FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 8, April, 2026, pp 269 – 286
DOI: https://doi.org/10.33003/fjs-2026-1008-5331

EVALUATION OF THE SUB-CHRONIC TOXICITY OF METHANOL EXTRACT OF *URARIA PICTA* (LEAVES) AND *LEPTODERRIS TRIFOLIOLATA* (ROOT) IN WISTAR RATS

***Kolawole Oluwadamilola Moromoke and Omoregie Ehimwenma Sheena**

Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin, Edo State, Nigeria.

*Corresponding authors' email: moromoke.kolawole@uniben.edu

ABSTRACT

Medicinal plants are important sources of bioactive compounds used in the treatment of several diseases, although their traditional use may present safety concerns. This study evaluated the sub-chronic toxicity of methanol extracts of *Leptoderris trifoliolata* and *Uraria picta* in female Wistar rats. Forty-five (45) rats weighing 100 ± 20 g were divided into nine groups and orally administered 50, 300, 1000, and 2000 mg/kg body weight of the extracts for 28 days, while the control group received no extract. Biochemical, haematological, oxidative stress, lipid profile, and histopathological parameters were evaluated. No major treatment related alterations were observed in body weight, organ/body weight ratio, oxidative stress indices, haematological parameters, or histopathology of the liver, kidney, and uterus. However, significant ($p < 0.05$) increases in creatinine levels were observed in *Uraria picta* 50 mg/kg (2.17±0.16 mg/dL), 300 mg/kg (2.67±0.17 mg/dL), and *Leptoderris trifoliolata* 1000 mg/kg (1.17±0.17 mg/dL) groups compared with the control (1.00±0.06). Total cholesterol increased significantly ($p < 0.05$) at *L. trifoliolata* 1000 mg/kg (74.33 mg/dL), while triacylglycerol decreased significantly across all treated groups compared with the control (103.33±0.33). Alterations were also observed in HDL-C, LDL-C, AST, ALT, total bilirubin, and direct bilirubin levels at specific doses. Despite these changes, histopathological examination revealed no tissue abnormalities. Overall, both extracts appeared relatively safe following sub-chronic administration, although prolonged use at certain doses may cause metabolic disturbances, warranting further chronic toxicity studies.

Keywords:
Methanol extract, Wistar Rats, Medicinal plants, Biochemical parameters, Haematological parameters

INTRODUCTION

Medicinal plants continue to play a central role in primary healthcare systems, particularly in developing countries where access to conventional medicine may be limited. In Africa, approximately 70–80% of the population relies on traditional medicine for the management of various diseases (Tilahun *et al.*, 2020). In addition to their ethnomedicinal relevance, plants have contributed significantly to modern drug discovery, with nearly 25% of currently used pharmaceutical agents originating from plant sources (Kale *et al*., 2012). Despite their therapeutic importance, the safety and efficacy of many medicinal plants remain scientifically underexplored. Several studies have demonstrated that some traditionally used herbs may possess toxic constituents capable of inducing adverse effects when consumed indiscriminately (Ertekin *et al*., 2005; Koduru *et al.*, 2006). Since bioactive phytochemicals may exert both therapeutic and toxic effects depending on dose and duration of exposure, there is a critical need for toxicological and pharmacological evaluation of medicinal plants with established ethnomedicinal claims.

The present study focuses on *Uraria picta* (Jacq.) and *Leptoderris trifoliolata*, two medicinal plants belonging to the family Fabaceae, selected based on their rich phytochemical composition and extensive traditional usage. *Uraria picta*, a perennial herb distributed across Africa, Asia, and Australia, is an important component of the Ayurvedic formulation "Dashmula/Dasamoola" used in traditional Indian medicine (Kritika Hem *et al*., 2017). Phytochemical investigations have shown that *U. picta* contains biologically active secondary metabolites including flavonoids, alkaloids, phenols, terpenoids, tannins, saponins, steroids, and cardiac glycosides, as well as amino acids and fatty acids (Saxena *et al*., 2014; Naik *et al*., 2018). These compounds are associated with several reported pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, antithrombotic, antipyretic, acaricidal, and aphrodisiac effects (Kale *et al*., 2012; Rahman *et al.*, 2007; Naik *et al*., 2018). The high flavonoid and phenolic contents, in particular, suggest strong antioxidant potential capable of modulating oxidative stress related pathological conditions.

Similarly, *Leptoderris trifoliolata*, a climber plant commonly found in swampy regions of Sierra Leone and Nigeria, is widely utilized in Nigerian traditional medicine, where it is locally known as "wowo." Herbal practitioners employ the plant in the management of uterine disorders and breast cancer, indicating possible bioactive effects on reproductive and cellular systems. Preliminary phytochemical investigations on species within the *Leptoderris* genus and related Fabaceae plants have revealed the presence of biologically active compounds such as alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, steroids, and cardiac glycosides, many of which possess antioxidant, anti-inflammatory, antimicrobial, cytotoxic, and antiproliferative activities (Owolabi *et al*., 2020; Olaleye *et al*., 2024). Although both plants are widely used in traditional medicine and possess diverse bioactive compounds, there is limited scientific information regarding their comparative biological activities, and safety profiles, particularly in relation to their therapeutic claims. This represents an important research gap, especially considering the increasing reliance on herbal remedies and the potential risks associated with prolonged or uncontrolled use.

MATERIALS AND METHODS

All chemicals, reagents and assay kits used were of analytical grade.

Plant Collection and Identification

Plant materials *Uraria picta* (leaf) and *Leptoderris trifoliolata* (root) were collected from their natural habitats. They were

taxonomically identified and authenticated by a botanist in the department of plant biology and Biotechnology, University of Benin, Benin City, Edo state, Nigeria, with Voucher specimens, (UBH_U-434) and (UBH_4- 435) respectively were deposited in the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Plant Preparation and Extraction

Plant materials were cut into small pieces, air-dried, and pulverized using a mechanical grinder. Weighed portion of *Uraria picta* (leaf) (500 g) and *Leptoderris trifoliolata* (500 g) respectively was soaked in methanol (1500 mL) and kept in a shaker at room temperature for 72hrs. thereafter, the extract was filtered using a muslin cloth, and the filtrates were placed in a rotary evaporator (RE 300, Bibby Scientific, UK) to allow evaporation of the solvents and subsequent concentration of the extract was done using a freeze- dryer

Experimental Design

Experimental Animals

Healthy female Wistar albino rats weighing 100±20 g was used for the study. The animals were gotten from the Department of Biochemistry, University of Benin, Nigeria. Animals were kept under standard acclimatization conditions with room temperature, 12 h light: 12 h dark cycle and were provided food and water *ad libitum*. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Ethical approval for this study was granted by the Institutional Ethics Review Committee, University of Benin (No: FLS/REC/2026/013)

Acute Toxicity Study

The acute toxicity study was conducted in accordance with a modified OECD guideline 420 (2001). Twenty-one (21) female Wistar rats (100-120 g) were randomly divided into seven groups of three animals each (n=3). Group 1 served as the control and received 0.5% carboxymethyl cellulose (CMC), while groups 2–7 received single oral doses of the test extracts at 10, 100, 1000, 1600, 2900, and 5000 mg/kg, respectively. All test substances were prepared in 0.5% CMC and administered once by oral gavage. Following dosing, animals were provided food and water ad libitum and observed continuously for 7 days for signs of acute toxicity, including tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma.

Sub-chronic Toxicity Study

The sub-chronic toxicity study was conducted with slight modification according to OECD guideline 407 (2008). Forty-five (45) female Wistar albino rats (100 ± 20 g) were randomly divided into nine groups (n = 5 per group). Group 1 served as the control and received 0.5% carboxymethyl cellulose (CMC) orally for 28 consecutive days. Treatment groups received graded oral doses selected as fractions of the LD_{50} to establish a dose-response relationship. Groups 2–5 were administered 50, 300, 1000, and 2000 mg/kg of methanol extract of *Uraria picta*, respectively, while groups 6–9 received corresponding doses of methanol extract of *Leptoderris trifoliolata* root. All administrations were carried out once daily by oral gavage for 28 days.

Sample Collection

All animals were fasted overnight and sacrificed by cervical dislocation after the last exposure to methanol extract of *Uraria picta* leaf and *Leptoderris trifoliolata* roots, the blood sample was collected via aortic puncture and allowed to clot for 1 h and then centrifuged at 2000 rpm for 10 min to obtain serum. EDTA bottle was used to collect blood samples for hematological parameters. Organs (Liver, kidney, and uterus) were harvested and weighed. A portion of the liver, kidney (1g) and uterus (0.5 g) were homogenized in 5 ml of 0.9% ice-cold physiological saline to obtain homogenate. Post-mitochondrial fractions were obtained by centrifuging the homogenates at 4000rpm for 20 min at 4°C. The post-mitochondrial fraction and serum were then used for biochemical assays. A small portion of the liver, kidney and uterus were fixed in 10% formalin for histological examination.

MATERIALS AND METHODS

Biochemical Analysis

Estimation of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

ALT activity was determined using the method of Reitman and Frankel (1957) with Randox diagnostic kits. Alanine aminotransferase (ALT) catalyses the conversion of L-alanine and α-ketoglutarate to pyruvate and L-glutamate at optimal pH. The pyruvate formed is reduced to L-lactate by lactate dehydrogenase (LDH) in the presence of NADH, resulting in oxidation of NADH to NAD^+ with a corresponding decrease in absorbance at 340 nm. The decrease in absorbance is proportional to ALT activity in serum. Briefly, 50 µL of sample was mixed with 500 µL of ALT reagent, and absorbance was measured at 340 nm after 1 min, followed by subsequent readings at 1-, 2-, and 3-min intervals. ALT activity was calculated using the expression: ALT activity (U/L) = 1746 × ΔA340 nm/min, where ΔA340 nm/min represents the change in absorbance per minute and 1746 is the extinction coefficient.

Aspartate aminotransferase (AST) catalyses the conversion of L-aspartate and α-ketoglutarate to L-glutamate and oxaloacetate. The oxaloacetate produced is subsequently reduced by malate dehydrogenase (MDH) in the presence of NADH, resulting in the oxidation of NADH to NAD^+ and a corresponding decrease in absorbance at 340 nm. Lactate dehydrogenase (LDH) present in the reagent minimizes interference caused by pyruvate in the sample. AST activity was determined using the same procedure described for ALT, except that AST reagent was used in place of ALT reagent. AST activity was calculated using the expression: AST activity (U/L) = 1746 × ΔA340 nm/min, where ΔA340 nm/min represents the change in absorbance per minute and 1746 is the extinction coefficient.

Estimation of Serum Total and Direct Bilirubin

Total bilirubin was determined using Randox commercial diagnostic kits according to the method of Jendrassik and Grof (1938). In alkaline medium, direct bilirubin reacts with diazotized sulphanilic acid to form a blue-coloured complex, while total bilirubin was measured in the presence of caffeine, which releases albumin-bound bilirubin for reaction with diazotized sulphanilic acid. Briefly, reagents 1, 2, and 3 were mixed with the sample and incubated for 10 min at 25°C. Thereafter, reagent 4 was added and the mixture was allowed to stand for 30 min at 25°C before absorbance was measured at 578 nm against the sample blank. Total bilirubin concentration was calculated using the expression: Total bilirubin (mg/dL) = 10.8 × A_{TB} (578 nm).

Direct bilirubin was determined using Randox commercial diagnostic kits based on the method of Jendrassik and Grof (1938). Briefly, reagents 1 and 2 (R1 and R2), 0.9% NaCl, and

the sample were mixed and allowed to stand for 10 min at 25°C. The absorbance of the reaction mixture was then measured at 546 nm against the sample blank. Direct bilirubin concentration was calculated using the expression: Direct bilirubin (mg/dL) = 10.8 × A_{DB} (546 nm).

