UNLOCKING SAFE REMEDIES: Preclinical Toxicological Evaluation of the Hydroethanolic Extract of Spondias mombin in Rodents

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Research Article

Keywords: Spondias mombin, hydroethanolic extract, oral toxicity, preclinical safety, hematology, immunomodulation

Posted Date: February 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8941887/v1

Additional Declarations: The authors declare no competing interests.

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ABSTRACT:

Background: Toxicity accounts for nearly one-third of candidate drug attrition, underscoring the need for robust preclinical toxicological evaluation early in the drug development pipeline. To the best of our knowledge, this is the first comprehensive toxicological profiling of *Spondias mombin* hydroethanolic leaf extract in rodent models in Nigeria. Traditionally used for the treatment of dysentery, gonorrhea, wounds, and anemia, *S. mombin* hydroethanolic extract warrants systematic evaluation to support its safe therapeutic use and guide the optimization of lead compounds.

Methods: Acute toxicity was assessed in mice using a limit test following a single oral dose and a 14-day observation period. Subacute and subchronic toxicity were evaluated in female Wistar rats (n = 6/group and n=10/group) administered 40, 200, or 1000 mg/kg/day of extract for 28 and 60 days, respectively. A control group received distilled water. Hematological and biochemical analyses were conducted post-treatment before humane euthanasia.

Results: No mortality or observable signs of toxicity were recorded at doses up to 2000 mg/kg. The extract significantly reduced white blood cell count, absolute lymphocytes, and total bilirubin (WBC: $5.60 \times 10^3/\mu L$ vs. $9.35 \times 10^3/\mu L$; lymphocytes: $2.08 \times 10^3/\mu L$ vs. $4.28 \times 10^3/\mu L$; bilirubin: 1.99 µmol/L vs. 2.39 µmol/L). *S.*

mombin extract also exhibited dose-dependent increases in red blood cell and hemoglobin counts, alongside reductions in AST, ALT, and ALP levels in the subacute phase.

In the subchronic study, *S. mombin* significantly reduced total cholesterol (40 mg/kg: 1.96 mmol/L vs. control: 2.85 mmol/L) and bilirubin (1000 mg/kg: 10.69 µmol/L vs. control: 22.58 µmol/L).

Conclusion: The validated safety profile of *S. mombin* hydroethanolic leaf extract supports its continued ethnomedicinal use and highlights its promise as a candidate for further pharmacological development—particularly as a potential hematinic, anti-infective, and hepatoprotective agent.

Keywords: *Spondias mombin*; hydroethanolic extract; oral toxicity; preclinical safety; hematology; immunomodulation

INTRODUCTION

BACKGROUND

Recent data from the U.S. FDA (2024) Drug Therapy Approvals (Figure 1) report highlights a consistent trend [1]. While the number of approved novel drugs per year remains stable, the cost of developing these drugs continues to rise significantly, with recent estimates ranging from $314 million to $2.8 billion [2]. An estimated average of 10,000 - 25,000 individual drug candidates are considered in the course of development of a new drug; however, toxicity has been reported to be responsible for the attrition of ~ 1/3 of drug candidates and is thus a major contributor to the high cost of drug development, particularly when not recognized until late in the clinical trials or post-marketing [3]. During the last century, the majority of drugs voluntarily withdrawn from the market or prohibited by regulatory agencies (Figure 2) were similarly reported to be related to adverse drug reactions, also known as toxic effects [4]. Understanding the underlying mechanisms of toxicity of natural products and potential drug candidates is therefore of utmost importance for current and future drug discovery [4].

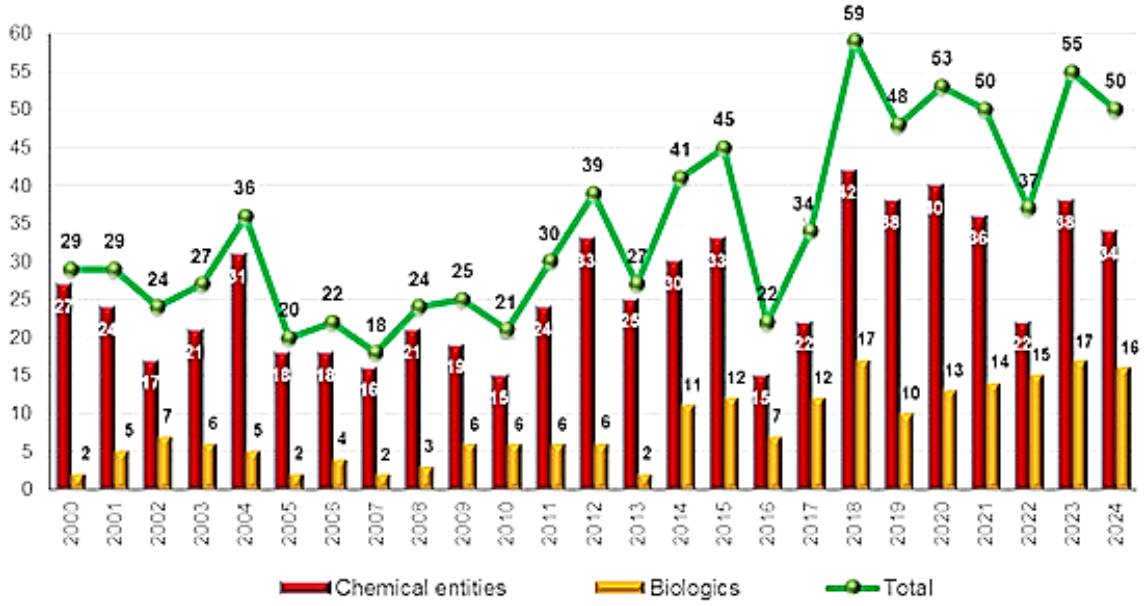

Figure 1. Drugs (new chemical entities and biologics) approved by the FDA in the last 25 years [5].

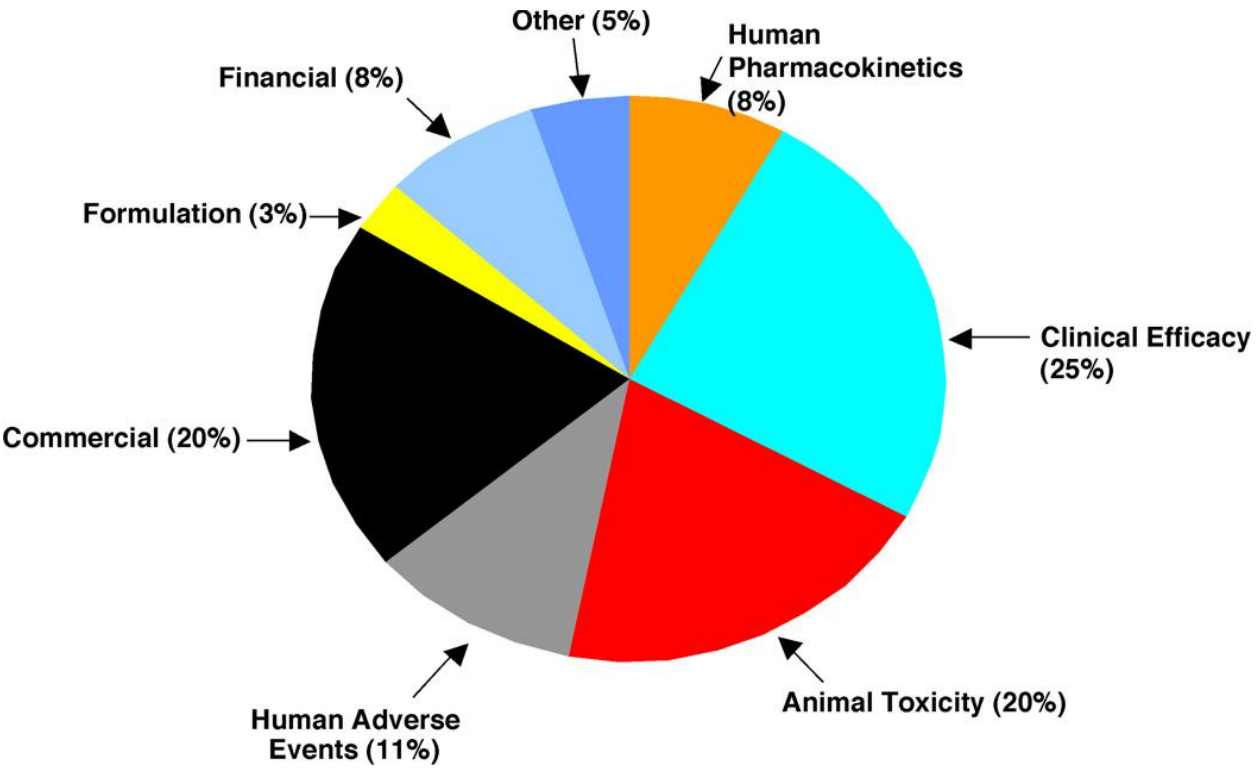

Figure 2: Reasons for Attrition of Drug Candidates in Pre-Clinical and Clinical Development (*ca. 2000*) [3].

