levels usually have no clinical significance and are not associated with unhealthy liver,[32] it has been reported that some medicinal plants possess hepatoprotective properties. *Laggera aurita* is reported to have such properties.[9] In addition to the liver transaminases, the total protein, bilirubin and albumin are also measures which are used to assess the health of the liver. There were no changes seen in their levels in the current study. Urea, creatinine, and electrolytes were evaluated for kidney function, with changes only noted in urea levels at 150 and 600 mg/kg doses in male rats compared to the control, as shown in Table 4. The renal system plays a vital role in the excretion of metabolic wastes, regulation of extracellular fluid volume, electrolyte composition and acid-base balance. The functional unit of the kidney is the nephron, which can also be affected by toxicants that can lead to the disruption of one or all its functions, consequently having a profound effect on total body metabolism.[33] Nephrotoxicity is indicated by a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (sCr), or urine output.[34] The parameters assessed in the current study were creatinine, urea, and electrolytes. Creatinine is a breakdown product of creatine phosphate in the muscles. It is a waste product which passes through the kidneys to be filtered and eliminated in the urine. Creatinine production is dependent on muscle mass thus, males have higher creatinine levels than females.[35] High levels of creatinine in the blood are reflective of kidney damage as the kidneys are no longer able to properly filter it. Elevated levels are also seen in muscular dystrophy paralysis, hyperthyroidism, anaemia, and leukaemia, while decreased levels are seen in glomerulonephritis, polycystic kidney disease, acute tubular necrosis, congestive heart failure, shock, and dehydration.[36] In this study, the creatinine level in both male and female rats was not significantly changed, which suggests that the EELA may not be toxic to the kidneys, although studies have shown that kidney damage can still occur without changing any established clinical marker of renal function.[34] Ammonia is a toxic byproduct of amino acid breakdown, is converted to urea, a nitrogenous end product of protein and amino acid that is usually produced by the liver and released into the blood, where it is filtered out by the kidneys and excreted in the urine.[37] Nearly 85% of urea is eliminated via the kidneys, while the rest is excreted through the gastrointestinal (GI) tract. Serum urea levels increase in conditions where renal function is compromised (acute and chronic impairment) and are seen in dehydration, vomiting, diarrhoea, consumption of large amounts of protein-rich foods and renal disorders such as chronic nephritis and glomerulonephritis. Although increased urea is associated with kidney disease or renal failure, higher levels may also be due to other factors unrelated to renal failure, such as upper GI bleeding.[37] Low levels of urea are seen in fluid excess, starvation, severe liver disease, inadequate protein diet, malnutrition, and anabolic steroid use.[38] In this research, significantly low levels of urea were noticed at 150 and 600 mg/kg doses in the male rats only, which was similar to previous studies.[16] This is further explained by the histopathology assay. The electrolytes sodium, potassium, chloride and bicarbonate assessed did not show any significant changes in both male and female rats treated with different doses of EELA in comparison with the control group

In Table 5, no changes were observed in oxidative stress biomarkers (GSH, SOD, MDA and CAT). The results of the oxidative stress test showed no significant changes in the levels of antioxidants between the treatment group and the control at all doses tested, suggesting that EELA did not affect the balance in the redox equilibrium. Oxidative stress is the imbalance between the antioxidant system and free radicals within the cellular environment, which may result from normal biochemical processes leading to the production of these reactive species (also called free radicals).[39] During normal cellular responses, reactive species such as hydrogen peroxide and superoxide are produced, which can cause damage to cells and DNA. Antioxidants such as glutathione peroxidase, catalase and superoxide dismutase, reduce these harmful reactive species to less harmful molecules by giving up their electrons to the free radicals; hence oxidation reaction chain is broken.[40]

A significant elevation of low-density lipoprotein (LDL) and cholesterol was detected in both male and female rats at 150-600 mg/kg doses compared to the control, this is shown in Figures 3 A and B. The lipid profile test indicated a significant elevation of cholesterol in both male and female rats at 300 and 600 mg/kg doses, as well as a significant elevation of LDL at 150 and 600 mg/kg doses in the male rats only. The role of cholesterol involves the synthesis of cell membranes of the nucleus, mitochondria and microsomes, which is obtained from exogenous sources such as diet and endogenous sources from acetate as a precursor for its synthesis. The cholesterol homeostasis is usually maintained by multiple feedback mechanisms that involve biosynthesis by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Coa) reductase, uptake by LDL, and catabolism by 7a-hydroxylase.[41] The biosynthesis and uptake of cholesterol are interdependent; as such, changes in cholesterol intake and cell