Estimation of Serum Albumin

Serum albumin concentration was determined using the method of Doumas and Biggs (1972) as described in the Randox albumin kit manual. The assay is based on the quantitative binding of albumin to bromocresol green (BCG), forming an albumin–BCG complex with maximum absorbance at 578 nm. Briefly, 0.01 mL of serum sample was mixed with 3 mL of BCG reagent (R1). Similarly, 0.01 mL of standard solution and 3 mL of BCG reagent were prepared as standard, while 0.01 mL of distilled water with 3 mL of BCG reagent served as the blank. The mixtures were incubated at 25°C for 5 min, and absorbance was measured at 578 nm against the reagent blank using a 1 cm light path cuvette. Albumin concentration was calculated using the expression: Albumin (g/L) = ($A_{sample}/A_{standard}$) × concentration of standard, where A_{sample} is the absorbance of the sample and $A_{standard}$ is the absorbance of the standard.

Lipid Profile

Estimation of Total Cholesterol

Serum total cholesterol concentration was determined using an enzymatic endpoint method described by Trinder (1969) and Richmond (1973) with Randox diagnostic kits. The method is based on enzymatic hydrolysis of cholesterol esters by cholesterol esterase to produce free cholesterol and fatty acids. Free cholesterol is subsequently oxidized by cholesterol oxidase to form cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with phenol and 4-aminoantipyrine to produce a red quinoneimine complex, the intensity of which is directly proportional to the cholesterol concentration in the sample. Briefly, 0.01 mL of serum sample and 0.01 mL of standard were dispensed into separate test tubes, followed by the addition of 1.0 mL of working reagent. A reagent blank containing distilled water and working reagent was also prepared. The mixtures were incubated at room temperature for 5 min, and absorbance was measured at 546 nm against the reagent blank within 60 min. Total cholesterol concentration was calculated using the expression: Total cholesterol (mg/dL) = ($A_{sample}/A_{standard}$) × 5.2

Estimation of Triglyceride

Serum triacylglycerol concentration was determined using an enzymatic method described by Trinder (1969) with Randox diagnostic kits. The assay is based on enzymatic hydrolysis of triacylglycerols by lipase to produce glycerol and fatty acids. Glycerol is subsequently phosphorylated by glycerol kinase and oxidized by glycerol phosphate oxidase to generate hydrogen peroxide, which reacts with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase to form a quinoneimine chromogen. The intensity of the coloured complex formed is proportional to the triacylglycerol concentration in the sample. Briefly, 0.01 mL of serum sample and 0.01 mL of standard were dispensed into separate test tubes, followed by the addition of 1 mL of enzyme reagent. The mixtures were incubated at 37°C for 5 min, while a reagent blank containing enzyme reagent alone was prepared separately. Absorbance was measured at 500 nm against the reagent blank within 60 min. Triacylglycerol concentration was calculated using the expression: Triacylglycerol (mg/dL) = ($A_{sample}/A_{standard}$) × 196.

Estimation of Serum High Density Lipoprotein (HDL) Cholesterol

High-density lipoprotein cholesterol (HDL-C) was determined using the precipitation method of Lopes-Virella *et al*. (1977) with Randox diagnostic kits. In this method, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and chylomicrons were precipitated using phosphotungstic acid in the presence of magnesium ions, leaving HDL cholesterol in the supernatant. Briefly, 0.5 mL of serum sample was mixed with 1.0 mL of precipitating reagent and incubated at 37°C for 10 min. The mixture was centrifuged at 4000 rpm for 10 min to obtain a clear supernatant. The cholesterol concentration of the supernatant was then determined enzymatically using the methods of Trinder and Richmond as previously described. HDL cholesterol concentration was calculated using the expression: HDL cholesterol (mg/dL) = ($A_{sample}/A_{standard}$) × concentration of standard.

Estimation of Serum Low Density Lipoprotein (LDL) Cholesterol

Low-density lipoprotein cholesterol (LDL-C) concentration was estimated using the Friedewald equation as described by Friedewald *et al*. (1972). The calculation is based on the relationship between total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C). LDL cholesterol (mg/dL) was calculated using the expression: LDL-C = Total cholesterol − (Triglycerides/5) − HDL-C.

Antioxidant Assay

Estimation Malondialdehyde (MDA)

Tissue malondialdehyde (MDA) concentration was determined spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) method as described by Biege and Aust (1978). The method is based on the reaction of MDA, a lipid peroxidation product, with thiobarbituric acid (TBA) under acidic and high-temperature conditions to form a pink chromogen that absorbs maximally at 535 nm, with absorbance proportional to MDA concentration. Briefly, 0.1 mL of serum sample was mixed with 2.0 mL of TCA-TBA-HCl reagent (25% trichloroacetic acid, 1% thiobarbituric acid, and 0.4 M HCl). A blank was prepared using 0.1 mL of distilled water in place of the sample. The mixture was heated in a boiling water bath at 95°C for 15 min, cooled, and centrifuged at 1000 rpm for 10 min to remove precipitates. The absorbance of the supernatant was measured at 532 nm against the blank. MDA concentration was calculated using the expression:

MDA (unit/mg protein) = (A × V × 1000) / (M × v × y), where A is absorbance, V is total reaction volume, M is the molar extinction coefficient, v is sample volume, and y is the protein content of the sample.

Estimation OF Glutathione Peroxidase (GPx)

Glutathione peroxidase activity was determined spectrophotometrically using a modification of the Chance and Maehly method as described by Andy and Goodman (1972). The assay is based on the enzymatic reaction involving the oxidation of pyrogallol in the presence of hydrogen peroxide to form purpurogallin, which absorbs maximally at 420 nm. Freshly prepared reagents included 5% pyrogallol solution (protected from light), 0.147 M hydrogen peroxide (H_2O_2), 0.1 M phosphate buffer (pH 6.0), and 2.0 N sulfuric acid (H_2SO_4). The reaction mixture consisted of distilled water (14.0 mL), 5% pyrogallol solution (2.0 mL), 0.147 M H_2O_2 (1.0 mL), and phosphate buffer (2.0 mL), which was equilibrated at 20°C for 5 minutes. The reaction

was initiated by the addition of 1.0 mL enzyme solution. After exactly 20 seconds, the reaction was terminated by adding 2.0 N H_2SO_4, and the optical density was measured at 420 nm against a water blank using a spectrophotometer. The increase in absorbance corresponding to purpurogallin formation was used to estimate GPx activity.

Estimation of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was determined using the method of Misra and Fridovich (1972). The assay is based on the ability of SOD to inhibit the auto-oxidation of adrenaline (epinephrine) to adrenochrome at alkaline pH (10.2), a reaction mediated by superoxide radicals (O_2^-). The degree of inhibition is proportional to SOD activity in the sample. Briefly, 0.2 mL of sample was mixed with 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and 0.3 mL of freshly prepared 0.3 mM adrenaline solution. The reference sample contained 0.2 mL of distilled water instead of the test sample, along with the same reagents. The absorbance was measured at 420 nm. Percentage inhibition was calculated as: % inhibition = [(OD test - OD reference) / OD test] × 100 SOD activity (U/mg protein) was then expressed as: SOD activity = % inhibition / (50 × Y), where Y represents the protein content (mg) of the sample.

Estimation of Catalase Activity

Catalase activity was determined using the method of Cohen *et al* (1970). The assay is based on the enzymatic decomposition of hydrogen peroxide (H_2O_2) by catalase into water and oxygen. The residual H_2O_2 is quantified indirectly through its reaction with potassium permanganate ($KMnO_4$), and the remaining $KMnO_4$ is measured spectrophotometrically at 480 nm. The rate of H_2O_2 decomposition is proportional to catalase activity in the sample. Briefly, 0.5 mL of sample was incubated with 5.0 mL of H_2O_2 and allowed to stand for 30 min. The reaction was terminated by the addition of 1.2 mL of 50 mM phosphate buffer (pH 7.4), 1.5 mL of 6 M H_2SO_4, and 7.0 mL of 0.01 M $KMnO_4$. The mixture was inverted, incubated for an additional 30 min, and absorbance was measured at 480 nm against distilled water within 30-60s. A reagent blank containing distilled water in place of H_2O_2 was processed similarly. Catalase activity was calculated using the expression:

Catalase activity (U/mg protein) = (OD/min × V) / (M × v × L × Y), where OD is absorbance, V is total reaction volume, M is molar extinction coefficient of H_2O_2, v is sample volume, L is path length, and Y is protein content of the sample.

Estimation of Reduced Glutathione (GSH) Concentration

Reduced glutathione (GSH) concentration was determined using the enzymatic method described by Tietze (1969). The assay is based on the reaction between GSH and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), resulting in the formation of 5-thio-2-nitrobenzoic acid (TNB), a yellow-colored product measured spectrophotometrically at 412 nm. Oxidized glutathione (GSSG) can be enzymatically recycled to GSH in the presence of glutathione reductase and NADPH. For the assay, 0.2 mL of sample was diluted with 1.8 mL of distilled water, followed by addition of 3 mL of precipitating solution. The mixture was allowed to stand for 5 min and then filtered. Subsequently, 4.0 mL of 0.1 M phosphate buffer and 1.0 mL of the filtrate were added, followed by 0.5 mL of Ellman's reagent (DTNB in sodium citrate). A reagent blank was prepared using phosphate buffer, diluted precipitating solution, and Ellman's reagent. Absorbance was read at 412 nm, and GSH concentration was determined to be directly proportional to the absorbance obtained.

Assessment of Kidney Function

Estimation of Serum Creatinine Concentration

Serum creatinine concentration was determined using the method of Bartels and Böhm (1972). The assay is based on the Jaffe reaction in which creatinine reacts with picric acid in an alkaline medium to form a coloured complex, the intensity of which is proportional to creatinine concentration. In the procedure, 1.0 mL of working reagent (picric acid and sodium hydroxide) was mixed with 0.1 mL of standard or serum sample in separate tubes. Absorbance was measured at 492 nm after 30 seconds (A_1) and again after 2 minutes (A_2). The change in absorbance (ΔA) was calculated as $A_2 - A_1$. All analyses were performed in triplicate. Creatinine concentration was calculated as:

Creatinine (mg/dL) = $(\Delta A_{sample} / \Delta A_{standard}) \times 2$ mg/dL

Estimation of Serum Urea Concentration

Serum urea concentration was determined using the method of Fawcett and Scott (1960). The assay is based on enzymatic hydrolysis of urea by urease to produce ammonia, which reacts via Berthelot's reaction with phenol and hypochlorite to form indophenol, a blue chromophore measurable spectrophotometrically. For the procedure, 0.01 mL of distilled water, standard, or serum sample was mixed with 0.1 mL of reagent 1 (urease, EDTA, and sodium nitroprusside) and incubated at 37°C for 10 minutes. Thereafter, 2.5 mL of reagent 2 (phenol) and 2.5 mL of reagent 3 (hypochlorite) were added, mixed, and incubated for 15 minutes at 37°C. Absorbance was read at 546 nm against a reagent blank. All determinations were carried out in triplicate. Urea concentration was calculated as:

Urea (mg/dL) = $(\Delta A_{sample} / \Delta A_{standard}) \times 2$ mg/dL

Determination of Serum Potassium Concentration

Serum potassium concentration was determined using the sodium tetraphenyl boron method as described by Terri and Sesin (1958). The principle is based on the formation of a colloidal suspension between potassium ions and sodium tetraphenyl boron, producing turbidity proportional to potassium concentration within the range of 2–7 mEq/L. In the procedure, 1.0 mL of potassium reagent was dispensed into all test tubes (blank, standard, control, and sample). Thereafter, 0.01 mL of serum sample was added to the sample tube, mixed, and incubated at room temperature for 3 minutes. Absorbance was measured at 500 nm against the reagent blank. Potassium concentration was calculated as:

Serum K^+ (mEq/L) = $(A_{sample} / A_{standard}) \times 4$ mEq/L

Estimation of Serum Sodium Concentration

Serum sodium concentration was determined using the method of Maruna (1958), based on precipitation of sodium as sodium magnesium uranyl acetate, followed by reaction of excess uranyl ions with ferrocyanide to form a chromophore whose absorbance is inversely proportional to sodium concentration. One millilitre (1.0 mL) of filtrate reagent was added to blank, standard, and sample tubes. Subsequently, 50 µL of sample and standard were added to their respective tubes, while distilled water was added to the blank. The mixtures were mixed for 3 minutes and centrifuged at 1500 g for 10 minutes. Then, 50 µL of supernatant was added to 1.0 mL acid reagent, followed by 50 µL sodium colour reagent. Absorbance was read at 550 nm. Sodium concentration was calculated as:

Serum Na^+ (mEq/L) = [(A_{blank} - A_{sample}) / (A_{blank} - $A_{standard}$)] × 150 mEq/L

Serum Chloride Concentration

Serum chloride concentration was determined based on the mercuric thiocyanate method (Maruna, 1958), in which chloride ions displace thiocyanate from mercuric thiocyanate, and the liberated thiocyanate reacts with ferric ions to form a red ferric thiocyanate complex with absorbance at 520 nm. The intensity of colour is directly proportional to chloride concentration. To 1.5 mL of chloride reagent, 0.01 mL of calibrator and sample were added in respective tubes (blank, calibrator, and sample), mixed, and incubated at room temperature for at least 5 minutes. Absorbance was then measured at 520 nm. Chloride concentration was calculated as: Cl^- (mEq/L) = (Asample / Acalibrator) × Calibrator concentration

Haematological Assay

Haematological parameters including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), and platelet count were determined using a Beckman Coulter JT series hematology analyzer as described by Jones et al. (1996).