Toxicity refers to the adverse effects of a substance on a living organism which is defined with reference to the quantity of substance administered or absorbed, how the substance is administered (inhalation, ingestion, topical application, injection) and distributed in time (single or repeated doses), the type and severity of injury, the time needed to produce the injury, the nature of the organism(s) affected, and other relevant conditions [6]. Toxicity is dependent on the chemical structure of a substance, its pharmacokinetics, and its metabolic reaction pathways, with the magnitude of the substance's toxic effect usually dose-dependent [7]. In contrast to side effects, which are primarily non-deleterious, [for example, dry mouth]; toxic effects are the undesirable results of a direct use of a substance on a living organism [6]. Current measures of *in vitro* toxicity assessments are based on acute, subacute, subchronic, and chronic experiments [6]. In 1985, Bruce developed an up-and-down procedure (UDP) for the determination of the acute toxicity of chemicals [8]. This procedure was subsequently adopted by the American Society for Testing and Materials (ASTM) in 1987 and the Organization for Economic Development (OECD) in 2001, respectively [8].

The acute toxicity testing procedure involves a limit test, which is primarily used in situations where the information indicating that the test material is likely to be nontoxic is available. In the limit test, an animal is given a starting test dose and observed. If the animal survives, four additional animals are sequentially dosed so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. For this test, the LD50 is greater than 2000 mg/kg if three or more animals survive. Additionally, testing in one sex (usually females) is generally considered sufficient for the acute toxicity testing [8].

In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute toxicity testing; such testing is referred to as subacute, subchronic, and chronic toxicity assessments, depending on the number of days the organism is exposed to the substance [6,9].

This toxicological evaluation provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on the nervous, immune, and endocrine systems[9]. The subacute and subchronic toxicity testing involves daily administration of graduated doses of the substance to several groups of experimental animals, one dose level per group. During the period of administration, the animals are observed closely, each day, for signs of toxicity and body fluids and organs are tested for biochemical and hematological toxicities at the end of the administration period [9,10]. Additionally, the preferred species for this assessment is the rat, although other rodent species (e.g., the mouse) may be used [9,10].

Given these regulatory standards and attrition risks, early-stage toxicity screening of natural products is crucial, particularly those with existing ethnomedical relevance. Natural products continue to serve as a vital starting point for the development of modern drugs [5]. In many regions of Africa, tropical South America, and Asia, the use of medicinal plants is primarily based on knowledge passed down through generations [11–13]. Fortunately, such traditional explorations have led to the continuous discovery of valuable drug leads from natural products [14]. For instance, in cancer research, between the 1940s and the end of 2014, out of 175 approved small-molecule drugs, 75% were not purely synthetic—over 49% were either natural products or direct derivatives, predominantly from plants [15]. Although medicinal plants have demonstrated significant therapeutic potential in treating various ailments [16,17] Research on them often ends at documenting ethnobotanical uses, culinary relevance, or economic value. This limits deeper investigation into mechanisms of action, compound characterization, toxicity profiling, and drug discovery [12,14]. Contemporary research on medicinal plants often remains focused on descriptive ethnobotanical and pharmacological screenings, without advancing toward mechanistic, toxicological, or translational studies [14]. However, many medicinal plants possess intrinsic toxicity due to their phytochemical constituents and may cause adverse effects if misused. As a result, there is growing concern regarding their safety [12], leading to ongoing debates and ambivalence about their clinical and traditional use.

One medicinal plant with rich ethnomedicinal utility is *Spondias mombin* Linn., widely known as yellow plum or hog plum [11,17,18]. Its various parts are traditionally used across Africa and South America to manage ailments ranging from leprosy, severe cough, and dysentery to postpartum haemorrhage and inflammation [17–19]. *S. mombin* (Figure 1) is also referred to as **Akika, Iyeye** (Yoruba), **Tsardar masar** (Hausa), **Ijikara, Uvuru** (Igbo), **Atoa, Akukor** (Ghana), **Caja, Cajazeira** (Brazil), **Ubo** (Peru), and **Jobo** (Central America) [17,19,20]. It belongs to the *Anacardiaceae* family and is distributed across tropical regions of South America, Africa, Asia, and Western India [20]. The crushed leaves emit a turpentine-like smell and are the most widely used part in traditional medicine, similar to other plants in Traditional African Medicine (TAM) [17]. Oral administration of *S. mombin* leaf decoction is used in the treatment of malaria, typhoid, male infertility, laryngitis, sore throat, tuberculosis, stomach disorders, and gonorrhea, among others [17,19,21].

Figure 3: Photographs of *S. mombin* tree, fruits and leaves [17,21].

Despite the documented uses of *S. mombin*, conclusions regarding its toxicity are often drawn from studies using organic solvent extracts (e.g., methanol, dichloromethane), which do not reflect traditional aqueous-based preparations [12].

For instance, Pereira *et al.* (2024) demonstrated that a hydroethanolic mixture (ethanol: water, 70:30%) yielded higher phytochemical content than single solvents during the extraction from dried and milled *S. mombin* leaves. Likewise, Ingrid *et al.* (2021) found that hydroethanolic extracts exhibited superior antibacterial activity compared to both aqueous and control groups. However, most prior toxicity studies on the leaves of *Spondias mombin* in Nigeria have focused on aqueous [22,23], methanolic [24,25] and ethanolic [23] extracts. This leaves a significant gap regarding the toxicological profile of the hydroethanolic leaf extract of *S. mombin*, which has shown promising potency and safety profiles in recent studies [26,27].

To address these limitations and build on recent findings, we investigated the following research questions:

1. What are the acute, subacute, and subchronic toxicity effects of the hydroethanolic leaf extract of *S. mombin* harvested at the University of Lagos, Nigeria?
2. What is the safety index of short-term and prolonged administration of this extract in rodents?
3. What hematological and biochemical changes are associated with its use in rodents?

To the best of our knowledge, this represents the first comprehensive toxicological profiling of the hydroethanolic leaf extract of *S. mombin* in rodent models in Nigeria.

Objectives

The ethnomedicinal knowledge on *S. mombin* is a rich source of insight into its therapeutic value and potential for scientific exploration [11]. This study aims to assess the oral acute, subacute, and subchronic toxicity of hydroethanolic leaf extract of *S. mombin* in albino mice and Wistar rats in Nigeria.

Study Significance, Novelty and Rationale

Notably, toxicity accounts for nearly one-third of candidate dropouts [3], underscoring the urgent need for robust preclinical toxicological evaluation early in the drug discovery pipeline.

The systematic profiling of *Spondias mombin* hydroethanolic leaf extract in rodent models provides critical preclinical evidence to support the ethnomedicinal use of hydroethanolic *S. mombin* leaf extract and to guide the identification and optimization of promising lead compounds.

The 2024 FDA report reveals that 66% of approved novel drugs utilized expedited regulatory pathways such as Fast Track and Accelerated Approval, processes that are highly dependent on well-documented safety data [1]. This study thus provides timely and relevant evidence aligned with global drug development priorities for the development of safe and efficacious medicines.

To date, no study in Nigeria has investigated the oral acute, subacute, and subchronic toxicity of the hydroethanolic extract of *Spondias mombin* leaves in rodent models, highlighting the novelty and importance of this investigation as a foundation for future translational studies, including target validation, formulation development, and clinical safety assessments.

METHODS

Study Design

This study employed an *in vivo* experimental design to assess the oral acute, subacute, and subchronic toxicity of *Spondias mombin* hydroethanolic leaf extract in rodent models. The study included randomized control groups, graded dose administration, observational tracking, and hematological/biochemical evaluations[8–10]. Only female rodent species were used in this study as conventional LD50 tests show that usually there is little difference in sensitivity between sexes, but in cases where differences are observed, females are generally slightly more sensitive [8].

Justification for Dose Selection

Acute Toxicity Dose Justification: The acute oral toxicity test was conducted using the Up-and-Down Procedure (UDP), following OECD Test Guideline 425.

Based on prior toxicity data on *Spondias mombin*, which demonstrated no mortality or significant toxicity at high doses [22–25,28], a limit dose of 2000 mg/kg was selected as the upper threshold for testing. In line with OECD recommendations, when prior evidence suggests low toxicity, a limit test at 2000 mg/kg is appropriate and sufficient without requiring a full dose-range study [8]. This approach minimizes animal use while ensuring scientifically valid outcomes.

Initial dosing at 100 mg/kg and 500 mg/kg was conducted to confirm tolerance and assess potential hypersensitivity or unexpected responses, followed by administration of the 2000 mg/kg limit dose to additional animals.

Subacute and Subchronic Dose Justification: For the 28-day (subacute) and 60-day (subchronic) studies, dose levels of 40, 200, and 1000 mg/kg/day were selected in line with OECD Test Guidelines 407 and 408 [9,10].

These guidelines recommend the selection of a high dose capable of eliciting signs of systemic toxicity (without inducing severe suffering or death), along with at least two lower doses to evaluate dose-response relationships [9,10].

The high dose of 1000 mg/kg/day was selected as the upper threshold based on OECD provisions for a limit dose, justified by the absence of observed toxicity in multiple prior studies on aqueous, methanolic, and ethanolic extracts of *S. mombin* at doses up to and exceeding this threshold [22–25,28]. Intermediate (200 mg/kg/day) and low (40 mg/kg/day) doses were chosen to provide rational spacing and enable detection of dose-dependent trends.