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

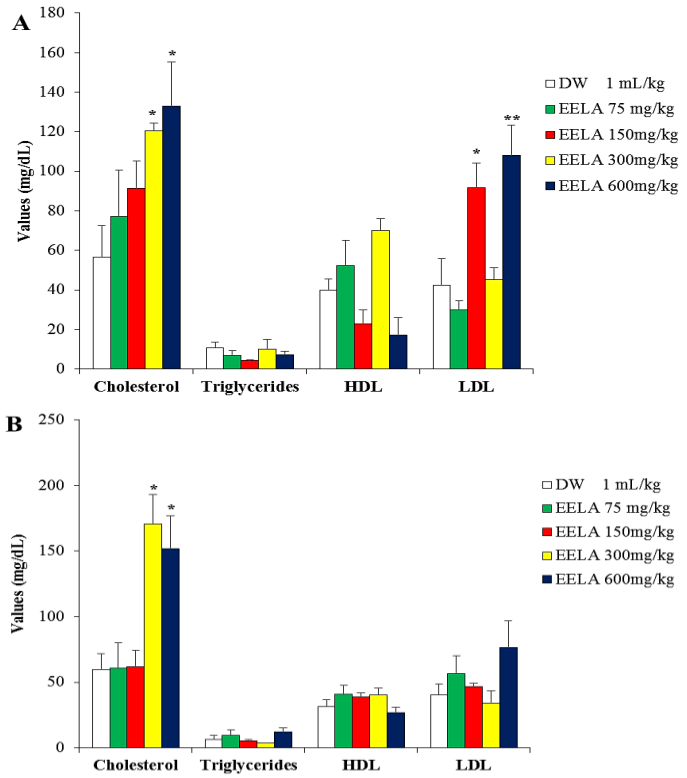

Figure 3: Effect of 90-days Daily Administration of Ethanol Extract of *Laggera aurita* on Serum Lipid Profile of Rats A= male, B= female

Values are Mean ± S.E.M, * =p<0.05, ** =p<0.01 as compared to DW group – One-way ANOVA followed by Bonferroni's post hoc test, n=5 (Male), n=5 (Female), DW = Distilled water, EELA = Ethanol Extract of *Laggera aurita,* HDL = High density lipoprotein, LDL = Low density lipoprotein.

requirement affect cholesterol synthesis through a complex feedback mechanism.[41] Elevated levels of cholesterol, triglycerides, and LDL are risk factors for cardiovascular diseases, whereas a high HDL level in the blood reduces the risk of cardiovascular diseases such as atherosclerosis. As such, an elevated level of cholesterol, triglycerides, and LDL with a corresponding decrease in HDL is associated with high risks of coronary heart disease and acute coronary syndrome.[42,12] This elevation of the cholesterol and LDL level can further be explained by the changes observed in the thyroid hormones.

Effect of Laggera aurita on Hormones

The hormonal assay revealed a significant reduction in thyroid hormones at 150-600 mg/kg doses in both male and female rats compared to the control (Figures 4 A and B); at the same time, no changes were noted in oestrogen, progesterone, and testosterone levels (Table 6). Thyroid hormones are involved in regulating several metabolic processes of the body, one of such is inducing the HMG-CoA reductase in cholesterol biosynthesis, therefore, it is involved in both the synthesis and breakdown of cholesterol. The thyroid hormones majorly produced are triiodothyronine (T3) and thyroxine (T4), which are produced in the thyroid glands when stimulated by the thyroid-stimulating hormone (TSH) located in the pituitary gland. The TSH stimulates the thyroid gland to release the T3 and T4 in the event of low levels of thyroid-stimulating hormones to keep them balanced. T3 is the active form of the thyroid hormone, which is utilized by the body in its original form, while T4 is usually converted to T3 by body enzymes. In this study, there was a reduction in the levels of TSH and T4 in both male and female rats. This could also be a possible reason why there was a significant elevation of the cholesterol and LDL levels observed, especially at higher doses. Low levels of thyroid hormones may lead to decreased LDL-receptor activity, resulting in decreased catabolism of LDL and consequently high levels of total cholesterol. Moreover, studies have shown that hypothyroidism is associated with an increase in total cholesterol and LDL.[43] The result of the assays of thyroid hormone suggests that LA could be a potential endocrine disruptor between doses of 150-600 mg/kg, as it can interfere with the activity of thyroid hormones. Phytoestrogens are plant hormones considered to be endocrine-disrupting chemicals, which are naturally found in plants. They are structurally and functionally similar to mammalian estrogens and their active metabolites.[44] Although there isn't confirmation of the presence of phytoestrogens in LA, Endocrine disruptors are toxic chemicals that interfere with the body's ability to function properly and maintain hormonal balance as they mimic hormones or block their action.[44] This suggests that LA has the potential to interfere with several metabolic processes of the body that are regulated by thyroid hormones.

