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Research Article

Multi-organ assessment of *Tamarindus indica* Linn., seed extracts in albino rats

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Background: *Tamarindus indica* seeds are widely used in traditional medicine, but their systemic safety remains inadequately characterized. This study evaluated the 28-day sub-acute toxicological and therapeutic effects of aqueous and ethanolic *T. indica* seed extracts in Wistar albino rats.

Methods: Following phytochemical screening and acute toxicity testing (Lorke's method), 40 rats received oral aqueous or ethanolic extracts (150, 350, or 600 mg/kg) daily for 28 days. Haematological, biochemical (hepatic, renal, lipid, and oxidative stress), and histopathological assessments were performed.

Results: Both extracts exhibited high acute safety (LD_{50} > 5000 mg/kg). The aqueous extract showed a favourable safety profile, preserving liver and kidney architecture while significantly reducing triglycerides and increasing high-density lipoprotein HDL levels. In contrast, the ethanolic extract enhanced intestinal superoxide dismutase activity but caused dose-dependent hepatic necrosis, renal damage, and reduced haemoglobin synthesis. 150, 350 and 600 mg/kg of both extracts produced cardiotoxic effects characterized by myocardial necrosis.

Conclusion: *T. indica* seed extracts possess solvent-dependent therapeutic potential but demonstrate narrow therapeutic windows. Their dose-dependent organ toxicity highlights the need for careful dose optimization and comprehensive safety evaluation before clinical application.

Keywords: *Tamarindus indica seeds, multi-organ toxicity, ethnopharmacology, hypolipidemic activity, cardiotoxicity, oxidative stress*

Introduction

Recent advancements in pharmacognosy have recognized the seeds of *Tamarindus indica* as a formidable reservoir of bioactive secondary metabolites. Comprehensive phytochemical profiling has demonstrated that the seeds are densely packed with phenolics, flavonoids, tannins, alkaloids, and saponins (Chacón-Fernández et al., 2019). Specific phenolic constituents, most notably epicatechin and caffeic acid, have been identified as primary drivers of the seeds' antioxidant efficacy (Natukunda et al., 2016). These compounds actively combat systemic oxidative stress by significantly reducing lipid peroxidation and restoring the critical cellular activity of endogenous enzymatic antioxidants, such as superoxide dismutase (SOD) and catalase (Natukunda et al., 2016). Consequently, *T. indica* seed extracts have been deeply integrated into ethnomedicine across African, Ayurvedic, and Unani healing systems. Traditionally, the seeds have been widely employed as potent natural remedies for treating conditions such as stomach ulcers, dysentery, and bladder stones, and

have even been utilized as an antidote for snakebites (Havinga et al., 2010). Despite the extensive history of traditional usage and the well-documented pharmacological properties of *T. indica* seeds, their consumption is not devoid of adverse physiological effects. Unrefined tamarind seeds contain high levels of dense tannins and dietary fiber, which, upon excessive ingestion, can act as potent laxatives and precipitate severe gastrointestinal discomfort (Kumar et al., 2008). Clinical and traditional observations have directly linked excessive consumption of the seeds to bloating, flatulence, and diarrhea, which can eventually culminate in systemic dehydration. Furthermore, idiosyncratic allergic reactions have been documented following the ingestion of the seed extracts. These allergic manifestations are typically characterized by transient itching and dermal rashes, and in rare, severe instances, can lead to dizziness or respiratory distress (Iskandar et al., 2017). While numerous fragmented studies have investigated the isolated impact of *T. indica* on specific biological systems, there is an alarming absence of comprehensive, single-study multi-organ toxicological evaluations (Iskandar et al., 2017). Current research fails to provide a consolidated systemic safety matrix detailing the concurrent haematological, cardiac, renal, hepatic, and intestinal responses to varying doses of *T. indica* extracts. The first objective of the study is to ascertain the percentage extraction yield and determine the qualitative phytochemical composition of the aqueous and ethanolic seed extracts. Secondly, to evaluate the acute toxicity and establish the median lethal dose (LD_{50}) of both extracts to define a baseline safety margin in the animal models. Thirdly, to assess the 28-day sub-acute physiological effects of the extracts by measuring haematological parameters, key biochemical markers governing liver and kidney function, systemic lipid profiles, and localized intestinal oxidative stress biomarkers (Krstic et al., 2016; Devi and Boruah, 2020). Finally, the study aims to investigate and correlate these biochemical alterations with detailed histopathological parameters in critical target organs, specifically the liver, heart, kidneys, and intestines.