Histopathological Examination of Tissues

Histopathological evaluation of liver, kidney, and uterus tissues was carried out according to the method of Humason (1962). Excised organs from control and treated rats were immediately fixed in 10% formol saline for morphological assessment. Tissue processing involved fixation, dehydration through graded alcohol concentrations (70% to absolute alcohol), clearing in toluene, and impregnation in molten paraffin wax. Embedded tissues were sectioned using a microtome at 5–15 µm thickness and mounted on slides. Sections were stained using Ehrlich's haematoxylin and eosin (H&E) technique, where haematoxylin-stained nuclei blue-black, while eosin counterstained cytoplasm and connective tissues in varying shades of pink to red. Slides were examined microscopically using ×100 and ×400 magnifications for histoarchitectural changes, and representative micrographs were documented for analysis.

Statistical Analysis

The statistical analysis was performed using the GraphPad Prism statistical package version 8 (SanDiego, CA, USA). The results obtained were expressed as were presented as Mean ± SEM. One way analysis of variance (ANOVA) test was used to determine significance differences between the groups and post hoc multiple comparison test was done using Tukey's HSD (honest significant difference). Statistical significance was declared when P value was less than 0.05.

RESULTS AND DISCUSSION

Acute Toxicity Study

Administrations of *Uraria picta* (leaf) and *Leptoderris trifoliolata* (root) methanol extracts to rats did not cause mortality up to a dose of 5000 mg/kg body weight. Furthermore, there were no visible signs of toxicity, i.e., food and water consumptions were unaffected; and convulsions, salivation, diarrhoea, lethargy, sleep, coma, aggression and rising furs, were not observed over a period of seven days. The administration of *L.trifoliolata* extracts at doses 10mg/kg, 100mg/kg and 2900mg/kg body weight resulted in a significant decrease ($p<0.05$) in body weight over the 7-day observation period when compared to day zero. For *U.picta*, reductions in body weight were insignificant ($p>0.05$) when compared to Day zero. However, rats in the group administered 1600mg/kg of the extract showed an insignificant increase ($P>0.05$) in body weight (Figure 2)

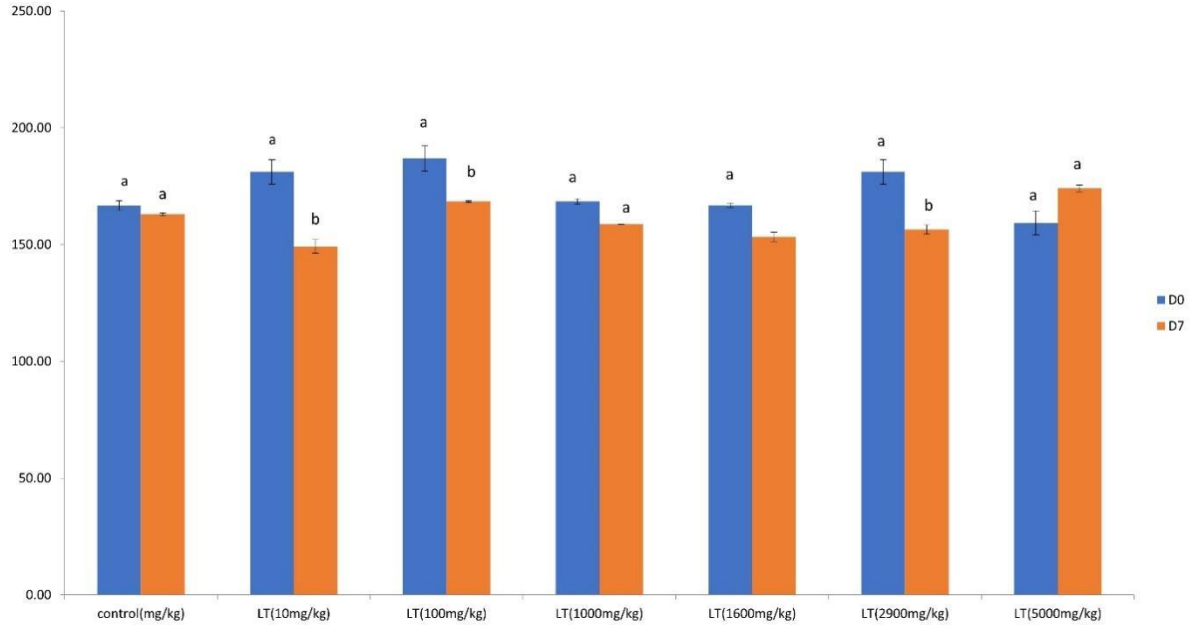

Figure 1: Body weight of rats administered varying doses (10 mg/kg, 100 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg, 5000 mg/kg body weight) of *L. trifoliolata* root extract. Weights were taken on day zero and day 7. The data illustrates the mean ± SEM of 5 rats per treatment groups (n = 5)

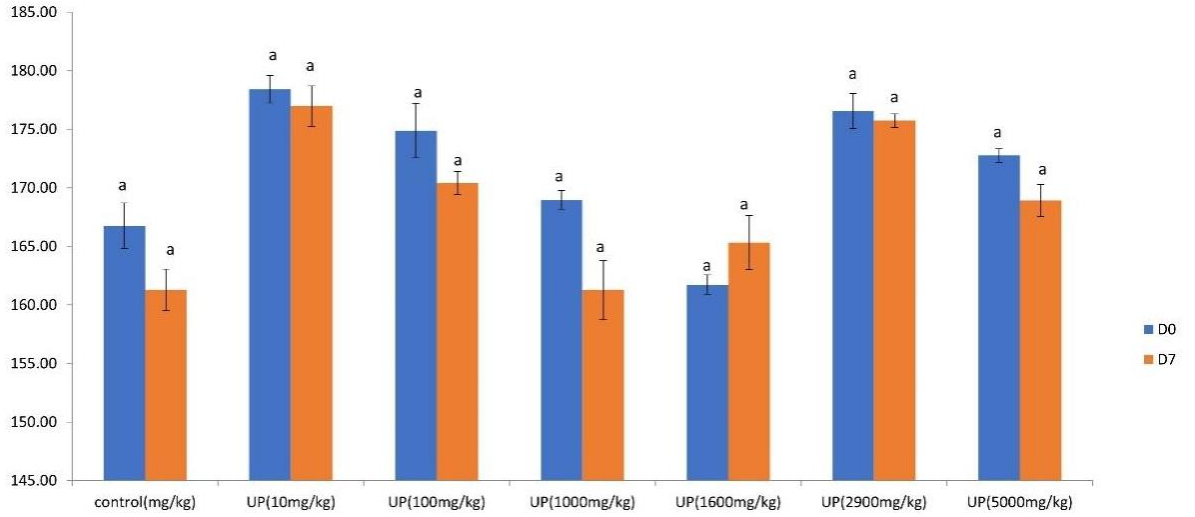

Figure 2: Body weight of rats fed oral doses (10 mg/kg, 100 mg/kg, 1000 mg/kg, 1600 mg/kg, 2900 mg/kg, 5000 mg/kg body weight) of *U. picta* methanol leaf extract. Weights were taken on day zero and day 7. Values are expressed as mean ± SEM of 5 rats per treatment groups (n = 5). Key: D0 = day zero, and D7 = day 7.

Sub-chronic Toxicity

Rats received different doses (50, 300, 1000, and 2000 mg/kg body weight) of both extracts for 28 days. After the treatment period, body weights were measured and compared with initial weights recorded on day 0. Rats treated with *Leptoderris trifoliolata* showed a significant increase in body weight ($p < 0.05$) compared to the control group, suggesting normal growth and no signs of toxicity.

Table 1: Effect of Methanol Extracts of *U. picta* Leaf and *L. trifoliolata* Root on Body Weight Changes after 28 Days

Group	Mean Body Weight (g)		
	Day 0	Day 28	Difference in Weight (%)
Control	103.93±13.87	137.13±8.21	33.20±3.20^a
50 mg/kgUP	122.96±4.71	146.31±9.93	23.35±1.27^a
300 mg/kgUP	113.63±3.67	144.43±8.05	30.8±0.26^a
1000 mg/kgUP	139.53±3.33	159.84±14.70	20.31±4.67^a
2000 mg/kgUP	116.21±3.38	142.33±8.65	26.12±3.22^a
50 mg/kg LT	116.68±1.33	144.76±2.86	28.08±1.59^b
300 mg/kg LT	108.17±2.04	155.77±5.64	47.6±3.46^b
1000 mg/kg LT	102.41±2.71	147.14±3.15	44.73±3.61^b
2000 mg/kg LT	108.47±2.85	143.41±1.77	34.93±3.80^b

Key: UP = *Uraria picta*, LT = *Leptoderris trifoliolata*, D0 = day zero and D28 = day 28. Values are expressed as mean ± SEM (n=5)

Haematological Indices

The results in Table 2 and table 3 shows that administration of *Uraria picta* and *Leptoderris trifoliolata* did not cause any significant change in the haematological parameters of the rats across all doses administered when compared to the control.

Table 2: Effect of Methanol Extracts of *U. Picta* Leaf and *L. Trifoliolata* Root on Haematological Parameters

Haematological Parameters	Normal Control	UP50(mg/kg)	UP300(mg/kg)	UP1000(mg/kg)	UP2000(mg/kg)
WBC ($\times10^3$/ųL)	9.0±1.30^a	7.1±0.60^a	5.7±0.50^a	5.3±0.80^a	6.2±0.80^a
RBC ($\times10^6$/ųL)	6.75±0.20^a	6.7±0.06^a	6.88±0.2^a	6.96±0.22^a	6.75±0.28^a
HGB (g/dL)	10.93±0.72^a	11.23±0.17^a	11.30±0.38^a	11.20±0.85^a	10.90±1.04^a
HCT (%)	37.8±2.51^a	39.3±1.00^a	40.9±1.41^a	41.1±1.20^a	40.1±2.20^a
MCV (fl)	55.97±1.83^a	58.63±1.01^a	59.73±3.16^a	59.17±1.25^a	59.40±0.86^a
MCHC(g/dL)	28.83±0.03^a	27.60±1.10^a	28.57±0.35^a	27.17±1.73^a	27.03±1.13^a
PLT (ul)	432.7±84^a	476.3±32.3^a	428.0±36.5^a	385.0±78.9^a	322.7±23.8^a
LYM (%)	91.23±1.52^a	92.73±0.43^a	92.93±0.23^a	90.33±2.22^a	85.90±1.65^a
PCT (%)	0.29±0.07^a	0.31±0.02^a	0.29±0.03^a	0.25±0.05^a	0.22±0.02^a
RDW-SD(Fl)	30.60±0.70^a	33.47±1.88^a	37.03±4.35^a	32.77±3.11^a	34.17±1.24^a
RDW-CV (%)	14.73±0.73^a	15.43±16.60^a	16.60±1.29^a	14.97±1.18^a	15.57±0.38^a
MPV (%)	6.7±0.00^a	6.7±0.1^a	7.0±0.1^a	6.8±0.3^a	6.9±0.1^a
PDW (%)	8.0±0.10^a	7.9±0.00^a	8.5±0.31^a	8.0±0.11^a	7.9±0.00^a