Table 4: Effect of Ethanol Extract of *Laggera aurita* on Kidney Function Parameters of Rats

Treatment (mg/kg)	Urea (mg/dL)	Creatinine (mEq/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Male						
DW 1 mL/kg	49.58±5.25	1.02±0.17	241.12±20.34	22.80±2.90	33.20±3.07	115.60±7.22
EELA 75	30.30±2.57	1.06±0.20	242.70±21.72	17.62±2.86	38.40±1.40	92.40±4.35
EELA 150	18.60±8.90*	1.10±0.06	242.70±20.41	21.93±6.52	33.00±4.00	93.67±5.93
EELA 300	43.43±5.21	1.27±0.12	244.43±21.48	18.50±8.60	31.33±6.44	111.67±6.67
EELA 600	20.15±7.31*	0.95±0.12	247.70±22.09	17.15±3.00	24.50±4.99	110.33±7.86
Female						
DW 1 mL/kg	35.86±8.39	1.20±0.1	248.86±18.81	24.60±2.52	30.40±5.87	101.20±10.90
EELA 75	26.93±5.27	0.88±0.1	245.53±16.13	31.63±5.66	26.00±1.00	82.50±12.53
EELA 150	33.08±8.44	1.06±0.1	246.26±17.80	24.74±6.45	29.20±4.63	112.60±8.03
EELA 300	25.73±5.91	1.18±0.1	256.85±21.84	24.53±2.42	35.00±3.56	109.25±6.83
EELA 600	35.62±6.09	1.12±0.1	265.12±24.06	27.84±3.39	33.00±3.10	99.80±1.20

Values are Mean ± S.E.M., * =p<0.05 compared to DW group – One way ANOVA followed by Bonferroni's post hoc test, n=5 (Male) n=5 female), DW = Distilled water, EELA = Ethanol Extract of *Laggera aurita*

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Table 5: Effect of Ethanol Extract of *Laggera aurita* on Oxidative Stress Markers of Rats

Treatment (mg/kg)	SOD (IU/L)	GSH (µg/mL)	MDA (mmol/mL)	CAT (IU/L)
Male				
DW 1 mL/kg	4.68±1.50	2.64±0.76	102.24±10.00	2.74±0.72
EELA 75	9.68±2.75	3.34±0.78	84.60±5.74	5.60±1.97
EELA 150	6.10±2.90	4.20±1.60	106.93±9.39	4.50±1.76
EELA 300	2.27±0.92	1.50±0.75	90.50±10.10	1.600±1.25
EELA 600	3.77±0.62	1.80±0.74	99.10±10.80	2.05±0.70
Female				
DW 1 mL/kg	7.02±2.88	4.54±1.16	92.52±12.29	3.80±1.45
EELA 75	4.97±2.45	3.58±0.93	56.18±11.72	4.53±2.19
EELA 150	6.72±1.65	5.16±0.93	76.94±8.92	3.56±1.37
EELA 300	5.13±1.73	2.78±1.40	76.83±13.17	2.20±0.82
EELA 600	5.28±2.35	1.96±0.65	95.12±9.41	2.22±0.79

Values are Mean ± S.E.M, no significant difference as compared to DW group – One way ANOVA followed by Bonferroni's post hoc test n=5 (Male), n=5 (Female), DW = Distilled water, EELA = Ethanol Extract of *Laggera aurita*, SOD = Superoxide dismutase, GSH = Reduced glutathione, MDA = Malondialdehyde, CAT = Catalase

The hypothalamic-pituitary-gonadal (HPG) axis regulates the reproductive system. It regulates the release of gonadotropin, which constitutes the follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The gonadotropins, in turn, act on the gonads, ovaries, and testes to stimulate the release of testosterone, estrogen, and progesterone. These hormones, in turn, initiate the production of sperm and the release of eggs. There is constant communication within the HPG axis to ensure normal function through a negative feedback mechanism; any disruption to this system may interfere with proper reproductive functions. Xenobiotics can interrupt reproductive processes in several ways, some of which include altering endocrine homeostasis, hormone antagonists and direct chemical reactions.[45,46] The estrogen, testosterone and progesterone are very important hormones in the reproductive system. These hormones are produced in the ovaries in females and the testes in males. The estrogen evokes the estrus in animals during which they are sexually active, while in human females, it contributes to the menstrual cycle. Furthermore, testosterone is the major male reproductive hormone, although estrogen also plays its role. Previous studies have shown that herbal medicinal plants can interfere with reproductive hormonal levels to cause imbalance or improve the hormone levels by mimicking the hormones.[47,48] No changes were observed in the reproductive hormones in both male and female rats treated with the plant extract, indicating it did not interfere with the level of estrogen, testosterone and progesterone.