Materials and Methods

Materials, Study Area, and Experimental Animals

The study was conducted at the Drug Development Division and the Central Diagnostic Laboratory of the National Veterinary Research Institute (NVRI), Vom, Nigeria. A total of forty (40) adult albino rats (Wistar strain), weighing between 100 and 180 g, were procured from the institute's small animal house. The animals were acclimatized and housed under germ-free, standardized environmental conditions (25 °C ambient temperature, 33% relative humidity). They were provided with standard commercial pelletized feed and access to clean drinking water *ad libitum*. All handling and experimental protocols were reviewed and approved by the NVRI Animal Use Ethical Committee and the Plateau State Ministry of Livestock Development (Approval No: MANR/SEC/47/S.6.VOL.I) prior to the study.

Plant Collection and Extraction

Mature, dried *Tamarindus indica* seeds were sourced from the Bukuru market in Jos South Local Government Area, Plateau State. The seeds were subjected to natural desiccation under direct sunlight, and their botanical identity was verified by the resident botanist at the National Veterinary Research Institute, Vom using the NVRI beneficial and poisonous plant inventory (Akpojosevbe et al., 2024). The seeds were pulverized into a fine powder. Following the standardized maceration technique described by Sofowara (1993) to preserve thermolabile phytochemicals, 1000 g of the powder was macerated separately in distilled water and 70% ethanol at a 1:10 (w/v) ratio. The mixtures were allowed to stand for 48 hours with intermittent agitation. The resulting crude extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and stored in sterile, airtight containers at 4 °C.

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of the *T. indica* seed extracts were conducted following the established protocols of Trease and Evans (2020) and Sofowara (1993). The extracts were evaluated for tannins (using H_2SO_4 and 5% ferric chloride), flavonoids (using 10% NaOH and dilute HCl), saponins (via the frothing test), cardiac glycosides (using pyridine and sodium nitroprusside), anthraquinones (using aqueous sulfuric acid, benzene, and 10% ammonia), alkaloids (using Dragendorff's reagent), and steroids (using Mayer's reagent).

Acute Toxicity Testing (LD_{50})

The median lethal dose (LD_{50}) for both the aqueous and ethanolic extracts was determined using the method described by Lorke (1983). In Phase 1, the rats were randomly divided into three groups and orally administered the extracts at doses of 10, 100, and 1000 mg/kg body weight, followed by observation for 24 hours to 7 days. In Phase 2, higher doses of 1600, 2900, and 5000 mg/kg were administered to evaluate mortality and systemic toxicity, definitively establishing the acute safety margin. The procedure was repeated separately for the aqueous and ethanolic extracts.

Sub-Acute Toxicity Experimental Design (28 Days)

For the 28-day sub-acute toxicological assessment, the albino rats were randomized into four groups of five animals each for both the aqueous and ethanolic extracts. Group 1 served as the control, receiving distilled water. The treatment groups (Groups 2, 3, and 4) were orally administered daily extract doses of 150 mg/kg, 350 mg/kg, and 600 mg/kg body weight, respectively.