Although OECD guidelines allow for a single-dose limit test in the absence of expected toxicity, a three-dose strategy was adopted to generate more granular toxicological data. This approach was deemed appropriate given the increasing therapeutic application of *S. mombin* in ethnomedicine and its potential for future pharmacological development.

Setting and Test Animals

The study was conducted at the Pharmacology and Toxicology Laboratory, College of Medicine, University of Lagos, Nigeria. Female albino mice (18–22 g) and Wistar rats (130–170 g) were obtained from the university's animal house. Animals were housed under controlled environmental conditions (22 ± 3 °C, 50–60% humidity, 12-hour light/dark cycle), fed commercial grower pellets (New Hope, Nigeria), and had unrestricted access to water. Animals were marked for identification and kept for 14 days to acclimatize before random assignment into experimental groups.

Aqueous suspensions of the extracts were administered in a consistent volume based on animal body weight, with doses not exceeding 0.04 mL/20g and 2 mL/100 g body weight for mice and rats, respectively. Animals were fasted before dosing: rats overnight and mice for 3-4 hours. After weighing, the extracts were administered, and a subsequent fasting period of 3-4 hours for rats and 1-2 hours for mice was observed.

Collection and Preparation of Plant Material

Fresh *Spondias mombin* leaves were collected from the University of Lagos Botanical Garden. Mr. Eze Tochukwu, the curator, authenticated the plant at the Department of Botany Herbarium, University of Lagos (Voucher No. LUH 9910). Leaves were rinsed, shade-dried at 25 ± 2 °C for five days, pulverized, and stored in airtight containers until use.

Extraction of Phytochemicals

The dried powdered plant material of *S. mombin* (~800 g) was extracted by cold maceration in 4 L of 50% ethanol-water (v/v) for 72 hours with intermittent stirring using a clean spatula. The macerate was filtered using muslin cloth and Whatman No. 2 filter paper, and subsequently concentrated at ≤45 °C using a rotary evaporator. The semi-solid extract was freeze-dried, weighed, and stored in sterile bottles at 4 °C.

Ethical Approval

The study received ethical approval from the University of Lagos Research Ethics Committee (Approval No. UNILAGREC/22/06/005).

Variables

- **Independent Variable**: Doses of *S. mombin* hydroethanolic extract (0, 40, 200, 1000, and 2000 mg/kg).
- **Dependent Variables**: Behavioral symptoms, mortality, body weight, hematological parameters (e.g., Mean White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin count (Hb)), and biochemical markers (e.g., Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), bilirubin, cholesterol).
- **Controlled Variables**: Animal species, diet, housing, administration volume, and fasting duration.

Data Sources and Measurement

Acute Toxicity Test [8]

Nulliparous, non-pregnant female mice (18–22 g) were selected for the study. A single mouse each was initially administered test doses of 100 mg/kg and 500 mg/kg and monitored for 24 hours for mortality and behavioral signs, including hyperactivity, circular movement, hind limb leaning, altered feeding habits, and scratching. With no lethality or abnormal behavior was observed, four additional mice were sequentially administered a limit dose of 2000 mg/kg and monitored. All animals were observed daily for 14 days for signs of toxicity or mortality. The LD_{50} value was determined at the end of the observation period.

A control group of five mice received 0.04 mL distilled water per 20 g body weight under the same conditions.

Subacute Toxicity Test [9]

Twenty-four nulliparous, non-pregnant female Wistar rats were randomly assigned to four groups (n = 6 per group). The treatment groups received oral doses of the extract at 40, 200, or 1000 mg/kg/day for 28 consecutive days. The control group received 2 mL of distilled water per 100 g body weight.

Rats were weighed weekly and monitored daily using standardized observation charts for clinical signs of toxicity. At the end of the treatment period, blood samples were collected via ocular puncture for hematological and biochemical analysis. Male rats were not included in this study, as evaluation of reproductive toxicity was beyond the scope of the research objectives.

Subchronic Toxicity Test [10]

An additional set of ten nulliparous, non-pregnant female Wistar rats was randomly assigned into four groups (n = 10 per group). The treatment groups received oral doses of the extract at 40, 200, or 1000 mg/kg/day for 60 consecutive days, while the control group received 2 mL of distilled water per 100 g body weight.

Rats were weighed weekly and monitored daily using standardized observation charts for any clinical signs of toxicity. At the end of the exposure period, blood samples were collected via ocular puncture for hematological and biochemical analyses.

Male rats were excluded from the study, as reproductive toxicity evaluation was outside the scope of this investigation. No interim kills were done before the completion of the study.

Reversibility Study [9]

A satellite group of six female adult Wistar rats was included in both the control and treatment arms to assess the reversibility, persistence, or delayed onset of any toxic effects. Following the 60-day treatment period,

these animals were monitored for an additional 14 days without further extract administration before being humanely euthanized via cervical dislocation for post-treatment evaluations.

Study Size and Sampling Criteria

Animals were randomly selected from acclimatized populations. A minimum of five animals per group ensured compliance with OECD guidelines and provided statistical power to detect dose-related trends.

- **Quantitative Variables**

At the end of each study, animals were fasted overnight before euthanasia via cervical dislocation. Blood samples for hematological and biochemical analyses were collected via ocular puncture immediately before euthanasia, following OECD guidelines.

- **Hematology**: Whole blood was collected into EDTA tubes. A volume of 13 µL of blood was mixed with 3.5 mL of diluent and 0.5 mL of lyse reagent, then analyzed using the *Mindray BC-3200® Auto Hematology Analyzer* to determine leukocyte, erythrocyte, hemoglobin, hematocrit, platelet, and plateletcrit values.
- **Biochemistry:** Lithium heparinized blood was centrifuged at 1600 rcf for 5 minutes using the *Eppendorf 5702® centrifuge*. Plasma (50 µL) was mixed with 100 µL of reagent and analyzed using the *Erba Mannheim XL-640® Auto Biochemical Analyzer* to evaluate cholesterol, bilirubin, urea, creatinine, total protein, albumin, and liver enzymes indicative of hepatocellular integrity, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Bias

To reduce selection bias, animals were randomized into groups. Observation and outcome assessments were performed using standardized forms. Personnel were blinded to group allocation during data collection and analysis.

Statistical Methods

Data were expressed as mean ± standard deviation (SD). One-way ANOVA was used to detect inter-group differences. Tukey's HSD test was employed for post hoc pairwise comparisons. Microsoft Excel® was used for plotting, and $p \leq 0.05$ was considered statistically significant.

Missing Data

No animals were lost during the study. Complete datasets were obtained for all animals across all parameters.

RESULTS

4.1 Extraction

Cold maceration of *Spondias mombin* leaves in 50% ethanol yielded 32.8% (w/w) of freeze-dried hydroethanolic extract with a distinct coffee-brown color (Figure 4).

Figure 2: Freeze-dried Hydroethanolic Extract of *Spondias mombin*

4.2 Acute Oral Toxicity Test

No statistically significant difference was observed in the relative weights of the treated (19.40 g $\pm$ 1.74) and control mice (20.40 g $\pm$ 1.67), as shown in Table 1. Oral administration of the hydroethanolic extract of *S. mombin* at doses up to 2000 mg/kg did not produce any signs of toxicity in mice over the 14-day observation period. No mortality, behavioral abnormalities, or clinical symptoms (e.g., changes in skin, eyes, salivation, or

excretion) were observed. LD_{50} of the hydroethanolic leaf extract of *Spondias mombin* in mice was >2000 mg/kg.

Table 1: Relative weights of mice treated with 2000 mg/kg of *S. mombin* extract

Serial Number of Mice	Weight of Treated Mice (g)	ight of Control Mice (g)
Mice 1	22.00	20.00
Mice 2	18.00	22.00
Mice 3	17.00	22.00
Mice 4	20.00	20.00
Mice 5	20.00	18.00
Mean ± S.D.:	**19.40 ± 1.74**	**20.40 ± 1.50**
Maximum Weight:	**22.00**	**22.00**
Minimum Weight:	**17.00**	**18.00**

4.3. Subacute Oral Toxicity Test

A statistically significant difference ($p<0.05$) was observed in the mean body weight changes of the treated and control rats in the subacute toxicity study, as shown in Chart 1 below;

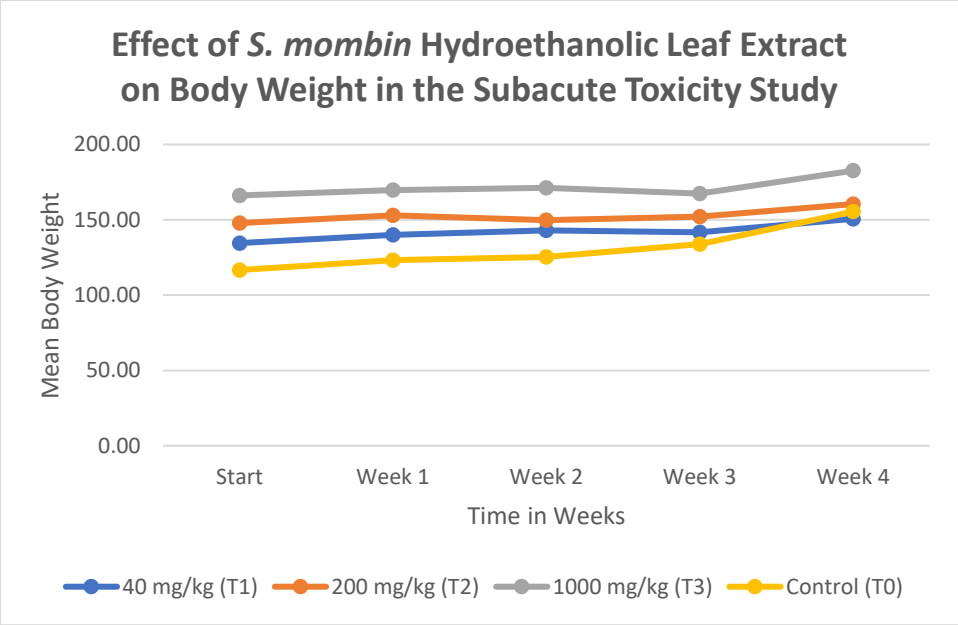

Chart 1: Effect of *S. mombin* Hydroethanolic Leaf Extract on Body Weight

S. mombin significantly reduced mean absolute lymphocytes and percentage Lymphocytes with peak reductions at 1000 mg/kg compared to control (Absolute Lymphocytes: 2.08 × 10^3/µL ± 0.13 vs. 4.28 × 10^3/µL ± 1.00; % Lymphocytes: 37.53 ± 0.84 vs 45.40 ± 3.41) as seen in Table 2 and Table 3 below.