Key: WBC = White Blood Cells; RBC(Red Blood Cells); HGB (Haemoglobin); HCT(Hematocrit) (also called Packed Cell Volume, PCV); MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Haemoglobin); MCHC(Mean Corpuscular Haemoglobin Concentration); RDW-SD (Standard Deviation in Red Cell Distribution Width); RDW-CV (Coefficient of Variation in Red Cell Distribution Width); PDW (Platelet Distribution Width); MPV (Mean Platelet Volume); PLT (Platelet); PCT (Procalcitonin); LYM(Lymphocyte Count); pg(picograms). Values are represented as Mean ±SEM (n = 4). (P< 0.05) indicate statistically significant changes in comparison with the normal control

Table 3: Effect of Methanol Extracts of *L. Trifoliolata* Root on Haematological Parameters

Haematological Parameters	Normal Control	LT50(mg/kg)	LT300(mg/kg)	LT1000(mg/kg)	LT2000(mg/kg)
WBC($\times10^3$/uL)	9.0±1.30^a	6.5±0.90^a	9.3±1.50^a	8.6±0.50^a	6.4±0.10^a
RBC ($\times10^6$/uL)	6.75±0.20^a	5.58±0.86^a	6.11±0.50^a	7.02±0.03^a	7.16±0.03^a
HGB (g/dL)	10.93±0.72^a	8.87±1.10^a	10.17±1.07^a	11.30±0.25^a	11.63±0.83^a
HCT (%)	37.8±2.51^a	33.3±2.41^a	37.0±1.60^a	39.4±1.30^a	38.9±0.62^a
MCV(fl)	55.97±1.83^a	60.67±6.60^a	61.10±2.80^a	55.63±0.80^a	55.13±0.66^a
MCHC(g/dL)	28.83±0.03^a	26.37±1.79^a	27.33±1.64^a	28.90±0.21^a	29.40±1.17^a
PLT(ul)	432.7±84^a	444.0±38.8^a	397.0±90.7^a	441.7±22.4^a	521.3±36.8^a
LYM (%)	91.23±1.52^a	89.43±1.56^a	91.00±1.57^a	90.50±0.35^a	90.57±1.14^a
PCT (%)	0.29±0.07^a	0.31±0.01^a	0.27±0.07^a	0.30±0.02^a	0.40±0.04^b
RDW-SD(Fl)	30.60±0.70^a	29.90±2.10^a	35.60±4.32^a	29.90±1.21^a	29.90±1.21^a
RDW-CV (%)	14.73±0.73^a	14.80±1.4^a	15.80±1.34^a	14.40±0.46^a	14.43±0.49^a
MPV (%)	6.7±0.00^a	7.2±0.4^a	6.9±0.20^a	6.8±0.11^a	7.7±0.72^a
PDW (%)	8.0±0.10^a	8.5±0.60^a	8.6±0.71^a	8.0±0.12^a	8.6±0.71^a

Key: WBC = White Blood Cells; RBC(Red Blood Cells); HGB (Haemoglobin); HCT(Hematocrit) (also called Packed Cell Volume, PCV); MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Haemoglobin); MCHC(Mean Corpuscular Haemoglobin Concentration); RDW-SD (Standard Deviation in Red Cell Distribution Width); RDW-CV (Coefficient of Variation in Red Cell Distribution Width); PDW (Platelet Distribution Width); MPV (Mean Platelet Volume); PLT (Platelet); PCT (Procalcitonin); LYM(Lymphocyte Count); pg(picograms). Values are represented as mean ±SEM of triplicates (n = 4). (P< 0.05) indicate statistically significant changes in comparison with the normal control

Effect of Methanol Extracts of *U. Picta* Leaf and *L.Trifoliolata* Root on Oxidative Stress Markers in the Liver of Wistar Rats

The *in vivo* antioxidant activity of *U. picta* and *L. trifoliolata* in the liver is shown in Figure 3. *L. trifoliolata* treatment produced no significant changes ($p < 0.05$) in SOD, catalase, and GPx activities compared to the control group. However, MDA levels increased significantly at doses of 50 and 300 mg/kg, while GSH activity decreased significantly at doses of 300, 1000, and 2000 mg/kg. Other doses showed no significant differences compared to the control. Administration of *U. picta* resulted in significant increases ($p < 0.05$) in SOD and catalase activities at doses of 300, 1000, and 2000 mg/kg. GPx activity also increased significantly at doses of 300 and 1000 mg/kg. In contrast, the 50 mg/kg dose showed no significant difference compared to the control group.

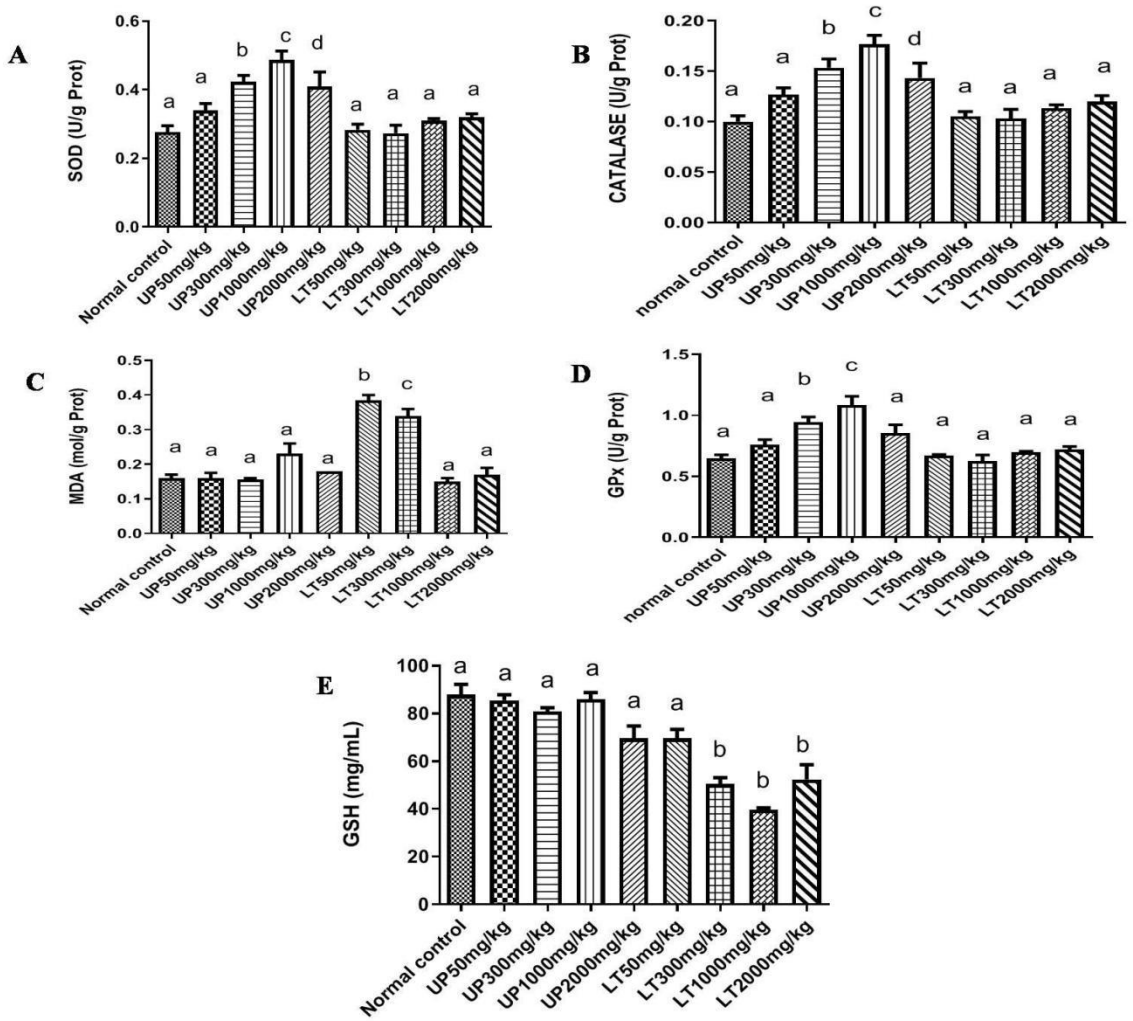

Figure 3: Effect of *Uraria picta* (leaf) (UP) and *Leptoderris trifoliolata* (root) (LT) on oxidative stress markers in rat liver. (A) Superoxide dismutase, (B) catalase, (C) malondialdehyde, (D) glutathione peroxidase and (E) glutathione reductase. Values are presented as mean ±SEM (n = 3). values with different alphabet indicate significant changes ($P < 0.05$) and values with similar alphabet indicate insignificant difference when compared to control.

Effect of Methanol Extracts of *U. Picta* Leaf and *L. Trifoliolata* Root on Oxidative Stress Markers in the Kidney of Wistar Rats

Figure 4 presents the effects of *Uraria picta* (UP) and *Leptoderris trifoliolata* (LT) on oxidative stress parameters in the kidney after treatment at different doses (50, 300, 1000, and 2000 mg/kg). The parameters assessed include superoxide dismutase (SOD), catalase, malondialdehyde (MDA), glutathione peroxidase (GPx), and reduced glutathione (GSH). Bars with different superscript letters indicate significant differences ($p<0.05$).

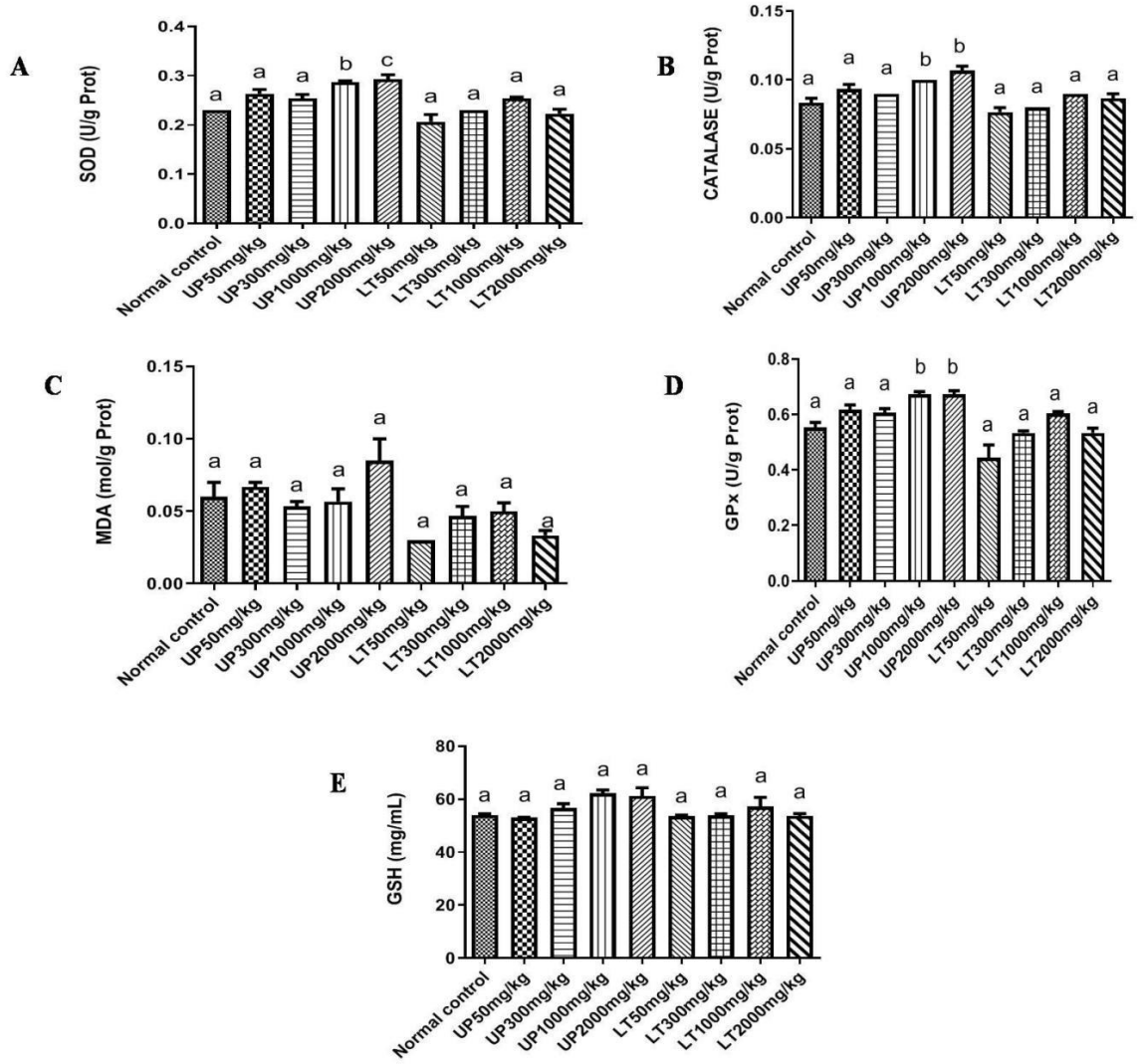

Figure 4: Effect of *U. picta* (leaf) (UP) and *L. trifoliolata* (root) (LT) on antioxidant parameters in rat kidney. (A) superoxide dismutase (SOD), (B) catalase, (C) malondialdehyde (MDA), (D)glutathione peroxidase (GPx) and (E) reduced glutathione (GSH). Values are presented as mean ±SEM (n = 3). Values with different alphabet indicate significant difference ($P < 0.05$) and values with similar alphabet indicate insignificant difference when compared to control