Haematological and Biochemical Assays

Following the 28-day regimen, blood and tissues were collected for systemic evaluation. Packed Cell Volume (PCV), Haemoglobin (Hb) concentration, Red Blood Cell (RBC) counts, and White Blood Cell (WBC) counts were quantified using standard manual haemocytometer methods and micro-hematocrit capillary techniques as described by Decie and Lewis (1991). Serum Aspartate transaminase (AST) and Alanine transaminase (ALT) levels were determined according to the colorimetric methods of Reitman and Frankel (1957). Serum total protein (TP) was also evaluated. Blood Urea Nitrogen (BUN), Creatinine, and Sodium levels were analyzed spectrophotometrically based on established clinical methods (Hosten, 1990) to determine renal excretory integrity and electrolyte homeostasis. Serum total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were quantified using fully automated enzymatic protocols (Devi and Boruah, 2020). Intestinal tissue homogenates were assayed for malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) to evaluate localized lipid peroxidation and cytoprotective mechanisms (Devi and Boruah, 2020).

Gross and Histopathological Examination

Following humane sacrifice at the conclusion of the study, the liver, kidneys, heart, and intestines were harvested and visually inspected. The tissues were promptly fixed in 10% neutral buffered formalin, dehydrated through a graded alcohol series, cleared in xylene, and embedded in paraffin wax. Microtome sections of 5-8 µm thickness were prepared and stained with Haematoxylin and Eosin (H&E). Microscopic evaluations were conducted to document tissue necrosis, cellular degeneration, or architectural changes at X400 magnification (Bancroft and Gamble, 2008; Luna, 1968)

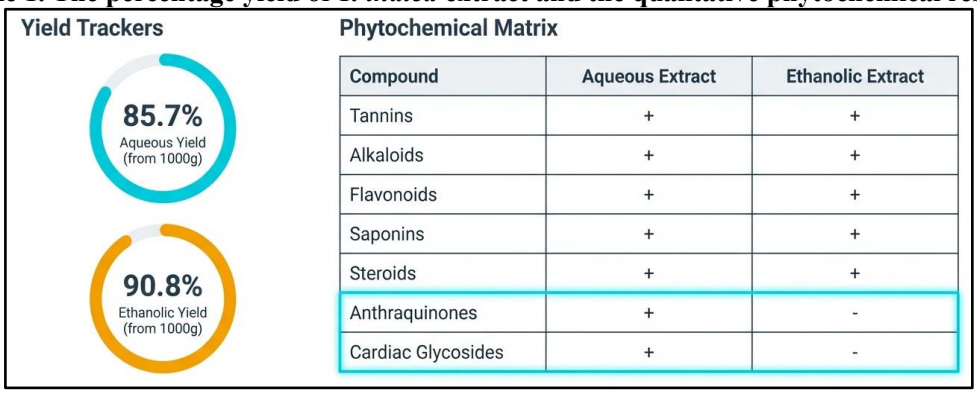
Statistical Analysis

All quantitative data were expressed as the standard mean ± standard deviation (SD) and evaluated using the Statistical Package for the Social Sciences (SPSS), version 25. Group comparisons were executed via one-way Analysis of Variance (ANOVA), followed by applicable post-hoc tests to identify significant dose-dependent variations. Statistical significance was defined as $p < 0.05$.

Results

Extraction Yield and Phytochemistry

Table 1. The percentage yield of *T. indica* extract and the qualitative phytochemical results



Compound	Aqueous Extract	Ethanolic Extract
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	+	+
Anthraquinones	+	-
Cardiac Glycosides	+	-

Table 1 shows the extraction yield and qualitative phytochemical screening results of *T. indica* seed extract. The ethanolic extract yielded 90.8%, slightly outperforming the aqueous extract, which yielded 85.7% from the initial 1000 g of plant material. Qualitative phytochemical screening revealed a robust matrix of secondary metabolites; both the aqueous and ethanolic extracts contained tannins, alkaloids, flavonoids, saponins, and steroids. The aqueous extract uniquely contained

anthraquinones and cardiac glycosides, which were completely absent in the ethanolic fraction, granting the aqueous extract a broader overall phytochemical profile.