As illustrated in Chart 2, relative to the control group, the extract also exhibited a dose-dependent increase in mean red blood cell (8.79 × 10^6/µL ± 0.19 vs. 7.93× 10^6/µL ± 0.42) and hemoglobin (14.75 ± 0.24 g/dL vs 13.98 ± 0.75 g/dL) levels. No statistically significant difference in aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels was observed (Chart 3) between the treatment and control groups in the subacute study.

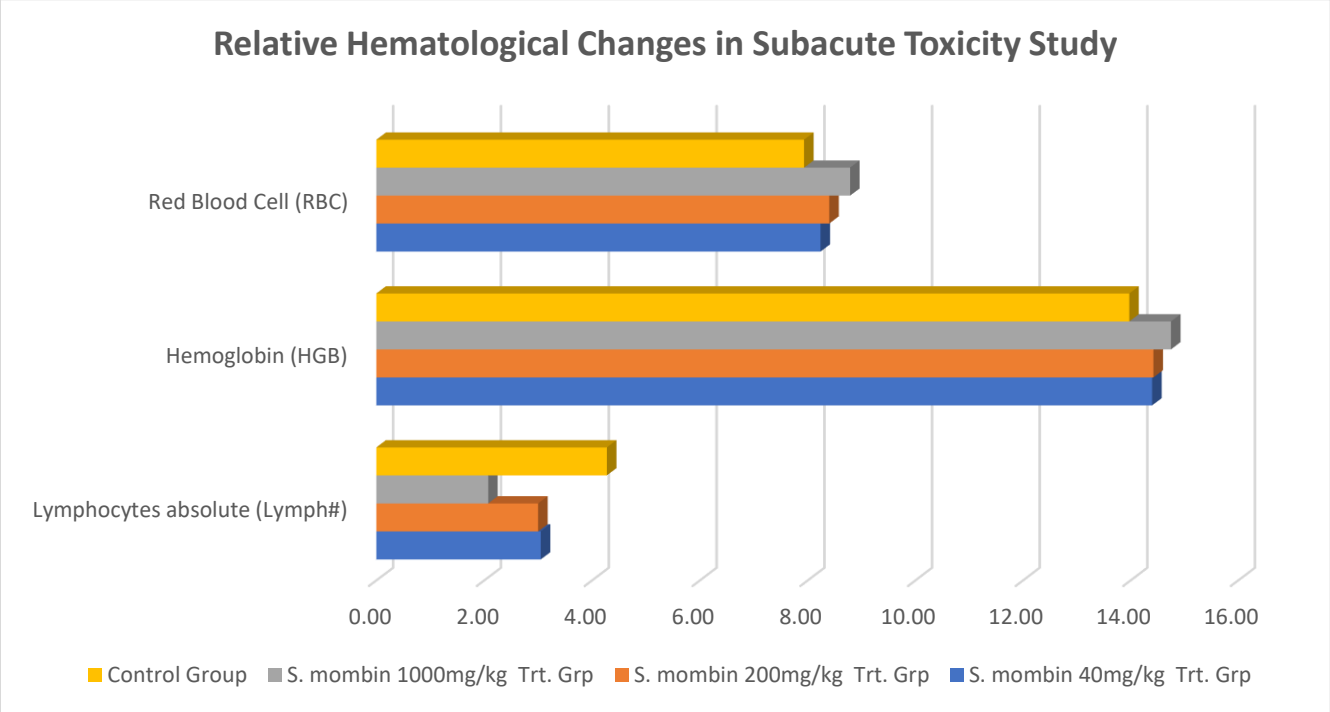

Chart 2: Relative Hematological Changes in Subacute Toxicity Study

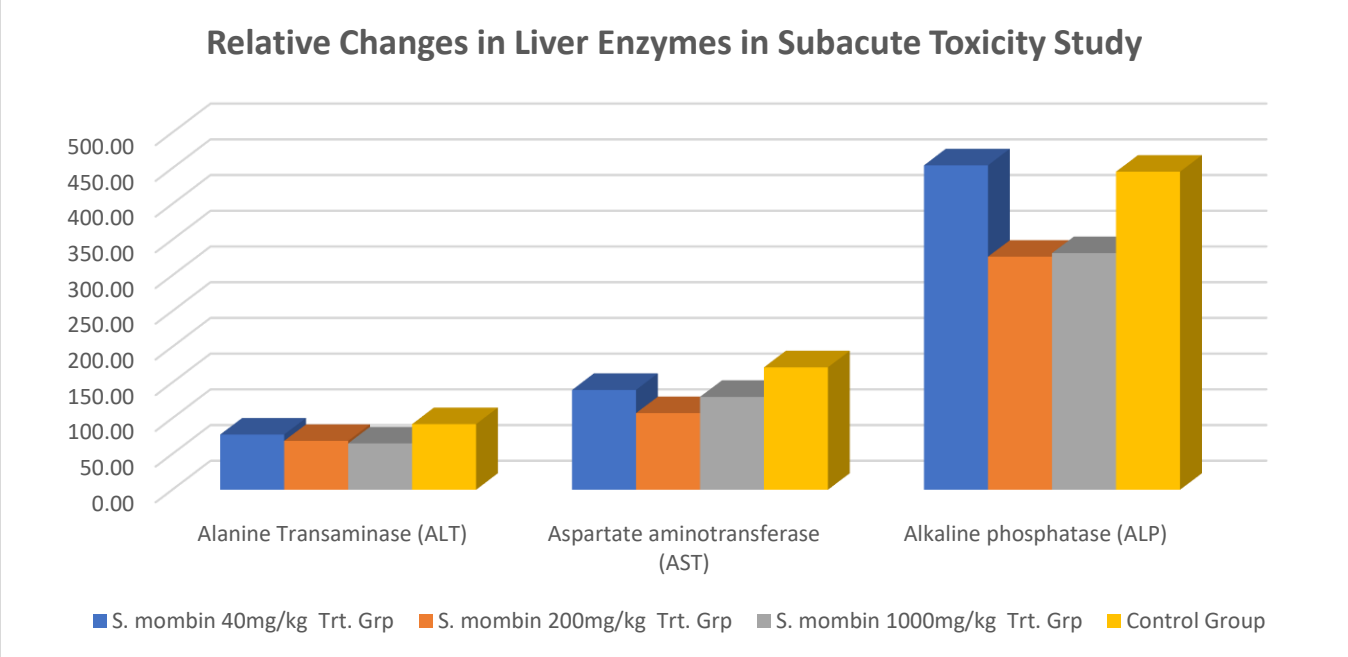

Chart 3: Relative Changes in Liver Enzymes in Subacute Toxicity Study

Chart 3: Mean Red Blood Cell Count of Rats in Treatment & Control Groups

Table 2: Subacute Toxicity Study of *S. mombin* Hematological Assessment

Hematological Parameter	Unit	40 mg/kg Treatment Group	200 mg/kg Treatment Group	1000 mg/kg Treatment Group	Control Group
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
White Blood Cells (WBC)	$10^3/\mu L$	7.30 ± 2.47	7.55 ± 1.64	5.60 ± 0.22	9.35 ± 1.75
Lymphocytes absolute (Lymph#)	$10^3/\mu L$	3.05 ± 0.91	3.00 ± 0.74	*2.08 ± 0.13	4.28 ± 1.00
Mid-sized Cell absolute (Mid#)	$10^9/\mu L$	1.08 ± 0.51	1.00 ± 0.45	0.85 ± 0.13	1.40 ± 0.35
Granulocytes absolute (Gran#)	$10^9/\mu L$	3.18 ± 1.11	3.55 ± 0.54	2.68 ± 0.05	3.68 ± 0.53
Lymphocytes percentage (Lymph%)	%	42.48 ± 3.99	39.80 ± 1.54	*37.53 ± 0.84	45.40 ± 3.41
Mid-sized cell percentage (Mid%)	%	14.28 ± 1.80	12.68 ± 3.34	14.23 ± 2.01	15.08 ± 1.20
Granulocytes percentage (Gran%)	%	43.25 ± 4.36	*47.53 ± 4.12	*48.25 ± 2.45	39.53 ± 3.21
Hemoglobin (HGB)	g/dL	14.40 ± 0.80	14.43 ± 1.12	14.75 ± 0.24	13.98 ± 0.75
Red Blood Cell (RBC)	$10^6/\mu L$	8.24 ± 0.42	8.40 ± 0.44	8.79 ± 0.19	7.93 ± 0.42
Hematocrit - Pack Cell Volume (HCT)	%	42.68 ± 1.79	42.43 ± 3.22	44.28 ± 0.66	42.15 ± 2.55
Mean Cell Volume (MCV)	fL	51.88 ± 1.14	50.55 ± 2.52	50.43 ± 0.49	53.20 ± 0.41
Mean Cell Hemoglobin (MCH)	pg.	17.45 ± 0.70	17.13 ± 0.82	16.75 ± 0.13	17.55 ± 0.21
Mean Cell Hemoglobin Concentratio (MCHC)	g/dL	33.70 ± 0.76	33.95 ± 0.17	33.25 ± 0.26	33.10 ± 0.45
Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV)	%	14.30 ± 0.58	14.20 ± 0.36	14.60 ± 0.38	14.18 ± 0.35
Red Blood Cell Distribution Width Standard Deviation (RDW-SD)	fL	26.00 ± 0.80	24.95 ± 1.34	25.38 ± 0.45	26.53 ± 0.45