In the uterus, administration of *L. trifoliolata* produced no significant changes in catalase, MDA, GPx, and SOD activities, except for a significant decrease ($p < 0.05$) in SOD activity at 1000 mg/kg compared with the control. Conversely, treatment with *U. picta* caused a significant decrease ($p < 0.05$) in SOD activity at 300 and 2000 mg/kg, while catalase activity significantly increased at 300, 1000, and 2000 mg/kg. A significant increase ($p < 0.05$) in MDA levels was observed at 300 and 1000 mg/kg, indicating enhanced lipid peroxidation. GPx activity was also significantly elevated at 1000 and 2000 mg/kg. However, GSH activity significantly decreased ($p < 0.05$) across all tested doses (50, 300, 1000, and 2000 mg/kg) of *U.picta.*

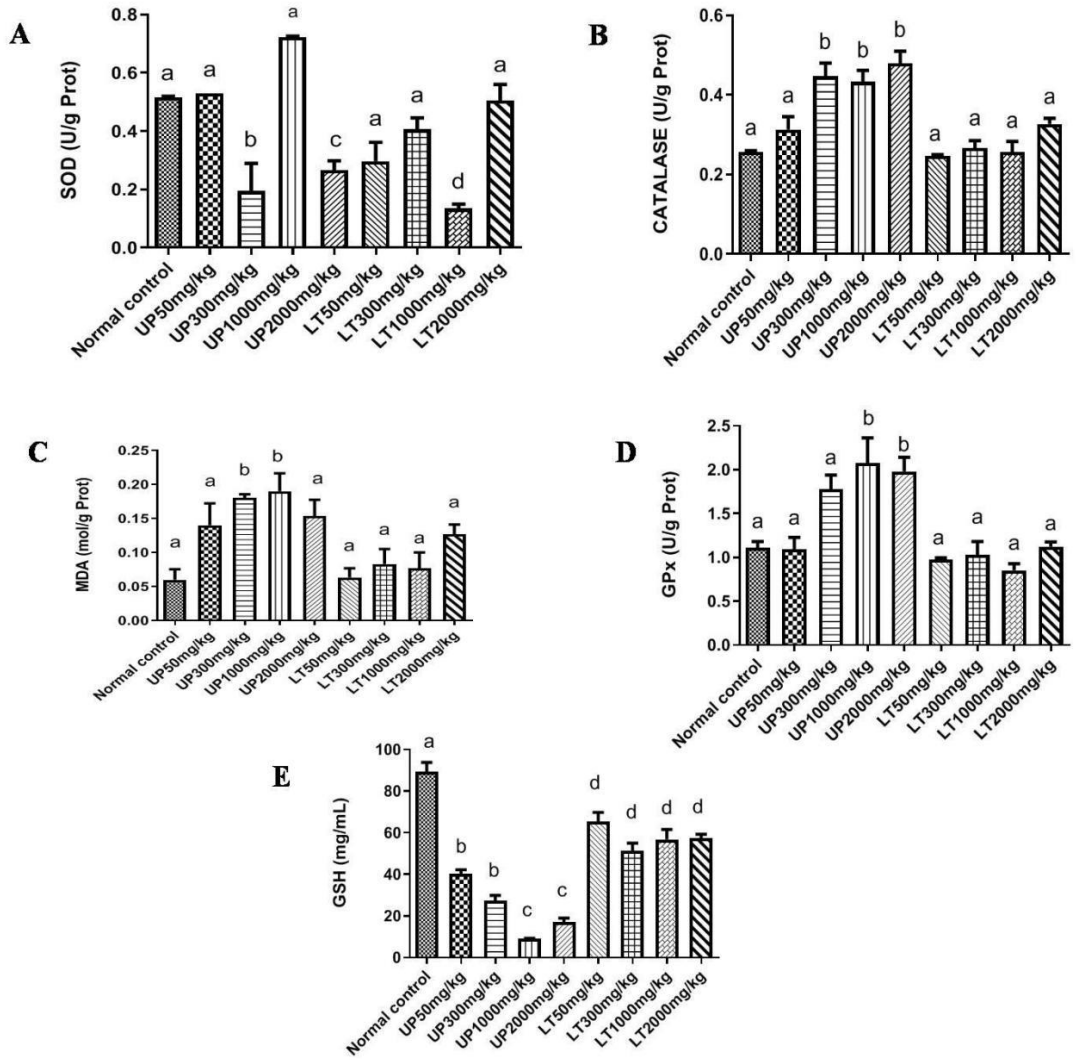

Figure 5: Effect of *Uraria picta* (leaf) (UP) and *Leptoderris trifoliolata* (root) (LT) on oxidative stress parameters in the uterus. (A) superoxide dismutase, (B) catalase, (C) malondialdehyde, (D)glutathione peroxidase and (E) glutathione reductase. Values are presented as mean ±SEM (n = 3). ($P < 0.05$) Values with different alphabet indicate significant difference ($P < 0.05$) and values with similar alphabet indicate insignificant difference when compared to control

Organ Function Test (Liver, Kidney)

The results (table 4) showed that administration of *U. picta* for 28 days caused significant increases ($p < 0.05$) in AST activity at 50 and 2000 mg/kg and ALT activity at 1000 and 2000 mg/kg, suggesting possible hepatic stress. A significant decrease in albumin levels was observed across all administered doses, while total bilirubin remained unchanged compared to the control. In contrast, *L. trifoliolata* produced no significant changes in AST and ALT activities at most doses, except for a significant increase in ALT at 2000 mg/kg. Significant increases in albumin and total bilirubin were observed at some doses, whereas direct bilirubin significantly decreased at 50 mg/kg.

Figure 6 revealed the effect of the extracts on kidney function parameters. Administration of *U. picta* caused no significant changes in urea levels and most creatinine concentrations, except for a significant increase in creatinine at 300 mg/kg. Significant reductions in serum sodium and chloride levels were observed at 50, 300, and 2000 mg/kg, while potassium significantly increased at 50 mg/kg. Conversely, *L. trifoliolata* showed no significant alterations in urea, creatinine, sodium, or chloride concentrations, although potassium levels significantly increased at 50, 300, and 2000 mg/kg.

Table 4: Effect of *Uraria Picta* (Leaf) (UP) and *Leptoderris Trifoliolata* (Root) (LT) on Liver Function Parameters

Parameters	AST (U/L)	ALT (U/L)	Albumin (g/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Total Protein(mg/dL)
Normal Control	12.00±0.00^{a}	12.00±0.00^{a}	3.23±0.44^{a}	0.73±0.03^{a}	0.32±0.01^{a}	2.80±0.08^{a}
UP50(mg/kg)	18.33±1.33^{b}	15.33±1.67^{a}	1.20±0.04^{b}	0.97±0.02^{a}	0.30±0.01^{a}	2.78±0.19^{a}
UP300(mg/kg)	6.67±0.67^{c}	13.67±1.67^{a}	0.90±0.46^{c}	0.63±0.10^{a}	0.46±0.04^{a}	4.56±0.25^{b}
UP1000(mg/kg)	12.00±0.00^{a}	21.00±0.00^{b}	1.10±0.36^{b}	0.56±0.04^{a}	0.63±0.02^{b}	3.98±0.08^{b}

Parameters	AST (U/L)	ALT (U/L)	Albumin (g/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Total Protein(mg/dL)
UP2000(mg/kg)	17.33±0.33^{b}	17.00±0.00^{b}	1.74±0.36^{b}	0.46±0.04^{b}	0.61±0.03^{b}	3.19±0.15^{b}
LT50(mg/kg)	13.67±1.67^{a}	12.00±0.00^{a}	0.78±0.22^{c}	0.88±0.04^{a}	0.08±0.00^{c}	1.92±0.07^{a}
LT300(mg/kg)	12.00±0.00^{a}	12.00±0.00^{a}	2.04±0.11^{b}	0.85±0.27^{a}	0.20±0.02^{a}	2.71±0.16^{a}
LT1000(mg/kg)	12.00±0.00^{a}	12.00±0.00^{a}	2.57±0.59^{b}	0.76±0.14^{a}	0.22±0.02^{a}	3.45±0.80^{b}
LT2000(mg/kg)	12.00±0.00^{a}	25.00±0.00^{b}	3.16±0.89^{b}	1.29±0.10^{b}	0.36±0.08^{a}	3.31±0.19^{b}