Acute Toxicity (LD_{50})

Zero mortality was recorded across all tested groups after 24 hours and up to 7 days of continuous observation, even at the maximum administered dose of 5000 mg/kg body weight. Despite the lack of fatal toxicity, transient idiosyncratic reactions characterized by severe itching and dermal rashes were observed in the animal subjects at specific lower doses (100 mg/kg and 1000 mg/kg) upon ingestion, though these symptoms resolved without inducing mortality.

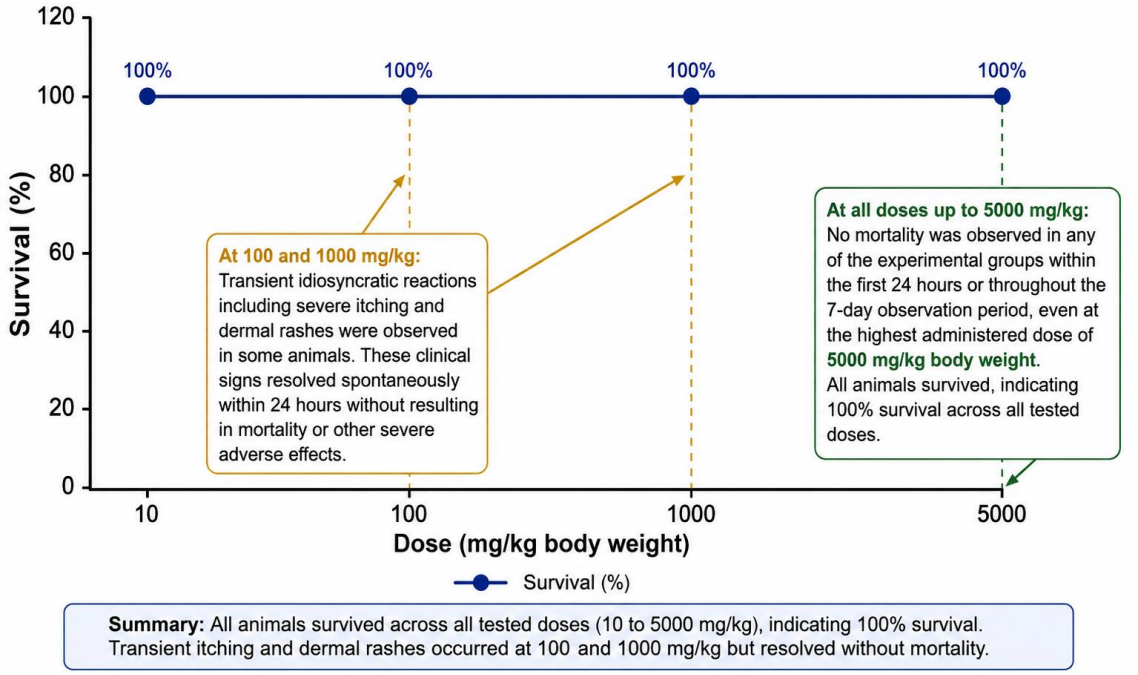


Figure 1. Acute toxicity test results for both aqueous and ethanolic *T. indica* seed extracts tested in albino Wistar rats

Histopathological Findings

Liver: The aqueous extract maintained entirely normal hepatic tissue architecture across all administered doses (150, 350, and 600 mg/kg). Conversely, the ethanolic extract induced severe hepatic damage, explicitly characterized by massive hepatic congestion and cellular necrosis at the 350 mg/kg and 600 mg/kg thresholds (Figure 2).