Platelet (PLT)	10/L	680.75 ± 84.99	808.50 ± 247.16	756.50 ± 69.29	640.00 ± 150.39
Mean Platelets Volume (MPV)	fL	7.15 ± 0.31	7.03 ± 0.40	7.38 ± 0.21	7.23 ± 0.59
Platelet Distribution Width (PDW)	%	16.08 ± 0.45	15.88 ± 0.64	16.38 ± 0.13	15.93 ± 0.43
Plateletcrit (PCT)	%	0.49 ± 0.06	0.38 ± 0.29	0.56 ± 0.06	0.46 ± 0.08

**p is statistically significant ($p<0.05$)*

Table 3: Subacute Toxicity Study of *S. mombin* Biochemical Assessment

Biochemical Parameter	Unit	40 mg / kg Treatment Group	200 mg/kg Treatment Group	1000 mg/kg Treatment Group	Control Group
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
High Density Lipoprotein (HDL)	mmolL	1.15 ± 0.17	1.35 ± 0.24	1.07 ± 0.31	1.34 ± 0.28
Creatinine (CRE)	umol / L	67.43 ± 4.47	67.25 ± 2.98	69.78 ± 3.26	63.61 ± 4.46
Alanine Transaminase (ALT)	IU / L	77.25 ± 8.81	68.40 ± 6.69	64.90 ± 13.36	91.93 ± 29.14
Aspartate aminotransferase (AST)	IU / L	139.73 ± 43.35	107.25 ± 8.71	130.00 ± 29.58	171.43 ± 83.18
Alkaline phosphatase (ALP)	IU / L	454.20 ± 87.72	326.23 ± 42.20	331.30 ± 31.10	445.30 ± 143.57
Urea	mmol / I	5.60 ± 0.92	6.79 ± 0.86	6.28 ± 1.70	5.28 ± 0.22
Triglyceride (TG)	mmol / I	0.70 ± 0.26	0.79 ± 0.19	0.76 ± 0.06	0.93 ± 0.28
Cholesterol (CHOL)	mmolL	1.60 ± 0.16	1.88 ± 0.33	1.61 ± 0.37	1.89 ± 0.33
Low Density Lipoprotein (LDL)	mmolL	0.23 ± 0.04	0.25 ± 0.06	0.20 ± 0.10	0.23 ± 0.13
Protein (PRO)	g / L	72.43 ± 3.13	73.98 ± 2.22	75.68 ± 3.48	72.48 ± 4.92
Albumin Blood test (ALB)	g / L	33.40 ± 0.88	33.30 ± 0.57	33.15 ± 2.91	34.48 ± 1.60

4.4 Subchronic Oral Toxicity Test

Following 60 days of repeated graded oral dosing of *S. mombin* hydroethanolic extract to the treatment rats and the control rats' group, a statistically significant difference was noted in the body weight changes between the groups, as shown in Chart 4 below;

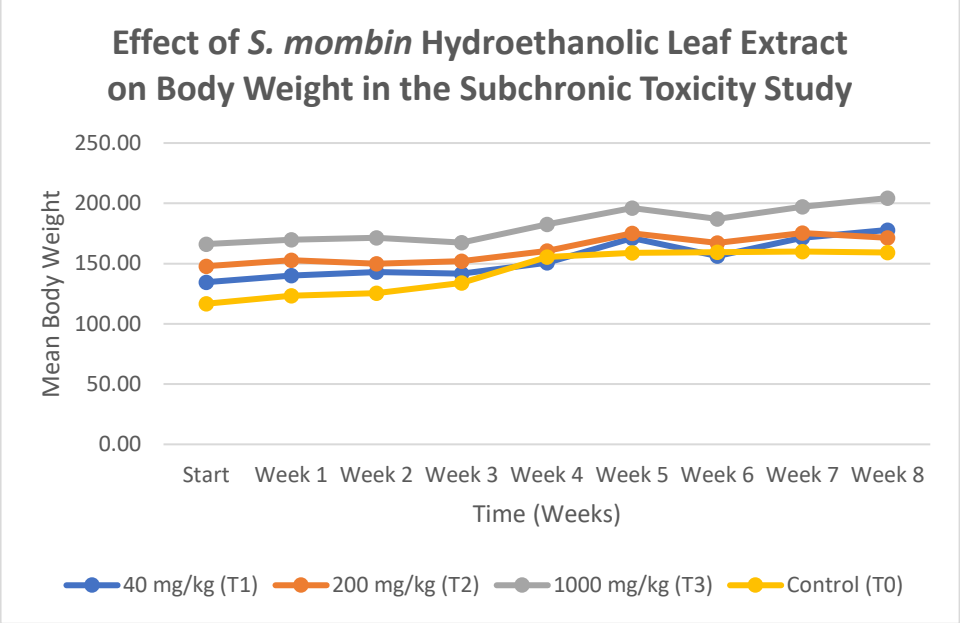

Chart 4: Effect of *S. mombin* Hydroethanolic Leaf Extract on Body Weight in the Subchronic Toxicity Study

S. mombin hydroethanolic extract exhibited statistically significant dose-dependent increase in mean white blood cells and absolute granulocytes with a peak reduction at a dose of 200 mg/kg (6.80×10^3/uL ± 1.53 and 3.40×10^9/uL ± 1.13 respectively) compared to control (4.30×10^3/uL ± 1.47 and 1.58×10^9/uL ± 0.50 respectively) as shown in Table 4 below.