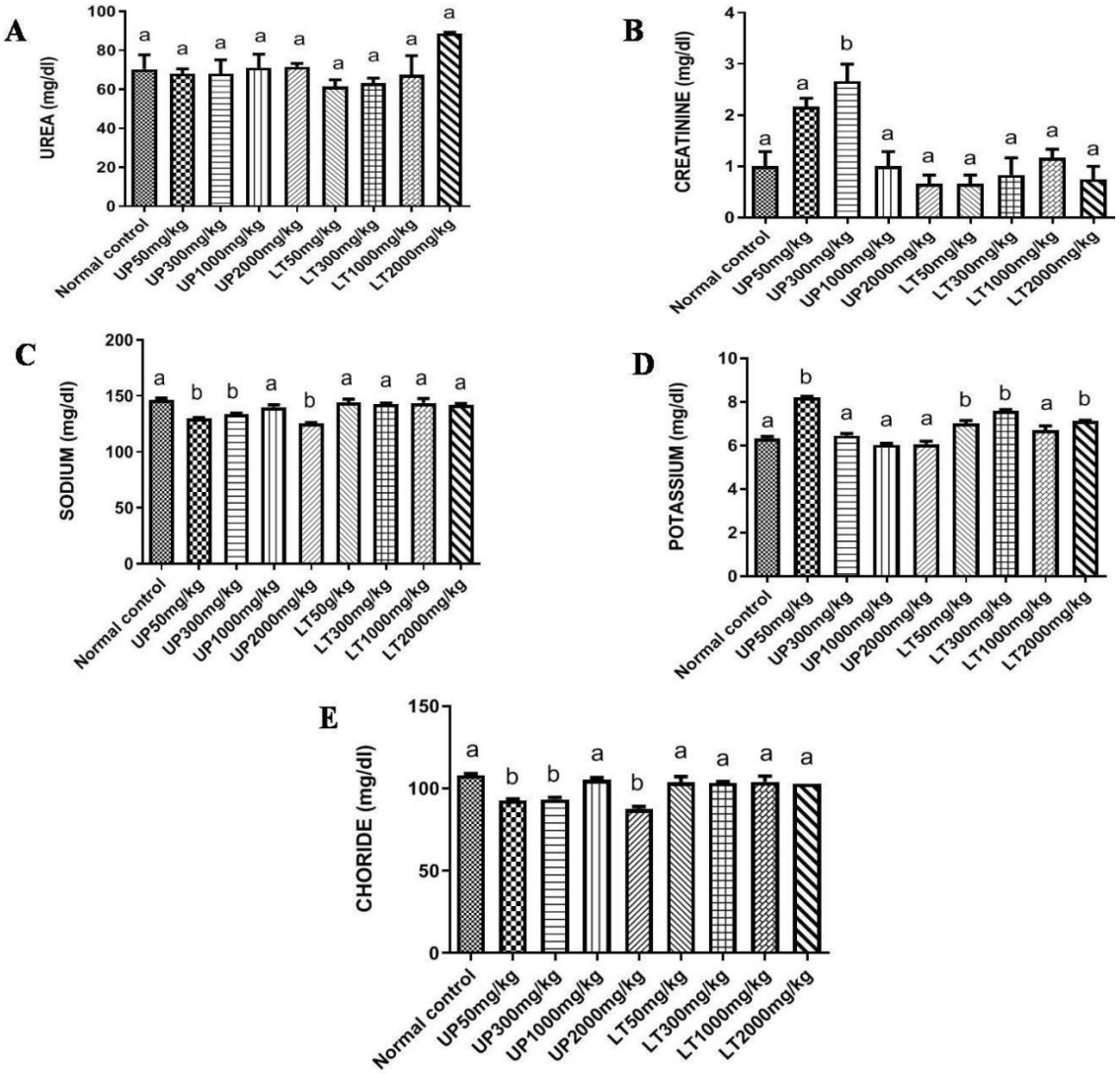

Figure 6: Effect of methanol extracts of *Uraria picta* (leaf) (UP) and *Leptoderris trifoliolata* (root) (LT) on kidney function parameters for 28 days (A) urea, (B) creatinine, (C) sodium, (D) potassium and (E) chloride. Values are presented as mean ±SEM (n = 4). (P < 0.05) values with different alphabet indicate significant changes in comparison with the normal control, and values with similar alphabet indicate insignificant difference when compared to control

Lipid Profile of Rats Exposed to Methanol Extracts of *U. Picta* Leaf and *L. Trifoliolata* Root for 28-Days

The administration of *U. picta* caused a significant increase (p<0.05) in Total cholesterol at 300mg/kg, and LDL-Cholesterol concentration at 50, 300 and 2000mg/kg. While a significant decrease (p<0.05) in triglyceride concentration (50, 300 and 2000mg.kg) and HDL-Cholesterol (50mg/kg) was observed. The administration of *L. trifoliolata* caused a significant decrease (p<0.05) in total cholesterol (50, 300 and 2000mg/kg), triglycerides (50, 300, 1000 and 2000mg/kg), HDL-Cholesterol (50 and 300mg/kg) and LDL-Cholesterol (50mg/kg) concentration when compared to the control. However, no significant difference (p<0.05) was observed in HDL-Cholesterol (1000 and 2000mg/kg) and LDL-cholesterol (50 and 2000mg/kg) concentration.

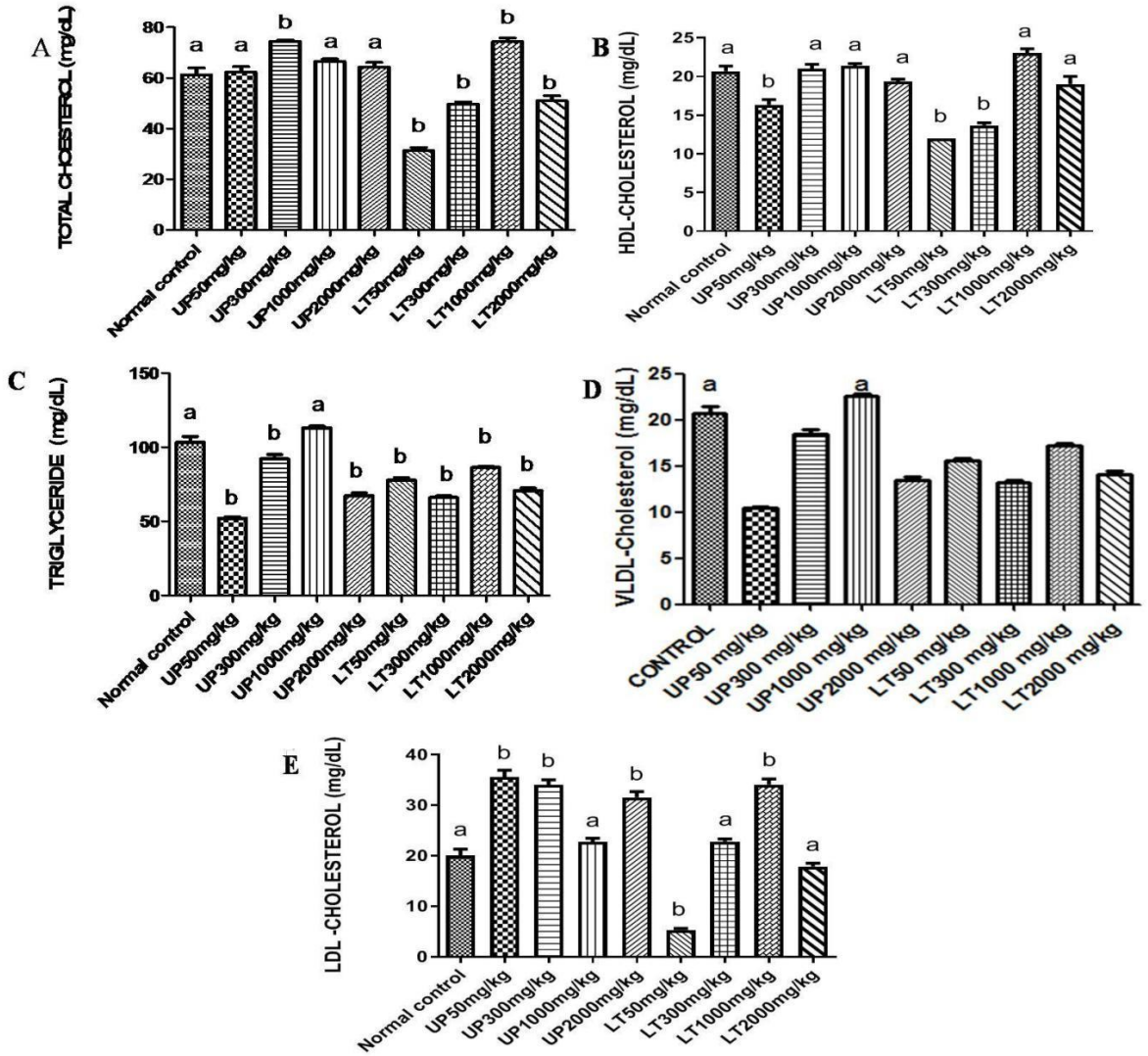

Figure 7: Effect of 28-day exposure to methanol extracts of *Uraria picta* (leaf) (UP) and *Leptoderris trifoliolata* (root) (LT) on lipid profile of rats. (A)Total-cholesterol (B) Triglyceride (C) HDL-Cholesterol, (D) VLDL-Cholesterol and (E) LDL-Cholesterol. Values are represented as mean ±SEM (n = 4). ($P < 0.05$) values with different alphabet indicate significant difference in comparison with the normal control, and values with similar alphabet indicate insignificant difference when compared to control

Histopathological Examination of Rat Liver, Kidney and Uterus Tissues exposed to Methanol Extracts of *U. Picta* Leaf and *L. Trifoliolata* Root

Liver

The effect of the administration of *U. picta and L. trifoliolata* extracts on the section of the liver tissue as shown in plate A-I show hepatocytes (arrow) with centrally placed normochromic to vesicular nuclei surrounded by eosinophilic cytoplasm. These cells are separated by a fibroconnective tissue stroma. Generally, the liver tissue maintained a normal architecture in comparison to the control.

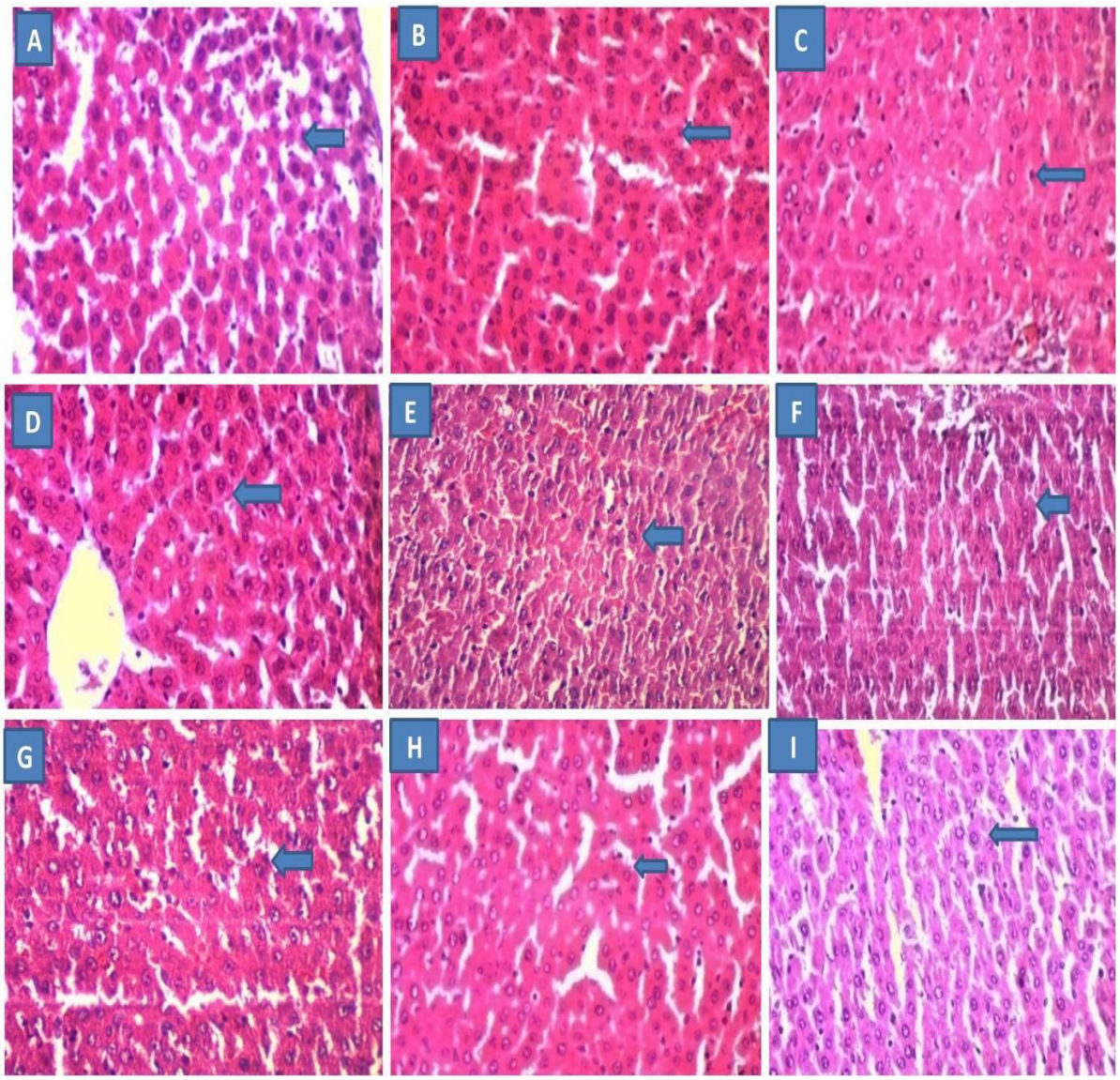

Figure 8: Photomicrograph sections of the liver tissues. (A) The control showed normal architecture with normal hepatocytes, centrally placed normochromic to vesicular nuclei surrounded by eosinophilic cytoplasm. These cells are separated by a fibroconnective tissue stroma. (B-I) Liver tissues from rats administered (50mg/kg, 300mg/kg, 1000mg/kg, and 2000mg/kg *U. picta* and *L. trifoliolata* extracts) respectively shows same features in comparison with the control. Photomicrographs were taken at 400x magnification using light Microscope, and 5 µm thick paraffin sections. Staining was done with Haematoxylin and Eosin stain.