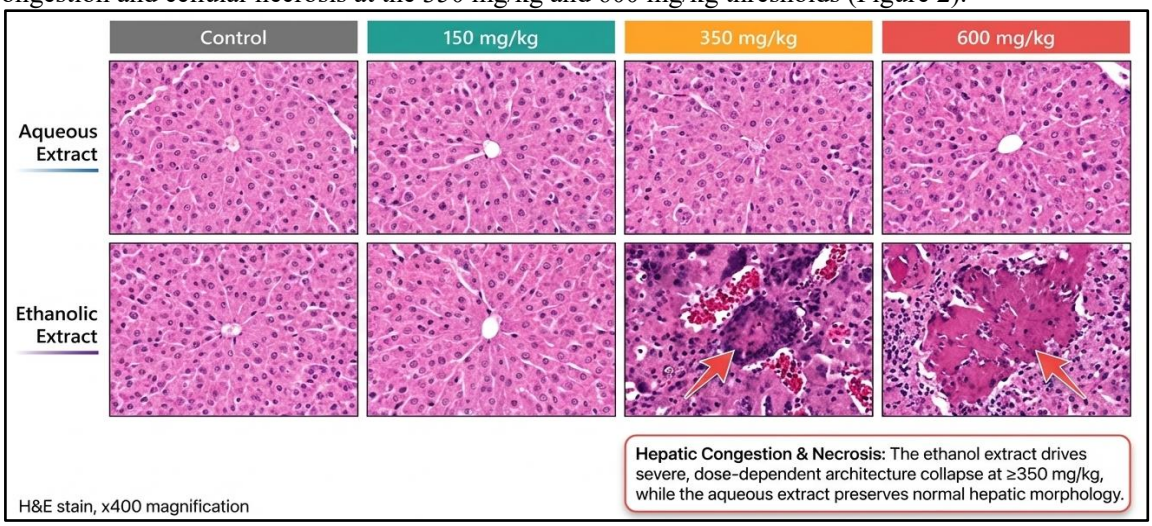


Figure 2. The histopathological micrographs of the liver of albino Wistar rats exposed to different doses of aqueous and ethanolic seed extracts of *T. indica*. (H & E, x400)

Kidney: Similar to the liver, kidney tissues from all groups administered the aqueous extract displayed normal, healthy morphology. The ethanolic extract, however, induced marked architectural damage at higher doses (350 mg/kg and 600 mg/kg), evidenced by congested renal tubules and oedematous renal vessels (Figure 3).

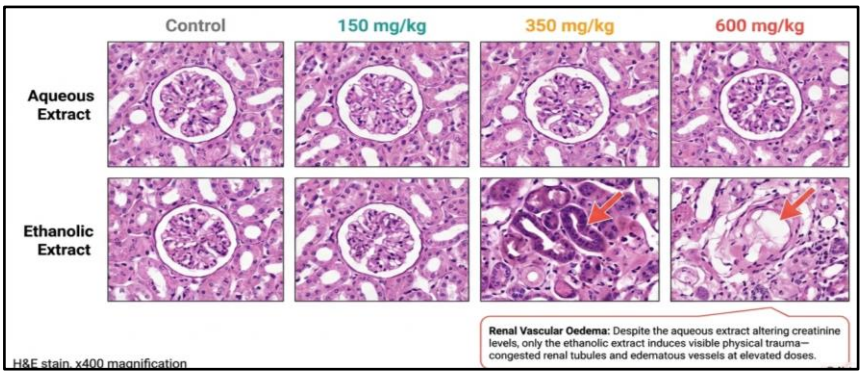


Figure 3. The histopathological micrographs of the kidneys of albino Wistar rats exposed to different doses of aqueous and ethanolic seed extracts of *T. indica*. (H & Ex400)

Heart: The heart emerged as highly susceptible to extract-induced stress. Doses (100 mg/kg, 350 mg/kg and 600 mg/kg) of both the aqueous and ethanolic extracts caused distinct myocardial necrosis. This indicates a universal, solvent-independent cardiac vulnerability when exposed to different concentrations of the seed extract (Figure 4).