Histopathology

Photomicrograph sections of the brain and heart in the extract-treated and control animals showed normal cells. However, Kupfer cell hyperplasia and hepatic necrosis were seen in the livers at 150 - 600 mg/kg, tubular adhesion and hyperplasia in the kidneys at 150-600 mg/kg, and mucosal necrosis in the stomachs at 600 mg/kg in comparison with the control (plate I-VI). Histological assays help identify target organs of toxicity, although morphological changes can occur in tissues and organs without accompanying significant changes in biochemistry. This makes histological assays important in identifying target organs of toxicity. Changes were observed in the liver, kidney, and stomach tissues only at the higher doses (300-600 mg/kg) of the extract. In the liver, hepatic necrosis, Kupffer cells hyperplasia and inflammatory hyperplasia were observed. Necrosis is simply cell death, which could be either apoptic necrosis, which is characterized by cell shrinkage or oncotic necrosis, which is characterized by swelling, leakage of cellular contents, and influx of inflammatory cells.[49] It is possible that the necrosis observed in the liver was apoptic necrosis; hence, there was no release of inflammatory cells, and as a result, no changes were observed in the liver transaminases. Furthermore, the Kupffer cell hyperplasia indicates that there was an influx of foreign substances, which were trapped by the Kupffer cells. Part of the function of the Kupffer cells is to break down blood cells and remove debris or foreign bodies that come from the hepatic portal vein into the liver from the intestines. The changes observed in the histological assay of the liver may explain why there was urea reduction, as the liver is responsible for the production of urea. Tubular adhesion was observed in the kidney, which could be because of the repair mechanism. Scar tissues are formed from the repair mechanism, which leads to the joining together of tubules in the kidney that are otherwise supposed to be separated. From the results of the 90-day repeated oral chronic toxicity study, no adverse effects were seen at 75 mg/kg in all the parameters tested. The no observed Adverse effect level (NOAEL) is the lowest dose where no adverse effects are seen, and one of the objectives of the repeated dose toxicity study was to determine the NOAEL in this study. The results indicated that 75 mg/kg is the NOAEL.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: Effect of Ethanol Extract of *Laggera aurita* on Reproductive Hormones Profile of Rats

Treatment (mg/kg)	Estrogen (pg/mL)	Progesterone (pg/mL)	Testosterone (pg/mL)
Male			
DW 1 mL/kg	10.17±0.64	0.93±0.06	1.63±0.05
EELA 75	9.86±0.45	0.94±0.02	1.58±0.12
EELA 150	10.78±0.33	0.89±0.04	1.41±0.11
EELA 300	11.17±0.43	0.95±0.04	1.56±0.05
EELA 600	9.90±0.77	1.08±0.09	1.60±0.14
Female			
DW 1 mL/kg	10.13±0.61	0.89±0.04	1.58±0.08
EELA 75	10.11±0.50	0.93±0.08	1.51±0.07
EELA 150	9.76±0.32	0.90±0.01	1.47±0.04
EELA 300	9.54±0.20	0.89±0.03	1.38±0.07
EELA 600	9.65±0.66	0.89±0.04	1.59±0.14

Values are Mean ± S.E.M, no significant differences as compared to DW group – One way ANOVA followed by Bonferroni's post hoc test n=5 (Male), n=5 (Female), DW = Distilled water, EELA = Ethanol Extract of *Laggera aurita*

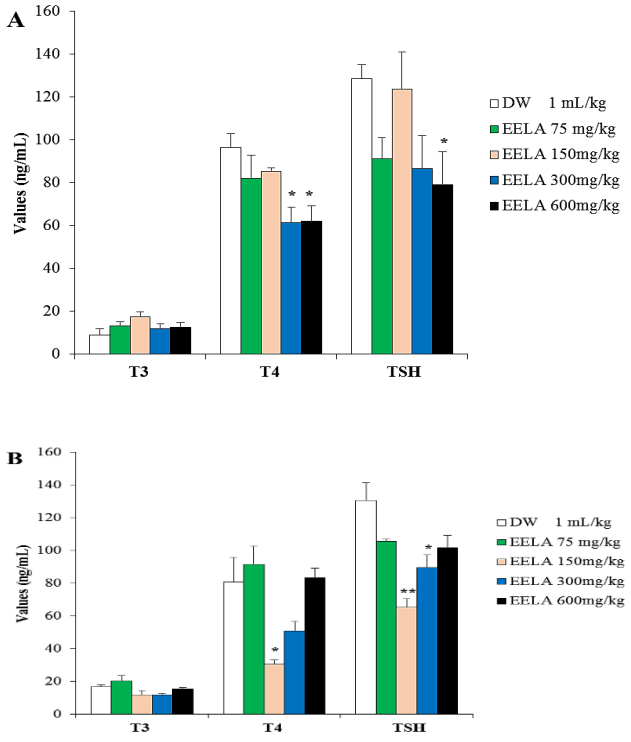

Figure 4: Effect of Ethanol Extract of *Laggera aurita* on Thyroid Hormonal Level in Rats A= male, B= female

Values are Mean ± S.E.M, * =p<0.05, ** =p<0.01 as compared to DW group – One-way ANOVA followed by Bonferroni's post hoc test n=5 (Male), n=5 (Female), DW = Distilled water, EELA = Ethanol Extract of *Laggera aurita,* T3=Triiodothyronine, T4=Thyroxine, TSH=Thyroid stimulatory hormone.