Kidney

The effect of the administration of *U. picta and L. trifoliolata* extracts on the kidney as shown in plate 2 (A-I) revealed that the kidney contained normal glomeruli (thick arrow) and normal mesangial cells. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Also present are normal blood vessels. Generally, the kidney tissue maintained a normal architecture in comparison to the control.

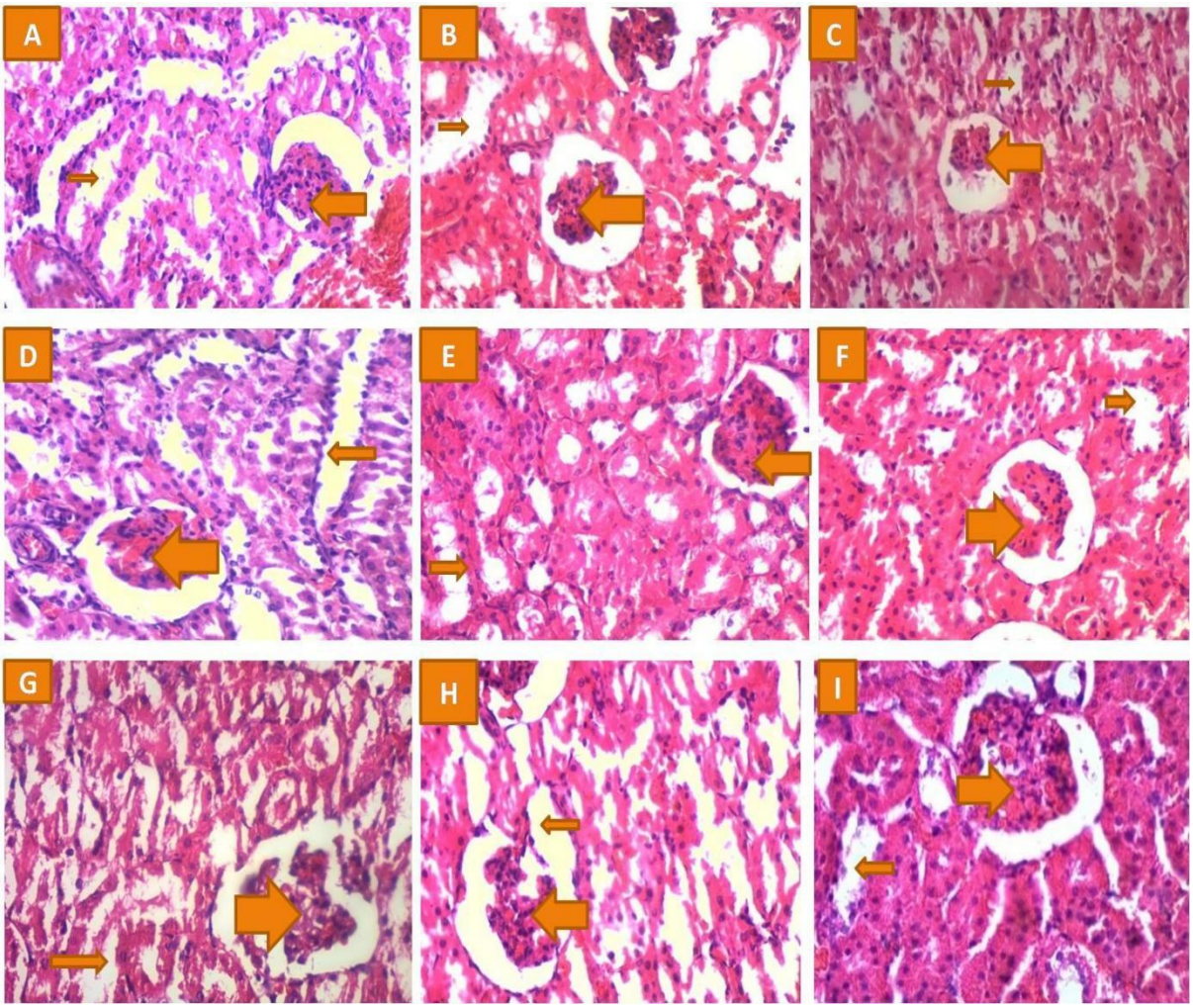

Figure 9: Photomicrograph sections of the kidney tissues of female Wistar rats. (A) the control showed a normal architecture of the kidney; normal glomeruli (thick arrow) containing normal mesangial cells. The tubules are oval shaped and lined by cuboidal epithelium (thin arrow) with some tubules containing pale eosinophilic material. Also present are normal blood vessels**.** (B-I) Kidney tissues from rats administered (50mg/kg, 300mg/kg, 1000mg/kg, and 2000mg/kg *U. picta* and *L. trifoliolata* extracts) respectively shows same features in comparison to the normal. Photomicrographs were taken at 400x magnification using light Microscope, and 5 µm thick paraffin sections with Haematoxylin and Eosin stain.

Uterus

The effect of the administration of *U. picta and L. trifoliolata* extracts on the uterus as shown in plate 3 shows a normal endometrial lining (thick arrow) composed of columnar epithelium and also some variably sized endometrial glands lined by columnar epithelium. The myometrium is normal (thin arrow). The Features observed are characteristics of a normal uterus.

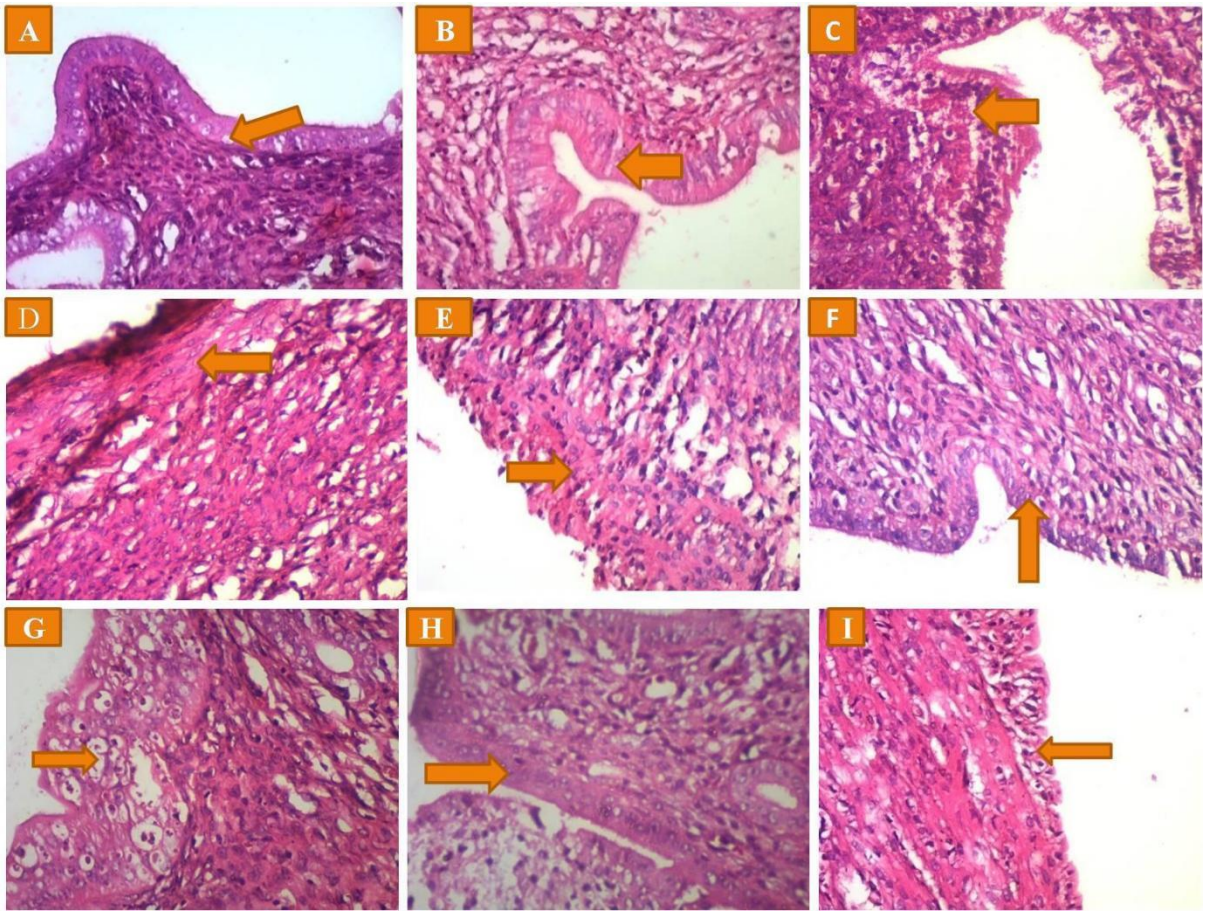

Figure 10: Photomicrograph sections of the uterus of female rats. (A) Uterus tissues from control rats had normal architecture showing a normal endometrial lining composed of columnar epithelium, also some variably sized endometrial glands lined by columnar epithelium and a normal myometrium**.** (B-E) Uterus tissues from rats administered (50 mg/kg, 300 mg/kg, 1000 mg/kg, and 2000 mg/kg *U. picta* and *L. trifoliolata* extracts) respectively shows normal architecture of the uterus in comparison with the control. Photomicrographs were taken at 400x magnification using light Microscope, and 5 µm thick paraffin sections. Staining was done using Haematoxylin and Eosin stain.

Discussion

This study provides a comprehensive toxicological evaluation of methanol extracts of *Uraria picta* and *Leptoderris trifoliolata*, integrating acute and sub-chronic systemic endpoints with biochemical, haematological, oxidative stress, and histopathological assessments. Collectively, the findings indicate that both extracts exhibit a wide margin of acute safety, while inducing mild, dose-dependent and organ-specific biochemical perturbations under repeated exposure, without corresponding structural tissue damage. The absence of mortality or overt neurobehavioural toxicity following administration of both extracts up to 5000 mg/kg suggests a low acute toxicity profile. In accordance with OECD guideline 423, substances with LD_{50} values above 5000 mg/kg are classified as practically non-toxic or of low toxicological concern (OECD, 2008). The lack of autonomic and behavioural toxicity indicators further supports minimal acute systemic or neurotoxic effects. These findings are consistent with reports on several Fabaceae-derived medicinal plants, which often demonstrate high acute safety margins attributed to polyphenol-rich phytochemical profiles (Ekor, 2014; Zhang *et al*., 2018). However, acute safety does not preclude sub-chronic biochemical or organ-specific effects, underscoring the importance of repeated-dose evaluation. Transient body weight reduction observed following acute administration of *L. trifoliolata* likely reflects short-term metabolic adaptation or transient perturbation of nutrient utilization. Body weight is a sensitive systemic toxicity marker, as xenobiotics may influence appetite regulation, nutrient absorption, or basal metabolic rate (Akhila *et al*., 2007). Notably, sustained administration over 28 days resulted in progressive weight gain across treated groups, suggesting physiological adaptation rather than progressive toxicity. Similar adaptive responses have been reported in repeated-dose studies of phytochemical-rich plant extracts, where initial metabolic stress is followed by restoration of homeostasis and improved growth indices (El Kabbaoui *et al.*, 2017). Importantly, this should not be interpreted as anabolic activity in the absence of metabolic flux or endocrine profiling. The stability of erythrocytic indices (RBC, Hb, HCT) and most leukocyte parameters indicates that neither extract exerted significant suppressive or stimulatory effects on haematopoiesis. This suggests preserved bone marrow function and red blood cell membrane integrity during exposure. Flavonoid-rich plant extracts have been reported to stabilize erythrocyte membranes and mitigate oxidative haemolysis through antioxidant mechanisms (Olorunnisola *et al.*, 2012). Given the sensitivity of the haematopoietic system to xenobiotic injury, the absence of marked alterations supports a low hematotoxic risk profile (Adebayo *et al*., 2005). An insignificant reduction ($p>0.05$) in WBC counts in some *U. picta* groups may reflect mild immunomodulation rather than immunotoxicity. Polyphenolic compounds are known to modulate cytokine signalling and leukocyte activity