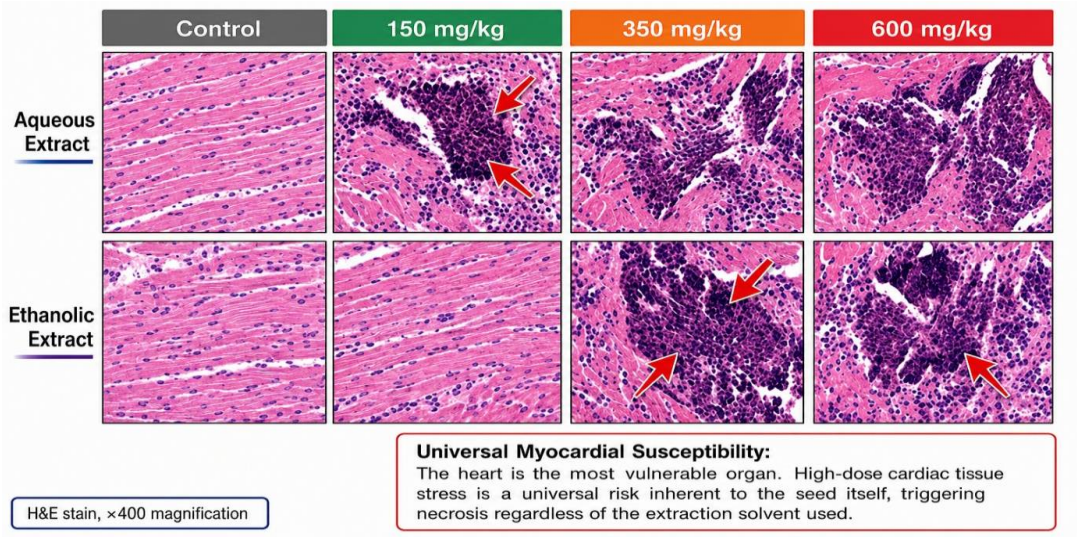


Figure 4. The histopathological micrographs of the heart of albino Wistar rats exposed to different doses of aqueous and ethanolic seed extracts of *T. indica* (H & Ex400)

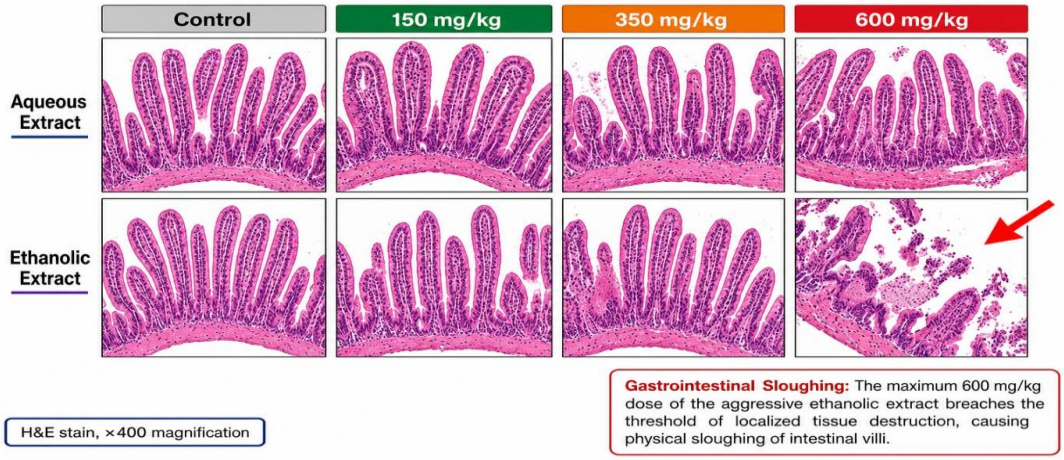


Figure 5. The histopathological micrographs of the small intestine of albino Wistar rats exposed to different doses of aqueous and ethanolic seed extracts of *T. indica* (H&E x400)

Intestines: The aqueous extract was proven safe for the gastrointestinal tract, maintaining normal morphological architecture across all doses. The ethanolic extract, however, reached a threshold of localized tissue destruction, causing the physical sloughing of the intestinal villi exclusively at the maximum 600 mg/kg dose (Figure 5).