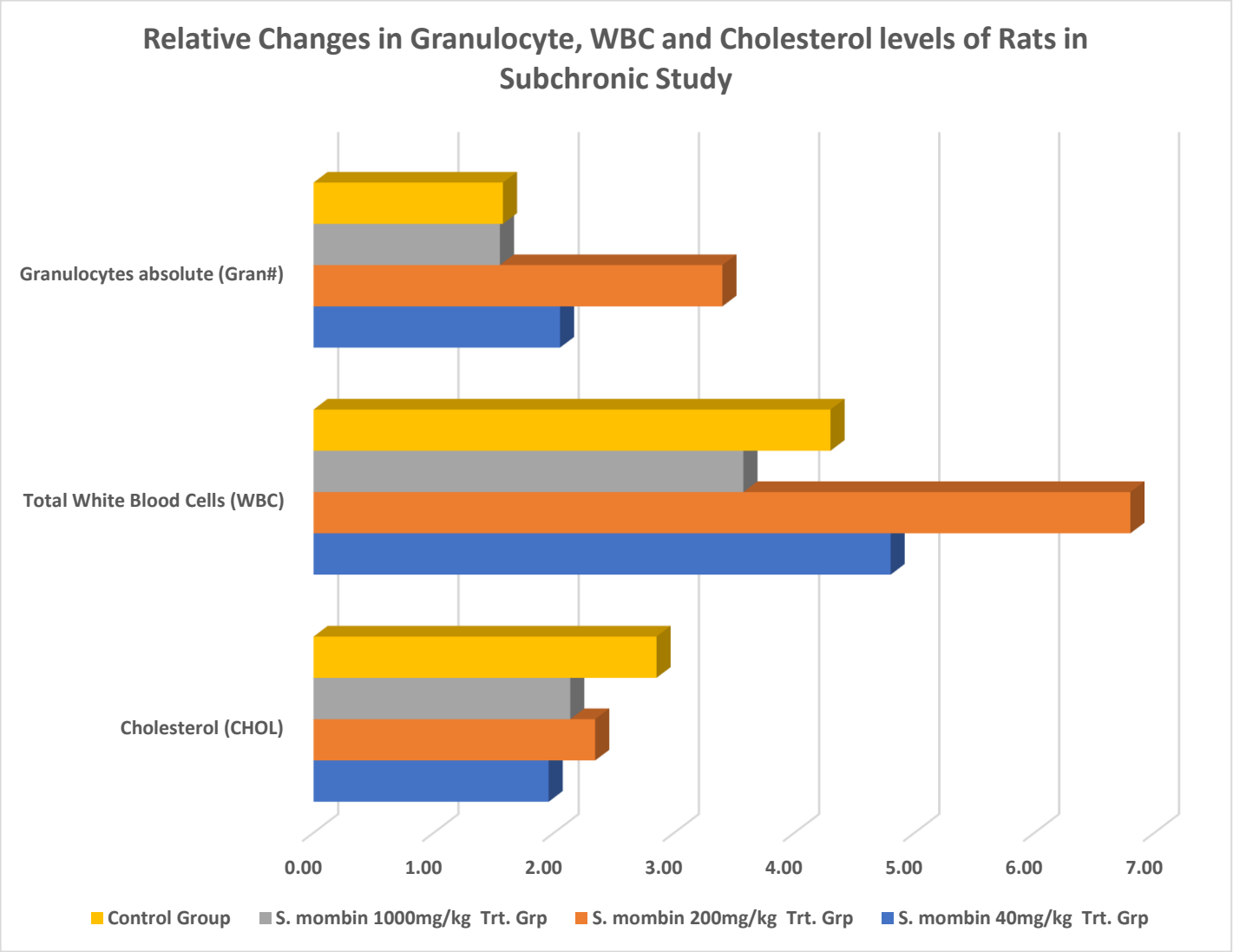

Chart 5: Relative Changes in Granulocyte, WBC and Cholesterol levels of Rats in Subchronic Study

A statistically significant decrease in total cholesterol and alkaline phosphatase (ALP) (Charts 5 and Table 5) was observed at a dose peak of 40 mg/kg of *S. mombin* hydroethanolic extract (1.96 mmol/L ± 0.30 and 97.30 IU/L ± 16.97, respectively) relative to the control group (2.85 mmol/L ± 0.36 and 152.03 IU/L ± 26.04, respectively).

Table 4: Subchronic Toxicity Study of *S. mombin* Hematological Assessment

Hematological Parameter	Unit	40mg/kg Treatment Grou	200mg/kg Treatment Grou	1000mg/kg Treatment Grou	Control Group
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
White Blood Cells (WBC)	$10^3/\mu L$	4.80 ± 0.93	*6.80 ± 1.53	3.58 ± 1.09	4.30 ± 1.47
Lymphocytes absolute (Lymph#)	$10^3/\mu L$	1.95 ± 0.59	2.43 ± 0.38	1.65 ± 0.51	1.95 ± 0.73
Mid-sized Cell absolute (Mid#)	$10^9/\mu L$	0.80 ± 0.18	0.98 ± 0.17	0.63 ± 0.17	0.78 ± 0.31
Granulocytes absolute (Gran#)	$10^9/\mu L$	2.05 ± 0.30	*3.40 ± 1.13	1.55 ± 0.68	1.58 ± 0.50
Lymphocytes percentage (Lymph%)	%	40.03 ± 5.14	*36.30 ± 4.58	43.53 ± 3.40	44.73 ± 2.50
Mid-sized cell percentage (Mid%)	%	16.35 ± 1.65	14.45 ± 1.88	17.35 ± 2.99	18.38 ± 3.90
Granulocytes percentage (Gran%)	%	43.63 ± 4.86	*49.25 ± 4.96	39.13 ± 5.23	36.90 ± 3.77
Hemoglobin (HGB)	g/dL	15.20 ± 0.32	15.80 ± 0.66	12.58 ± 4.92	15.80 ± 0.20
Red Blood Cell (RBC)	$10^6/\mu L$	8.37 ± 0.38	8.76 ± 0.33	7.03 ± 2.54	8.69 ± 0.37
Hematocrit - Pack Cell Volume (HCT)	%	44.93 ± 0.70	43.98 ± 4.50	37.78 ± 14.37	47.20 ± 0.84
Mean Cell Volume (MCV)	fl	54.05 ± 1.74	53.15 ± 1.55	53.33 ± 2.28	54.43 ± 1.78
Mean Cell Hemoglobin (MCH)	pg.	18.20 ± 0.67	18.00 ± 0.75	16.83 ± 1.56	18.15 ± 0.66
Mean Cell Hemoglobin Concentration (MCHC)	g/dL	33.78 ± 0.29	33.95 ± 0.51	33.03 ± 1.00	33.43 ± 0.31
Red Blood Cell Distribution Width - Coefficient of Variation (RDW-CV)	%	13.48 ± 0.80	14.93 ± 1.44	17.20 ± 9.14	14.53 ± 0.79
Red Blood Cell Distribution Width - Standard Deviation (RDW-SD)	fL	26.00 ± 0.80	27.63 ± 2.06	27.88 ± 4.21	26.98 ± 1.22
Platelet (PLT)	10^3	613.03 ± 423.47	795.50 ± 20.73	634.75 ± 220.82	756.25 ± 108.85
Mean Platelets Volume (MPV)	fL	7.13 ± 0.67	7.45 ± 0.19	7.30 ± 0.48	7.05 ± 0.49
Platelet Distribution Width (PDW)	%	15.68 ± 0.38	16.00 ± 0.14	15.73 ± 0.39	15.75 ± 0.24
Plateletcrit (PCT)	%	0.57 ± 0.08	0.59 ± 0.03	0.46 ± 0.14	0.53 ± 0.04

**p is statistically significant ($p<0.05$)*

Table 5: Subchronic Toxicity Study of *S. mombin* Biochemical Assessment

Biochemical Parameter	Unit	40mg/kg Treatment Group Mean ± SD	200mg/kg Treatment Group Mean ± SD	1000mg/kg Treatment Group Mean ± SD	Control Group Mean ± SD
Protein (PRO)	g/L	*74.78 ± 5.84	81.63 ± 5.89	84.58 ± 1.63	79.43 ± 1.42
Cholesterol (CHOL)	mmolL	*1.96 ± 0.30	2.34 ± 0.39	2.14 ± 0.26	2.85 ± 0.36
High Density Lipoprotein (HDL)	mmolL	*1.61 ± 0.21	1.78 ± 0.26	1.78 ± 0.23	2.19 ± 0.19
Albumin Blood test (ALB)	g/L	39.33 ± 2.10	39.58 ± 3.10	40.63 ± 0.64	39.53 ± 0.98
Urea	mmol / L	8.02 ± 1.40	8.14 ± 0.73	8.71 ± 0.99	8.51 ± 1.41
Alanine transaminase (ALT) test	IU/L	37.85 ± 3.57	82.00 ± 79.66	37.68 ± 1.11	50.65 ± 5.47
Aspartate transaminase (AST)	IU/L	95.53 ± 9.42	154.33 ± 100.31	109.80 ± 21.22	113.03 ± 8.19
Alkaline phosphatase (ALP)	IU/L	*97.30 ± 16.97	117.25 ± 15.99	136.38 ± 33.20	152.03 ± 26.04
Creatinine (CRE)	umolL	81.01 ± 3.50	80.07 ± 2.51	80.30 ± 2.83	79.81 ± 5.70
Triglyceride (TG)	mmol/ L	0.51 ± 0.09	*0.66 ± 0.11	0.47 ± 0.10	0.47 ± 0.04
Low-Density Lipoprotein (LDL)	mmol/ L	0.27 ± 0.27	0.38 ± 0.18	0.15 ± 0.04	0.46 ± 0.18

**p is statistically significant ($p<0.05$)*

4.5 Reversibility Study

No statistically significant difference was observed in the hematological parameters between the treatment and control groups, as shown in Table 6 below.

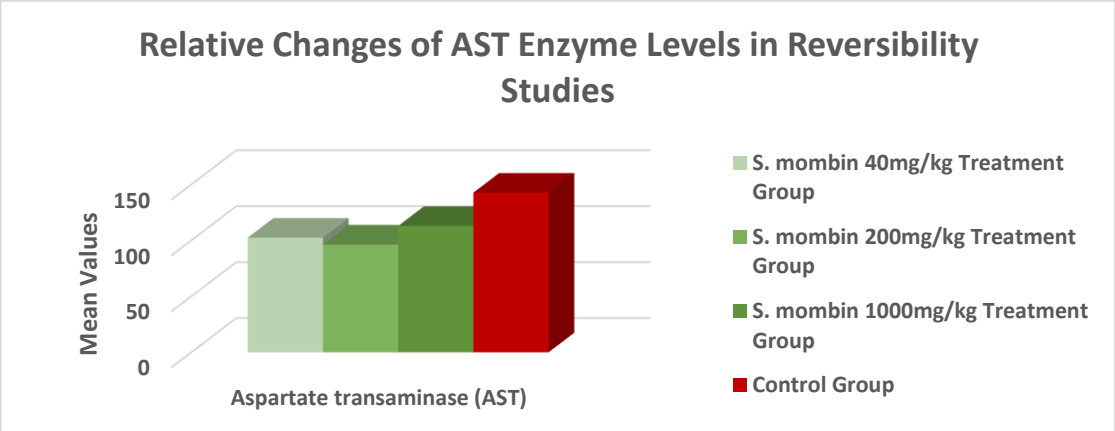

Chart 6: Relative Changes of AST Enzyme Levels in Reversibility Studies

In charts 6 and 7, prolonged treatment of the hydroethanolic extract of *S. mombin* is shown to led to a statistically significant reduction in Aspartate transaminase (AST) and Urea levels across all treatment groups (AST 200 mg/kg: 96.33 IU/L ± 12.14, Urea 1000 mg/kg: 7.26 mmol/L ± 0.63) relative to the control group (AST: 142.68 IU/L ± 38.86 and Urea: 10.80 mmol/L ± 0.40). No statistically significant difference was observed in all other biochemical parameters as shown in Table 7 below.