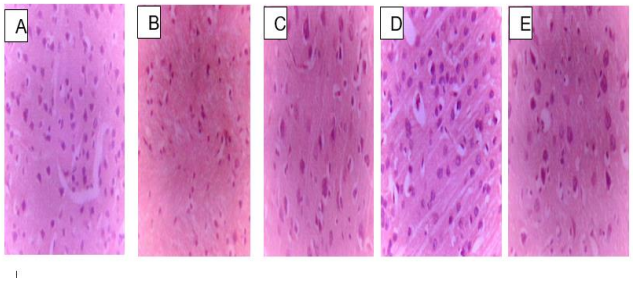

Plate I: Effects of ethanol leaf extract of *Laggera aurita* for 90 days on the brain of rats (magnification x 400) a = distilled water, b = 75 mg/kg, c= 150 mg/kg, d = 300 mg/kg, e = 600 mg/kg.

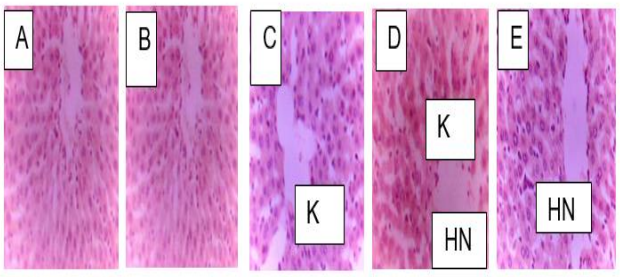

Plate II: Effects of ethanol leaf extract of *Laggera aurita* for 90 days on the liver of rats (magnification x 400). LH = hyperplasia, KH = Kupfer cell hyperplasia, HN = hepatic necrosis, a = distilled water, b = 75 mg/kg, c = 150 mg/kg, d = 300 mg/kg, e = 600 mg/kg.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

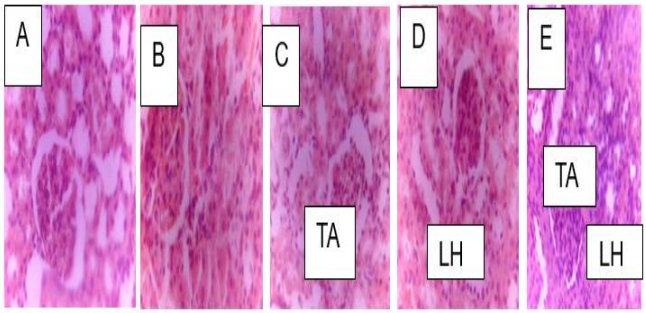

Plate III: Effects of ethanol leaf extract of *Laggera aurita* for 90 days on the kidney of rats (magnification x 400). TA = tubular adhesion, LH = hyperplasia, a = distilled water, b = 75 mg/kg, c= 150 mg/kg, d = 300 mg/kg, e = 600 mg/kg.

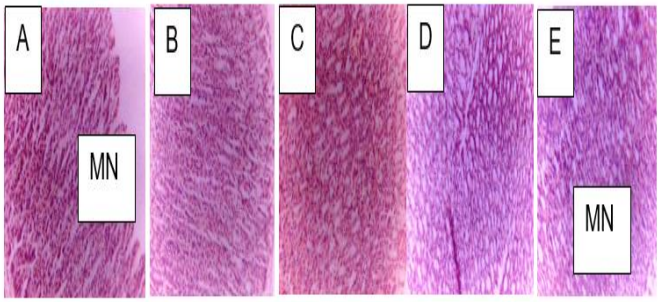

Plate IV: Effects of ethanol leaf extract of *Laggera aurita* for 90 days on the stomach of rats (magnification x 400). MN = mucosal necrosis, a = distilled water, b = 75 mg/kg, c= 150 mg/kg, d = 300 mg/kg, e = 600 mg/kg.

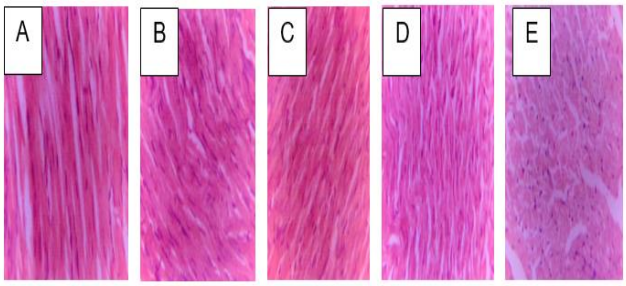

Plate V: Effects of ethanol leaf extract of *Laggera aurita* for 90 days on the heart of rats (magnification x 400). a = distilled water, b = 75 mg/kg, c= 150 mg/kg, d = 300 mg/kg, e = 600 mg/kg.

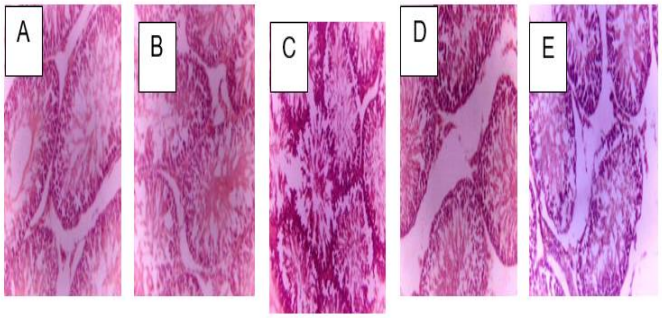

Plate VI: Effects of ethanol leaf extract of *Laggera aurita* for 90 days on the testes of rats (magnification x 400). a = distilled water, b = 75 mg/kg, c= 150 mg/kg, d = 300 mg/kg, e = 600 mg/kg.