through redox-sensitive inflammatory pathways (Ekor, 2014). In contrast, the observed insignificant increase ($p>0.05$) in platelet indices in *L. trifoliolata* treated groups may indicate subtle modulation of platelet turnover or thrombopoietic activity. Platelet parameters such as MPV and PCT are increasingly recognized as dynamic indicators of bone marrow platelet output (Korniluk *et al*., 2019). However, in the absence of accompanying pathological or inflammatory changes, this is more consistent with adaptive physiological modulation than pathological thrombocytosis. Oxidative stress represents a central mechanism of xenobiotic-induced tissue dysfunction, particularly in metabolically active organs such as the liver and kidney (Ighodaro and Akinloye, 2018). In this study, *U. picta* enhanced antioxidant enzyme activities (SOD, CAT, GPx), suggesting reinforcement of endogenous redox defense systems. This may involve activation of redox-sensitive pathways such as Nrf2-mediated transcription; however, this remains inferential in the absence of molecular validation (Bellezza *et al.,* 2018). Conversely, *L. trifoliolata* induced increased malondialdehyde (MDA) levels and reduced glutathione (GSH) at selected doses, indicating mild lipid peroxidation and attenuation of intracellular antioxidant buffering capacity. MDA elevation reflects membrane oxidative damage, while GSH depletion indicates compromised thiol-dependent detoxification systems (Ayala *et al.,* 2014). These findings align with the recognized biphasic nature of polyphenolic compounds, which may exert antioxidant or pro-oxidant effects depending on concentration, redox environment, and tissue context (Bouayed and Bohn, 2010; Zhang *et al*., 2018). The observed compensatory upregulation of catalase and GPx in some tissues further suggests an adaptive response to oxidative challenge rather than irreversible oxidative injury. Reproductive tissues appeared particularly sensitive, as evidenced by altered uterine oxidative markers. This is biologically plausible given the high mitochondrial activity and hormonal responsiveness of reproductive organs, which makes them vulnerable to redox imbalance (Agarwal *et al*., 2012). Importantly, oxidative perturbations did not translate into histological abnormalities, suggesting subclinical oxidative stress without structural degeneration. Dose-dependent increases in serum AST and ALT, particularly in *U. picta* treated groups, suggest mild hepatocellular stress. ALT elevation is generally more indicative of hepatocellular integrity disruption due to its cytosolic localization in hepatocytes (Ozer *et al*., 2008). Reduced serum albumin further suggests subtle impairment in hepatic synthetic capacity. However, the absence of marked bilirubin elevation and the preservation of normal histoarchitecture indicate that these biochemical changes likely reflect early functional perturbations rather than overt hepatotoxicity. Such dissociation between biochemical and histological findings is well documented in sub-chronic toxicity models, where serum biomarkers detect early hepatocellular stress preceding structural injury (El Kabbaoui *et al.,* 2017). Renal function markers (urea and creatinine) remained largely unchanged, indicating preserved glomerular filtration. Mild alterations in serum electrolytes suggest possible modulation of tubular ion transport processes or hormonal regulation of renal homeostasis. Similar electrolyte fluctuations without structural kidney damage have been reported in studies involving plant-derived bioactive compounds with mild diuretic or nephroactive properties (Kamel and Halperin, 2017; Nwidu *et al*., 2018). The extracts exhibited divergent effects on lipid metabolism. *U. picta* increased LDL-cholesterol and total cholesterol at selected doses, which may indicate a potential atherogenic risk if sustained, given the established role of LDL in endothelial dysfunction and atherogenesis (Libby, 2021). In contrast, *L. trifoliolata* demonstrated more consistent lipid-lowering effects, reducing total cholesterol and triglycerides. Such hypolipidaemic activity is consistent with flavonoid- and saponin-rich plant extracts that modulate lipid metabolism through inhibition of cholesterol biosynthesis, enhanced bile acid excretion, and reduced intestinal lipid absorption (Nazaruk and Borzym-Kluczyk, 2015). These contrasting lipid profiles highlight species-specific phytochemical effects and underscore the importance of chemical characterization for mechanistic interpretation. Despite biochemical and oxidative alterations, histopathological examination of liver, kidney, and uterine tissues revealed preserved architecture without evidence of necrosis, inflammation, fibrosis, or cellular degeneration. This indicates that functional biochemical disturbances did not progress to structural organ damage within the study period. Such divergence between biochemical and histological findings is common in sub-chronic toxicology, reflecting the higher sensitivity of serum biomarkers to early cellular stress compared with morphological endpoints (El Kabbaoui *et al.,* 2017). This reinforces the interpretation that observed changes represent early or reversible physiological responses rather than irreversible toxicity.

CONCLUSION

Methanol extracts of *Uraria picta* and *Leptoderris trifoliolata* showed high acute safety with no toxicity up to 5000 mg/kg. However, sub-chronic exposure caused mild, dose-dependent biochemical and oxidative changes without corresponding tissue damage, suggesting adaptive rather than toxic effects. *U. picta* enhanced antioxidant activity but showed mild liver and uterine stress at higher doses, while *L. trifoliolata* maintained stable haematological and renal functions with mixed oxidative and lipid-modulating effects. Overall, both extracts appear relatively safe under short- to mid-term exposure, though further detailed and long-term studies are needed to confirm their safety and mechanisms.

ACKNOWLEDGMENT

This research was funded by TETFUND Institution Based Research Fund 2024/2025 Batch 20 with reference code TETF/DR&D/CE/UNI/BENIN/IBR/2024/VOL.I

REFERENCES

Adebayo, A. H., Abolaji, A. O., Kela, R., Ayepola, O. O., Olorunfemi, T. B., & Taiwo, O. S. (2005). Antioxidant activities of the leaves of Chrysophyllum albidum. Pakistan *Journal of Biological Sciences*, 8(3), 428–431.

Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A., & Gupta, S. (2012). The effects of oxidative stress on female reproduction: *A review. Reproductive Biology and Endocrinology*, 10, 49.

Akhila, J. S., Deepa, S., & Alwar, M. C. (2007). Acute toxicity studies and determination of median lethal dose. *Current Science*, 93(7), 917–920.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, 2014, 1–31.

Bellezza, I., Giambanco, I., Minelli, A., & Donato, R. (2018). Nrf2-Keap1 signaling in oxidative and reductive stress.

Biochimica et Biophysica Acta (*BBA) - Molecular Cell Research*, 1865(5), 721–733.

Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state. *Oxidative Medicine and Cellular Longevity*, 3(4), 228–237.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, 52, 302–310.

Cohen, G., Dembiec, D., & Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, 34, 30–38.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 177.

El Kabbaoui, M., Chda, A., El-Akhal, J., Azdad, O., & Mejrhit, N. (2017). Evaluation of the acute and subchronic toxicity of herbal medicine in rodents. *Journal of Toxicology*, 2017, 1–9.

Ertekin, V., Selimoglu, M., & Altinkaynak, S. A. (2005). Combination of unusual presentations of Datura stramonium intoxication in a child: Rhabdomyolysis and fulminant hepatitis. *Journal of Emergency Medicine*, 28, 227–228.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative centrifuge. *Clinical Chemistry*, 18(6), 499–502. https://doi.org/10.1093/clinchem/18.6.499

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants—Superoxide dismutase, catalase and glutathione peroxidase: Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, 54(4), 287–293.

Kale, R. H., Halde, U. K., & Biyani, K. R. (2012). Protective effect of aqueous extract of *Uraria picta* on acetaminophen-induced nephrotoxicity in rats. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *3*(1), 110–113.

Kamel, K. S., & Halperin, M. L. (2017). Fluid, electrolyte, and acid-base physiology (5th ed.). *Elsevier.*

Koduru, S., Grierson, D., & Afolayan, A. (2006). Antimicrobial activity of Solanum aculeastrum. *Pharmaceutical Biology*, 44(3), 283–286.

Korniluk, A., Koper-Lenkiewicz, O. M., Kamińska, J., Kemona, H., & Dymicka-Piekarska, V. (2019). Mean platelet volume (MPV): New perspectives for an old marker in the course and prognosis of inflammatory conditions. *Mediators of Inflammation*, 2019, 1–14.

Kritika, H., Narendra, K. S., & Manoj, K. S. (2017). Anti-inflammatory and hepatoprotective activities of the roots of Uraria picta. *International Journal of Green Pharmacy*, 11(1), S166–S170.

Kunal, N., & Krishnamurthy, R. (2018). Anti-inflammatory activity of methanolic extracts of Pseudarthria viscida and Uraria picta against carrageenan induced paw edema in albino rat. *Open Access Journal of Medicinal and Aromatic Plants*, 9(1), 1–11.

Kwan, Y. P., Ibrahim, D., Yeng, C., Subramaniam, S., & Sreenivasan, S. (2013). Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. *BioMed Research International*. https://doi.org/10.1155/2013/182064

Libby, P. (2021). The changing landscape of atherosclerosis. Nature, 592, 524–533.

Lopez-Virella, M. F., Stone, P., Ellis, S., & Colwell, J. A. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry*, 23(7), 882–884. https://doi.org/10.1093/clinchem/23.7.882

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10), 3170–3175.

Nazaruk, J., & Borzym-Kluczyk, M. (2015). The role of triterpenes in the management of diabetes mellitus and its complications. *Phytochemistry Reviews*, 14, 675–690.

Nwidu, L. L., Elmorsy, E., Aprioku, J. S., Siminialayi, I., & Carter, W. G. (2018). Evaluation of the toxicity profile of herbal products. *Journal of Integrative Medicine*, 16(4), 245–252.

Olaleye, O. O., Kim, D. H., & Spriggs, K. A. (2024). Antiproliferative activities of some selected Nigerian medicinal plants against breast, liver, and cervical cancer cells. *BMC Complementary Medicine and Therapies*, 24, 110.

Olorunnisola, O. S., Bradley, G., & Afolayan, A. J. (2012). Protective effect of Tulbaghia violacea rhizome extract against hypercholesterolemia-induced oxidative stress. *Molecules*, 17(5), 6033–6045.

Owolabi, M. S., Ogundajo, A., Ndukwe, N., Dosoky, N. S., & Setzer, W. N. (2020). Antimicrobial activities and chemical compositions of Daniellia oliveri and Leptoderris micrantha essential oils from Nigeria. *Journal of Evidence-Based Integrative Medicine*, 25.

Ozer, J., Ratner, M., Shaw, M., Bailey, W., & Schomaker, S. (2008). The current state of serum biomarkers of hepatotoxicity. *Toxicology*, 245(3), 194–205.

Rahman, M. M., Gibbons, S., & Gray, A. I. (2007). Isoflavanones from Uraria picta and their antimicrobial activity. *Phytochemistry*, 68, 1692–1697.

Reitman, S., & Frankel, S. (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *American Journal of Clinical Pathology*, 28, 56–58.

Richmond, W. (1973). Cholesterol enzymatic colorimetric test (CHOD-PAP method). *Clinical Chemistry*, 19(12), 1350–1356. https://doi.org/10.1093/clinchem/19.12.1350

Saxena, H. O., Soni, A., Mohammad, N., & Choubey, S. K. (2014). Phytochemical screening and elemental analysis in

different plant parts of Uraria picta. *Journal of Chemical and Pharmaceutical Research*, 6(5), 756–760.

Tilahun, A., Dinkenesh, C., Netsanet, F., Dawit, S., Tesfaye, S., Asfaw, D., & Beyene, P. (2020). Evaluation of acute and sub-acute toxicity of selected traditional antiurolithiatic medicinal plant extracts in Wistar albino rats. *Toxicology Reports*, 7, 1356–1365.

Trinder, P. (1969). A colorimetric method of determination of serum and plasma cholesterol. *Annals of Clinical Biochemistry*, 6, 24–27. https://doi.org/10.1093/clinchem/6.1.24

Zhang, J., Onakpoya, I. J., Posadzki, P., & Eddouks, M. (2018). The safety of herbal medicine: From prejudice to evidence. *Evidence-Based Complementary and Alternative Medicine.*