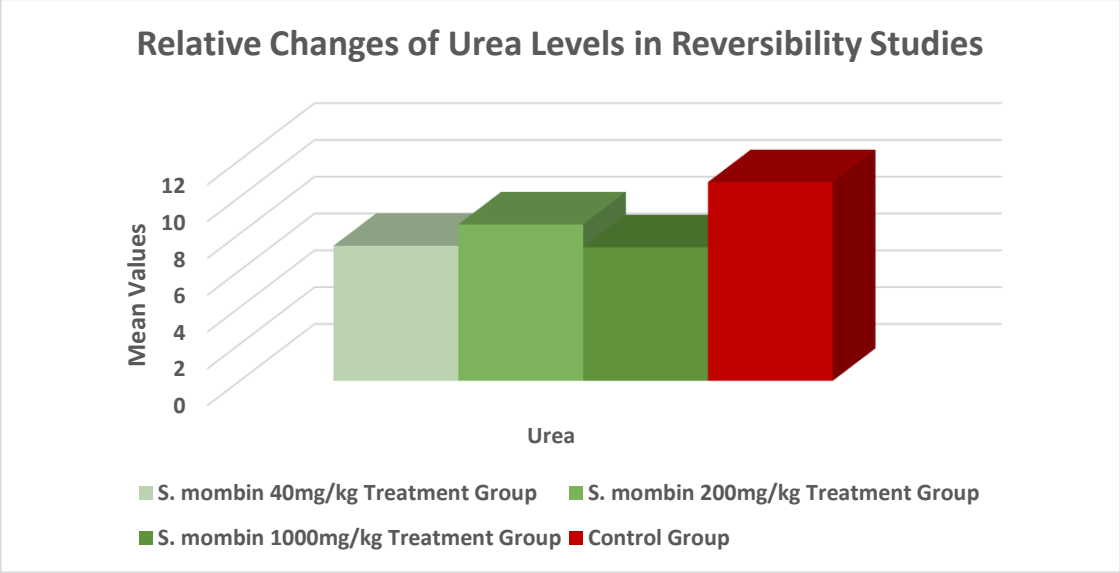

Chart 7: Relative Changes of Urea Levels in Reversibility Studies

Table 6: Hematological Assessment in Reversibility Study of *S. mombin*

Hematological assessment	Unit	40mg/kg Treatment Group Mean ± SD	200mg/kg Treatment Group Mean ± SD	1000mg/kg Treatment Group Mean ± SD	Control Group Mean ± SD
White Blood Cells (WBC)	$10^3/\mu L$	5.18 ± 1.15	5.55 ± 1.10	4.18 ± 1.83	6.03 ± 1.39
Lymphocytes absolute (Lymph#)	$10^3/\mu L$	2.13 ± 0.53	1.95 ± 0.37	1.78 ± 0.69	2.15 ± 0.19
Mid-sized Cell absolute (Mid#)	$10^9/\mu L$	0.93 ± 0.26	0.98 ± 0.15	0.63 ± 0.26	0.90 ± 0.08
Granulocytes absolute (Gran#)	$10^9/\mu L$	2.13 ± 0.65	2.63 ± 0.86	1.78 ± 0.91	2.98 ± 1.20
Lymphocytes percentage (Lymph%)	%	41.60 ± 5.61	35.75 ± 5.46	43.90 ± 4.20	36.23 ± 4.28
Mid-sized cell percentage (Mid%)	%	18.25 ± 1.97	17.53 ± 2.81	14.75 ± 1.47	16.15 ± 3.96
Granulocytes percentage (Gran%)	%	40.15 ± 7.20	46.73 ± 6.27	41.35 ± 5.45	47.63 ± 7.91
Hemoglobin (HGB)	g/dL	16.58 ± 1.09	16.03 ± 0.49	16.40 ± 0.32	16.10 ± 1.07
Red Blood Cell (RBC)	$10^6/\mu L$	9.20 ± 0.24	9.16 ± 0.32	9.13 ± 0.34	9.16 ± 0.58
Hematocrit - Pack Cell Volume (HCT)	%	48.58 ± 2.81	47.80 ± 1.37	48.48 ± 0.60	47.80 ± 2.83
Mean Cell Volume (MCV)	fl	52.85 ± 1.96	52.30 ± 2.48	53.20 ± 2.01	52.28 ± 1.35
Mean Cell Hemoglobin (MCH)	pg	17.98 ± 0.78	17.48 ± 0.73	17.95 ± 0.73	17.53 ± 0.34
Mean Cell Hemoglobin Concentration (MCHC)	g/dL	34.05 ± 0.37	33.48 ± 0.29	33.80 ± 0.23	33.63 ± 0.56
Red Blood Cell Distribution Width - Coefficient of Variation (RDW-CV)	%	13.03 ± 0.99	13.83 ± 2.25	13.33 ± 1.95	13.20 ± 1.60
Red Blood Cell Distribution Width - Standard Deviation (RDW-SD)	fL	25.00 ± 1.77	25.78 ± 3.51	25.55 ± 2.39	24.75 ± 2.88
Platelet (PLT)	10/L	768.75 ± 93.92	823.75 ± 131.16	805.25 ± 136.99	772.00 ± 126.39
Mean Platelets Volume (MPV)	fL	7.18 ± 0.35	7.15 ± 0.24	6.85 ± 0.38	7.05 ± 0.26
Platelet Distribution Width (PDW)	%	15.78 ± 0.31	15.90 ± 0.22	15.63 ± 0.21	15.80 ± 0.22
Plateletcrit (PCT)	%	0.55 ± 0.06	0.59 ± 0.08	0.55 ± 0.07	0.54 ± 0.08

Table 7: Biochemical Assessment in Reversibility Study of *S. mombin*

Biochemical Assessments	Unit	40mg/kg Treatment Group Mean ± SD	200mg/kg Treatment Group Mean ± SD	1000mg/kg Treatment Group Mean ± SD	Control Group Mean ± SD
Protein (PRO)	g/L	75.98 ± 5.42	76.80 ± 8.16	76.10 ± 1.69	73.90 ± 5.79
Cholesterol (CHOL)	mmolL	2.30 ± 0.30	1.81 ± 0.14	2.00 ± 0.22	1.87 ± 0.29
High Density Lipoprotein (HDL)	mmolL	1.75 ± 0.36	1.41 ± 0.12	1.68 ± 0.13	1.43 ± 0.25
Alanine transaminase (ALT) test	IU/L	57.48 ± 2.40	47.58 ± 7.95	47.38 ± 1.54	58.60 ± 16.69
Aspartate transaminase (AST)	IU/L	102.48 ± 5.99	96.33 ± 12.14	112.95 ± 6.72	142.68 ± 38.86
Alkaline phosphatase (ALP)	IU/L	276.08 ± 51.24	181.25 ± 65.18	188.83 ± 30.21	228.18 ± 56.97
Albumin Blood test (ALB)	g/L	32.73 ± 2.67	32.18 ± 0.85	32.88 ± 0.39	31.75 ± 1.46
Urea	mmol/L	7.34 ± 0.82	8.50 ± 0.76	7.26 ± 0.63	10.80 ± 0.40
Creatinine (CRE)	umolL	79.59 ± 4.61	79.11 ± 1.98	78.63 ± 1.45	82.23 ± 9.57
Triglyceride (TG)	mmol/L	0.74 ± 0.07	0.67 ± 0.09	0.69 ± 0.05	0.63 ± 0.11
Low Density Lipoprotein (LDL)	mmol/L	0.22 ± 0.09	0.10 ± 0.03	0.28 ± 0.10	0.15 ± 0.09

DISCUSSION

Despite the documented uses of *S. mombin*, three major barriers have limited its safe use as a medicinal remedy [29], including;

1. Lack of standardization in the preparation and dosing of *S. mombin* extracts, as the bioactive compounds in these extracts can vary widely depending on factors such as the species of plant used, the part of the plant used, and the method of preparation [17,19]. This makes it difficult to compare results across studies and establish consistent dosing guidelines.
2. A relatively small number of toxicity studies have been conducted on the traditional-based preparations of *S. mombin.* Current conclusions regarding *S. mombin's* toxicity are mainly drawn from studies using organic solvent extracts (e.g., methanol, dichloromethane), which do not reflect traditional aqueous-based preparations [12]. For instance, most prior toxicity studies on the leaves of *Spondias mombin* in Nigeria have focused on aqueous [22,23], methanolic [24,25] and ethanolic [23] extracts, thus leaving a significant gap regarding the toxicological profile of the hydroethanolic leaf extract of *S. mombin*, which has shown promising potency and safety matrices in recent studies [26,27].
3. Gaps in the literature also exist on the long-term safety of the hydroethanolic leaf extracts of *S. mombin*. While *S. mombin* extracts are generally considered safe when taken in recommended dosages, more research is needed to determine the potential risks of long-term use. This is especially important for people with underlying health conditions or who are taking medications that may interact with these supplements.