Haematological Assay

Table 2 revealed differential effects between the extracts. The ethanolic extract significantly impacted haemoglobin synthesis, demonstrating a marked decrease at higher doses (350 mg/kg and 600 mg/kg), while the aqueous extract left haemoglobin levels relatively stable. Conversely, the aqueous extract caused a statistically significant, dose-dependent decrease in the RBC count, whereas the ethanolic extract did not significantly alter RBC counts. The ethanolic extract caused significant fluctuations in the total WBC count, marked by an initial spike at the low dose (150 mg/kg) followed by suppression at higher doses. It also significantly increased the percentage of Lymphocytes at the maximum dose of 600 mg/kg. The aqueous extract, on the other hand, exhibited a very mild profile, showing no significant impact on WBCs, Neutrophils, or Lymphocytes across any of the administered concentrations. Both extracts showed no statistically significant impact on PCV.

Table 2. Haematological parameters in albino rats administered *T. indica* extracts

Parameters	Control	150 mg/kg	350 mg/kg	600 mg/kg	*p*-value
Aqueous extract PCV (%)	32.00 ± 2.65	35.00 ± 1.00	32.67 ± 3.52	34.33 ± 0.58	0.390
Hb (g/dL)	9.00 ± 0.52	8.70 ± 0.50	9.93 ± 1.01	8.43 ± 0.25	0.104
RBC ($x10^6/\mu L$)	4.53 ± 0.06	3.73 ± 0.42	3.77 ± 0.28	3.50 ± 0.10	0.011 *
WBC ($x10^3/\mu L$)	4.27 ± 0.15	4.77 ± 0.50	4.40 ± 0.30	4.43 ± 0.23	0.342
N (%)	62.33 ± 11.68	47.67 ± 2.08	53.00 ± 6.08	44.00 ± 4.36	0.448
L (%)	45.33 ± 10.26	50.00 ± 1.00	53.67 ± 0.58	53.67 ± 0.58	0.698
Ethanolic extract					
PCV (%)	38.33 ± 2.52	38.00 ± 1.00	36.67 ± 1.15	35.00 ± 1.00	0.105
Hb (g/dL)	13.00 ± 0.60	12.43 ± 1.27	9.93 ± 0.84	10.97 ± 0.59	0.007 *
RBC ($x10^6/\mu L$)	4.93 ± 0.40	4.57 ± 0.31	4.87 ± 0.64	5.40 ± 0.26	0.200
WBC ($x10^3/\mu L$)	4.37 ± 1.10	6.63 ± 0.40	3.30 ± 0.30	4.13 ± 0.55	0.002 *
N (%)	49.33 ± 10.50	52.67 ± 15.70	43.00 ± 4.58	57.67 ± 15.95	0.354
L (%)	37.67 ± 11.68	45.00 ± 15.13	60.33 ± 2.08	64.00 ± 3.61	0.032*

(Note: Data is represented as Mean ± Standard Deviation; * Significantly different from the control at $p < 0.05$; PCV= Packed Cell Volume, Hb= Haemoglobin, RBC= Red Blood Cells, WBC= White Blood Cells, N= Neutrophils, L= Lymphocytes).

Biochemical Assays

Table 3 shows the effects of both extracts on the liver function of albino rats. The aqueous extract showed no statistically significant changes in Aspartate transaminase (AST), Alanine transaminase (ALT), or Total Protein (TP) levels. Conversely, the ethanolic extract significantly elevated both AST and ALT levels in a dose-dependent manner, while simultaneously disrupting TP metabolism, particularly at the higher dosage tiers.

Table 3. Effects of *T. indica* extract on liver function parameters of albino rats

Parameters	Control	150 mg/kg	350 mg/kg	600 mg/kg	p-value
Aqueous extract					
AST (U/L)	44.67 ± 6.33	50.00 ± 7.81	41.67 ± 1.15	41.00 ± 0.00	0.198
ALT (U/L)	22.33 ± 2.31	21.00 ± 0.00	21.00 ± 0.00	18.33 ± 2.31	0.085
TP (g/L)	59.33 ± 4.20	55.03 ± 0.73	57.11 ± 2.05	55.71 ± 2.16	0.273
Ethanol extract					
AST (U/L)	53.00 ± 1.73^c	54.00 ± 1.00^bc	57.00 ± 1.73^ab	60.67 ± 3.06^a	0.007
ALT (U/L)	17.00 ± 0.00^c	18.33 ± 2.31^bc	21.00 ± 0.00^ab	24.33 ± 3.06^a	0.007
TP (g/L)	61.19 ± 3.90^a	54.80 ± 0.92^b	60.89 ± 2.90^a	53.58 ± 2.78^b	0.020