Conclusion

The ethanol leaf extract of *Laggera aurita* is generally safe on long-term administration at the 75 mg/kg dose, and the NOAEL was established to be 75 mg/kg. The use of the plant should be with caution in cardiovascular and thyroid disorders due to its potential risk of elevating serum lipids and lowering thyroid hormones at higher doses.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We wish to acknowledge that the research was funded by the Institutional Based Research (IBR) from TETFUND. We would like to acknowledge the assistance of Aliyu Ahmad, Abba Tati Abdulkadir and Nasiru Shehu of the Laboratory of Pharmacology and Therapeutics Department, Bayero University Kano for their assistance.

References

1. Kpemissi M, Metowogo K, Melila M, Veerapur VP, Negru M, Taulescu M, Potârniche AV, Suhas DS, Puneeth TA, Vijayakumar S, Eklu-Gadegbeku K, Aklikokou K. Acute and Subchronic Oral Toxicity Assessments of *Combretum micranthum* (Combretaceae) in Wistar rats. Toxicol Rep. 2020 18(7):162-168. doi: 10.1016/j.toxrep.2020.01.007. PMID: 31993335; PMCID: PMC6976914.
2. Oladipupo AR, Alaribe CS, Balogun OT, Akere HT, Ayanda FA, Toye ET, Coker HAB. Investigation of Chemical, Genotoxic and Haematological Properties of Secondary Metabolites From N-Hexane Extract of *Olax Subscorpioidea Oliv.* (Olacaceae) Leaves. Trp J Nat Prod Res. 2018; 2(12):506-511
3. Vaddi ANV Harita, Soumitra Sahana, Sumanta Mondal. Evaluation of Toxicity and Anticonvulsant Activities of *Solanum torvum* Sw., Fruits. Trop J Nat Prod Res, 2025; 9(2): 613 -627
4. Rosidah I, Renggani TN, Firdausi N, Ningsih S, Yunianto P, Permatasari D, Pongtuluran OB, Bahua H, Efendi J,

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Kusumastuti SA, Nuralih, El Muttaqien, S, Nizar, Kusumaningrum S, Agustini K. Acute and Subchronic Toxicological Study of the Cocktail Extract from *Curcuma xanthorrhiza Roxb, Phyllanthus niruri L*. and *Morinda citrifolia L*., J Toxicol 2024; 1-16 https://doi.org/10.1155/2024/9445226