Indeed, while there is some scientific literature on the potential health benefits of the hydroethanolic leaf extracts of *S. mombin*, these gaps in the current research necessitate further research to enhance scientific understanding of the effects of the extract on human health.

This evaluation provides critical and timely preclinical evidence to support the ethnomedicinal use of hydroethanolic *S. mombin* leaf extract, guide the dosing of the hydroethanolic leaf extract of *S. mombin,* and objectively quantify its long- and short-term biochemical and hematological effects, ultimately contributing to the discovery of new therapeutic leads. This study, to the best of our knowledge, represents the first investigation on the oral acute, subacute, and subchronic toxicity of *S. mombin* hydroethanolic extract in rodent models in Nigeria, highlighting the novelty and importance of this investigation.

In the acute toxicity study, no statistically significant difference was observed in the relative weights of treated and control mice. Additionally, oral administration of the hydroethanolic extract of *S. mombin* at doses up to 2000 mg/kg did not cause any signs of toxicity, including behavioral abnormalities, or clinical symptoms (such as changes in skin, eyes, salivation, or excretion) in mice over the 14-day observation period. Body weight changes are indicators of adverse effects of chemical drugs and products; thus, the comparable weights of treated and control mice suggest a desirable safety profile for the hydroethanolic leaf extract of *Spondias mombin.* This is similar to the findings of Mayur *et al* 2020, who investigated the toxicological profile and effects of *Spondias mombin* methanolic leaf extract in experimental rats in India and reported the absence of any signs of toxicity or mortality after acute administration of the extract at doses up to 2000 mg/kg [30]. In Nigeria, Nwaogwugwu *et al* (2018) also reported that oral administration of the aqueous leaf extract of *S. mombin* from 400 to 5000 mg/kg did not produce any toxicity, mortality in the experimental albino rats. This validates the safe acute use of *S. mombin* hydroethanolic extracts in ethnomedicine.

White blood cells, or leukocytes, are part of the immune system and participate in innate and humoral immune responses as they circulate in the blood and mediate inflammatory and cellular responses to injury or pathogens [31].

For instance, lymphocytosis, defined by an increase in absolute lymphocyte count (ALC) to more than 4000 lymphocytes/microL in adult patients, is a common hematologic abnormality associated with viral bacterial and parasitic infections [32]. In the subacute study, *S. mombin* significantly reduced mean absolute lymphocytes and percentage Lymphocytes with peak reductions at 1000 mg/kg compared to the control. Subsequently, *S. mombin* hydroethanolic extract exhibited statistically significant dose-dependent increase in mean white blood cells and absolute granulocytes with a peak reduction at a dose of 200 mg/kg compared to the control in the subchronic study, which highlights the immunomodulatory potential of the hydroethanolic leaf extract of *S. mombin* and its potential benefits in managing infectious disease. Our findings are in contrast to the results of an evaluation of the protective role of the aqueous extract of *Spondias mombin* against the spleenotoxic effect of Phenylhydrazine (PHZ) by Innih *et al* (2020); who reported no significant changes in the total white blood cell count across all the groups of Wistar rats treated with *S. mombin* as compared with the control group [33], further highlighting the potential impact of extraction solvent choice on the pharmacological and safety characteristics of natural product extracts.

Anemia is characterized by a reduction in red cell mass or a reduction in the quantity and quality of hemoglobin, leading to a decreased oxygen concentration in the tissues and ultimately affecting cellular metabolism [33]. In this study, the hydroethanolic extract of *S. mombin* also exhibited a dose-dependent increase in mean red blood cell (and hemoglobin count validating its ethnomedicinal use as a hematinic agent [17,19] and positioning the extract for discovery of the next generation of hematinics. Our findings also align with Innih *et al* (2020) who showed the ability of an aqueous extract of *S. mombin to* ameliorate Phenylhydrazine (PHZ) induced anemia [33].

In addition, the biochemical results of the reversibility study demonstrated significantly higher Aspartate transaminase (AST) and Urea levels in the control group relative to the treated groups. The observed reduction in urea and AST levels in the treated rats 14 days post-administration of extract suggests the

hepato- and reno-protective effects of the hydroethanolic stem extract of *S. mombin,* similar to the findings of Nwidu *et al* (2018) [25]. The impact of this finding is further demonstrated as an increase in urea, also known as Blood Urea Nitrogen (BUN), has been reported to correlate with decreased renal excretion [34], hence the mean reduction of urea by *S. mombin* opens up new horizons for novel reno-protective therapies.

Dyslipidemias significantly contribute to the onset of non-alcoholic fatty liver disease, insulin resistance, and obesity (among other diseases) and are often related to reduced levels of high-density lipoprotein (HDL) and/or the elevation of serum and/or tissue levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and very low-density lipoprotein cholesterol (VLDL) [35,36]. In the subchronic study, a statistically significant decrease in total cholesterol was observed at a dose peak of 40 mg/kg of *S. mombin* hydroethanolic extract relative to the control group. This aligns with the findings of Lucena *et al* (2022), who proved that the regular consumption of the lyophilized pulp and peel extract of *S. mombin* induced a cholesterol-lowering effect, reduced lipid accumulation in the liver, increased fecal lipid excretion, and protected the liver from oxidative damage caused by the consumption of a high-fat diet in Brazil [36]. Similarly, in Benue, Nigeria, Anebi *et al* (2025) investigated the effects of the ethanolic leaf extract of *S. mombin* on the lipid level of Wistar rats and showed that *in vivo* administration of *S. mombin leaf* extract significantly reduced total cholesterol, triglycerides, and LDL levels while increasing HDL [35]. The results from our finding, however show a more significant reduction (31.2%) in total cholesterol levels at 40 mg/kg compared to the results of Anebi *et al* (2025), which showed only an average of 8.6% reduction in total cholesterol levels of the treatment group following a 7-day administration of the 200 and 400 mg/kg of the ethanolic leaf extract of *S. mombin.* These striking differences may be due to the duration of therapy, dosage, and ultimately warrant further investigation on the lipid-lowering effects of *S. mombin,* preferably in human subjects. Overall, our findings position the hydroethanolic extract as an attractive and promising source of efficacious lipid-lowering compounds with minimal toxicity effects.

Limitations and Generalizability

In alignment with the objectives of this study, assessments such as the functional observation battery (FOB), urinalysis, thyroid hormone (T3, T4, TSH) profiling, organ weight measurement, and histopathological examination were not conducted. Gross necropsy did not reveal any observable organ abnormalities; however, histopathological analyses were not conducted in this study and are planned for follow-up studies. These endpoints are typically required for extended or definitive toxicity evaluations (e.g., OECD TG 408 or 90-day studies). However, the present study was designed as a preclinical screening investigation to provide preliminary insights into the acute, subacute, and subchronic systemic toxicity of *Spondias mombin* hydroethanolic leaf extract. Future studies are planned to incorporate these parameters in line with full regulatory toxicology requirements.

CONCLUSION

The ethnomedicinal use of *Spondias mombin*, combined with its demonstrated low toxicity in acute, subacute, and sub-chronic models, underscores its potential as a novel therapeutic lead. Validation of its safety profile not only supports its continued traditional use but also positions the hydroethanolic leaf extract as a promising candidate for further pharmacological development—particularly in the search for hematinics, anti-infectives, and hepatoprotective agents.

These findings are especially relevant in the context of evolving regulatory priorities, including the FDA's emphasis on diversity in compound origins, rare disease therapeutics, and novel mechanisms of action. While this study provides critical preclinical evidence, future investigations should incorporate chronic dosing studies, organ-specific histopathology, and therapeutic window assessments to comprehensively evaluate long-term safety and translational potential.

DECLARATIONS

Ethics approval and consent to participate: Ethical clearance was obtained from the Research Ethics Committee of the University of Lagos. *(REG NO: UNILAGREC/22/06/005)*

Consent for publication: Not Applicable

Availability of data and material: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests

Funding: This project was funded by the Tertiary Education Trust Fund (TETFund), registration number TETF/ES/DR&D-CE/NRF2021/SETHi/HSW/00290/VOL.1 with Gloria A. Ayoola as the Principal Investigator, and the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, as the hosting laboratory.

Authors' contributions: The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript. Specific author contributions are as follows: GAA is the Principal Investigator. She developed the research concept and design, analyzed and interpreted data, and reviewed and approved the manuscript; A.S.A. developed the research concept and design, collected, analyzed, and interpreted data, and prepared and reviewed the manuscript; IOI developed the research concept and design, analyzed and interpreted data, and reviewed and approved the manuscript; DBO developed the research concept and design, collected, analyzed, and interpreted data, and approved the manuscript; OOJ critically reviewed and approved the manuscript; DKA critically reviewed and approved the manuscript; MOO critically reviewed and approved the manuscript; AOA critically reviewed and approved the manuscript.

Acknowledgements: We acknowledge the numerous authors whose works are referenced in this study for laying the foundation and inspiring our research.

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ABBREVIATIONS

Abbreviation	Full Meaning
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
EDTA	Ethylenediaminetetraacetic Acid
FOB	Functional Observation Battery
LD_{50}	Median Lethal Dose
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organization for Economic Co-operation and Development
RBC	Red Blood Cell
UDP	Up-and-Down Procedure
WBC	White Blood Cell

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