5. Abdullah MT, Mohammed M, Abubakar A, Na'imatu AS, Mohammed MS, Mohammed HS, Emmanuel O. Acute, Sub-acute, Sub-chronic and Chronic Toxicity Studies of Four Important Ethnomedicinal Plants In Nigeria 2021; 7(1) 1-12
6. Burkil, HM. The Useful Plants of West Tropical Africa, Vol. 1 (2nd ed). Royal Botanical Gardens, New England, 1985. 452-453.
7. Njan NAM, Saotoing P, Tchouonkeu JC, and Messi J. Effects of Essential Oils of Six Local Plants Used as Insecticide on Adults of *Anopheles gambiae*. J Ethnopharmacol. 2007; 4(6): 444-450.
8. Okhale SE, Odiniya EO, and Kunle OF. Preliminary Phytochemical and Pharmacogonistical Investigation of Pediatrics Antimalarial Effects of *Laggera petrodonta* (DC) of Nigerian origin. Ethno Leaf 2010 (4): 457-466.
9. Malami S, Kyari H, Danjuma NM, Ya'u J, and Hussaini IM. Anticonvulsant Properties of Methanol Leaf Extract of *Laggera aurita* Linn. F (Astraceae). J Ethnopharmacol. 2016; 191(2016): 301-306
10. Julde SM, Borodo SB, Wada AS, Malam, S, and Bichi LA. Preliminary Phytochemical Screening and Quantitative Analysis of Crude Extract of *Laggera aurita* Linn (Asteraceae). Rec Chem Sci. 2024; 3(2); 22-27
11. OECD guideline 425 Acute Oral Toxicity Study- Up and Down Method
12. OECD guideline 408 Repeated Dose 90-Day Oral Toxicity Study In Rodents
13. Mubarak H A, Zezi UA, Anafi SB, Alshargi OY, Mohammed M. Mustapha S, Bala AA, Muhammad S, Julde SM, Wada AS, Jatau AI. Sub-Acute Toxicity Study on Hydromethanolic Leaves Extract of *Combretum Hypopilinum* (Combretaceae) Diels in Wistar Rats. Toxicol Res. 2022; 38(4):487-502
14. Amran N, Ermalyanti F, Muhammad F. R. H. Y, Muhammad Z. A. Disi, Yusri S, Muhammad SAS, Hasyrul H. Acute Toxicity Studies of Nutmeg (*Myristica fragrans* Houtt.) Pulp Ethanol Extractin Balb/c Mice Trop JNat Prod Res, April 2025; 9(4):1539 -1542
15. Shehu A. Temidayo O, Zezi AU, and Ahmad A. (2016). Acute Toxicological, Analgesic and Anti-inflammatory Effects of Methanol Extract of *Laggera aurita* Linn F (compositae) in Mice. Afri J Pharm and Thr. 2016; 5(2): 65-73.
16. Julde S, Kyari H, Malami S. Assessment of Serum Parameters After 28-Day Oral Administration of Methanol Leaf Extract of *Lagerra aurita* in Wistar Rats. Trop J Nat Prod Res. 2017; 1 (6): 251-254
17. Komlan M. D., Aboudoulatif D., Povi L. E., Yendube, T. K., Tchin D., Batomayena B., Kwashie E. G. A 90-Day Oral Toxicity of Hydroethanolic Root Extract of *Carissa spinarum* in Wistar Rats, J Toxicol. 2021; (1): 1-6
18. Ebohon O, Irabor F, Omoregie E.S. Sub-Acute Toxicity Study of Methanol Extract of *Tetrorchidium Didymostemon* Leaves Using Biochemical Analyses and Gene Expression in Wistar Rats, Heliyon. 2020; 6(2020): 1-9
19. Bailey SA, Zidell RH, Perry R.W. Relationships Between Organ Weight and Body/Brain Weight in the Rat: What Is the Best Analytical Endpoint? Toxicol Path. 2004; 32:448–466.
20. Nirogi R, Goyal VK, Jana S, Pandey SK, and Gothi A. (2014). What Suits Best for Organ Weight Analysis: Review of Relationship Between Organ Weight and Body/ Brain Weight for Rodent Toxicity Studies, Int J Pharm Sci and Res. 2014; 5(4): 1525-1532
21. Waller DG, Sampson AP. Medical Pharmacology and Therapeutics. Elsevier Philadelphia. 2018; Pp. 64-71.
22. Kaushansky K. Lineage-Specific Hemaopoetic Growth Factors. N Eng J Med. 2006; 354(19): 2034-2045.
23. Hervé E, Nomane E, Goze B, Léandre K, Angoué K, Yapo P. Effects of Subacute Oral Administration of Aqueous Extract of *Macaranga Barteri Müll* Arg (Euphorbiaceae) Leaf on Anthropometric and Haematological Parameters in Rats. Toxicol Res. 2020; 37(1):135-146
24. Kabak M, Çil B, Hocanli I. Relationship Between Leukocyte, Neutrophil, Lymphocyte, Platelet Counts, And Neutrophil to Lymphocyte Ratio and Polymerase Chain Reaction Positivity. Int Immunopharmacol. 2021; 93(2021): 1-6
25. Joseph T. Opeyemi, Joseph S. Oyepata, Zubairu A. Sabastine, Onyia F. Chukwuebuka. Haematological and Histopathological Effects of Ethanol Extract of *Bauhinia variegata* (Linn.) on the Brain, Lungs and Spleen of Wistar Rats Trop JNat Prod Res, 2025; 9(4):1672 – 167
26. Temple RJ, and Himmel MH. Safety of Newly Approved Drugs: Implications 4 presenting. JAMA. 2002; 287(17): 2273-2275.
27. Jon Farizal, Zamharira Muslim, Rahmad Abdillah. Acute Oral Toxicity Assessment of *Morinda citrifolia L.* (Noni) Leaves in Experimental Mice Trop JNat Prod Res, 2024; 8(12): 9586 - 9590
28. Almazroo OA, Miah MK, Venkataramanan R. Drug Metabolism in the Liver. Clin Liver Dis. 2017; 21(1):1-20
29. Farzaei MH, Bayrami Z, Farzaei F, Aneva I, Das SK, Patra JK, Das G, Abdollahi M. Poisoning by Medical Plants. 2020; Arch Iranian Med 23(2):117-127
30. Limdi JK, Hyde GM. Evaluation of Abnormal Liver Function Tests. Postgr Med J. 2003; 79(932): 307-312.
31. Kpomah ED, Arghoghro EM, Uwakwe A. (2012). Biochemical and Histopathological Changes in Wistar Rats Following Chronic Administration of Di herbal Mixture of *Zanthoxylum leprieurii* and *Piper guineense*. J Nat Sci Res. 2012; 2(6): 22-28
32. Rogério S. Chivodze, Atifa I. Sulemane, Isabel Magaia, Vanina N.C. Saete, Saquina C. Rugunate, Hercílio E. Zimila, François Munyemana. Phytochemical screening and Evaluation of Antioxidant and Antimicrobial Activity of *Solanum linnaeanum* Extracts. Trop JNat Prod Res, 2022; 6(2):194-201
33. Schnellman RG. Toxic Responses of Kidney In: C.D. Klassen (ed), Casarett and Doull's Toxicology: The Basic Sciences of Poisons, (7th ed), New York McGraw – Hill 2008; 583-608.
34. Oluwatosin A. Dosumu, Adelani I. Bababode, Solomon O. Rotimi, Adio J. Akamo, Oluwatosin O. Omotosho, Latifah O. Sani, Kehinde T. Osinuga, Odunayo A. Taiwo, Oluwafemi P. Owolabi, Oluwafemi A. Ojo. DMBA-Induced Kidney

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Dysfunction: Effects of Supplementary Dietary Vitamin K in Rats. Trop JNat Prod Res, 2021; 5(5):917-923

35. Anh N. Dang, Wang Yao-Guang, Zhao Xi, Zhang Jing, Hoang N. Nguyen. Effects of Tang Shen 2 Hao Fang, a Traditional Chinese Medicine, on Diabetic Nephropathy in Sprague–Dawley Rats Trop JNat Prod Res, 2021;5(10):1828-1834
36. Edmund L, David J. Kidney Function Tests. In: Carl AB, Edward R, David E. T. (eds). Textbook of Clinical Chemistry and Molecular Diagnostics. 4th Ed. New Delhi: Elsevier Inc.; 2006. 797-808.
37. Houda Z. Megherbi, Sara Aouissat, Farida O. Boukortt. Effect of Casein Hydrolysates and Zinc on Insulin Resistance, Renal Function, Pancreatic and Kidney Histopathology in Diabetic Rats, Trop JNat Prod Res, 2025; 9(4):1441 – 1448
38. Lippi G, Plebani M. Current Trends and Future Projections in the Clinical Laboratory Test Market: Implications for Resource Management and Strategic Planning. Clin Chem Lab Med 2025;63(4): e91-e93. https://doi.org/10.1515/cclm-2024-1424
39. Enaohwo T Mamerhi, Orororo C Osuvwe, Jaiyeoba-ojigho E Jennifer, Dunkwu C Cyril, Enyi C Kingsley, Oghaimeh O Grace, Mode, Joan. *Justicia carnea* Extract Mitigates TNBS-Induced Liver Inflammation by Ameliorating Oxidative Stress, Improving Liver Function Indices and Normalizing NF-κb/Caspase 3 Expression. Trop J Nat Prod Res 2025; 9(4): 1574 -1583
40. Espinoza SE, Guo H, Fedarko N, Fried PL, Xue QL, Leng S, Beamer B, Walston JD. Glutathione Peroxidase Enzyme Activity in Ageing, J. Gerontol Series A, Bioland Med Sci. 2008; 63(5): 505-509.
41. Chu Z, Fang L, Xiang Y, Ding Y. Research Progress on Cholesterol Metabolism and Tumour Therapy. Discov Onc 2025; (16):647. https://doi.org/10.1007/s12672-025-02430-5
42. Chapman, M.J., Ginsberg, H.N., Amarenco, P., Andreotti, F., Boren, J., Catapano, Al., Descamps, O.S., Fisher, E., Kovanen, P.T., Lesnik, J.P., Masana, L., Reiner, Z., Taskinen, M.R., Tokgozolu, A.H., And Watts, G.F. Triglyceride Rich Lipoproteins and High-Density Lipoprotein n Patients at High Risk of Cardiovascular Disease: Evidence and Guidance for Management. EHJ. 2011; 32(11):1345-1361
43. Rizos, C.V., Elisaf, M.S., and Liberopoulos, E.N. Effects of Thyroid Dysfunction on Lipid Profile. Open Cardiovasc Med J. 2011; (5): 76-84
44. Mandava, S., and Mandava, N.B. Endocrine Disrupting Chemicals and the Effects They Play in Plant Regulators J. Endocr Disord. 2021; 7(1): 1-6
45. Mattison D. R. The Mechanisms of Action of Reproductive Toxins. Am J Ind Med, 1983; 4(1-2): 65–79.
46. Smith C. G. Reproductive Toxicity: Hypothalamic-Pituitary Mechanisms. Ame J Ind Med. 1983; 4(1-2): 107–112.
47. Kuchi Manjeera, Raja Sundararajan. Toxicity Studies of *Abutilon crispum* and *Indigofera prostrata* Whole Plants on Wistar Rats, Trop JNat Prod Res, 2024; 8(11): 9073 – 9078
48. Chinnappan, S., George, A., Ashok, G., & Choudhary, Y. K. Effect of Herbal Extract *Eurycoma Longifolia* (Physta) On Female Reproductive Hormones and Bone Biochemical Markers: An Ovariectomised Rat Model Study. BMC Compl Med and Ther. 2020; 20(31): 1-12
49. Juliet N. Olayinka, Abigail M. Akhigbemen, Emmar E. Okpakpor, Dickson O. Uwaya, Gerald I. Eze, Raymond I. Ozolua. Toxicological Evaluation of the Aqueous Leaf Extract of *Blighia Sapida K.D. Koenig* (Sapindaceae) in Rodents. Trop JNat Prod Res, 2023; 7(12) 5690-5698

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License