(Mean ± SD along each row that do not share a superscript are significantly different at 5% level. AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, TP = Total Protein)

Table 4 shows the effects of both extracts on the renal function of albino rats. Both extracts altered key renal excretory markers but exhibited distinct pathogenic profiles. The ethanolic extract significantly elevated serum urea and creatinine levels exclusively at the maximum 600 mg/kg threshold, suggesting high-dose renal stress, while maintaining stable sodium levels across all groups. Alarmingly, the aqueous extract elevated creatinine levels across all administered doses and significantly disrupted systemic electrolyte homeostasis by elevating serum sodium at the 600 mg/kg dose.

Table 4. Effects of *T. indica* seed extracts on renal function parameters of albino rats

Parameters	Control	150 mg/kg	350 mg/kg	600 mg/kg	*p*-value
Aqueous extract					
Urea (mg/dL)	40.90 ± 1.94	40.55 ± 1.16	38.17 ± 0.72	41.93 ± 0.63	0.030*
Creatinine (mg/dL)	0.33 ± 0.05	0.61 ± 0.16	0.59 ± 0.04	0.67 ± 0.05	0.008*
Sodium (mEq/L)	36.59 ± 1.39	38.29 ± 0.27	35.79 ± 1.44	41.14 ± 2.05	0.008*
Ethanolic extract					
Urea (mg/dL)	40.17 ± 2.00	37.96 ± 1.17	39.34 ± 1.12	43.14 ± 0.69	0.008*
Creatinine (mg/dL)	0.60 ± 0.03	0.56 ± 0.03	0.53 ± 0.08	0.71 ± 0.03	0.009*
Sodium (mEq/L)	37.48 ± 0.07	37.50 ± 2.78	37.34 ± 1.83	37.52 ± 1.12	0.999

(Note: Data is represented as Mean ± Standard Deviation. * Significantly different from the control at $p < 0.05$.)

Table 5 revealed the effects of both extracts on the lipid profiles of albino rats. The ethanolic extract had no statistically significant impact on any lipid parameters (total cholesterol, triglycerides, HDL, or LDL) under the tested conditions. The aqueous extract, however, demonstrated active, dose-dependent hypolipidemic efficacy. It significantly lowered systemic triglycerides and simultaneously elevated beneficial High-Density Lipoprotein (HDL) levels, although it did cause a slight but significant elevation in total cholesterol at the maximum dose.

Table 5. The effects of *T. indica* seed extracts on the lipid profiles of albino rats

Parameters	Control	150 mg/kg	350 mg/kg	600 mg/kg	*p*-value
Aqueous extract					
CHOL (mg/dL)	116.97 ± 5.46	121.10 ± 2.52	119.03 ± 4.72	129.97 ± 3.86	0.026*
TG (mg/dL)	152.37 ± 5.48	140.77 ± 5.35	146.07 ± 5.83	138.20 ± 6.94	0.046*
HDL (mg/dL)	44.34 ± 1.77	48.81 ± 6.50	55.79 ± 6.74	55.32 ± 5.74	0.049*
LDL (mg/dL)	42.15 ± 4.43	44.13 ± 7.54	34.03 ± 10.40	47.01 ± 7.55	0.277
Ethanolic extract					
CHOL (mg/dL)	116.47 ± 6.20	120.67 ± 2.95	119.40 ± 2.10	126.37 ± 6.96	0.183
TG (mg/dL)	140.93 ± 5.50	148.43 ± 15.97	147.67 ± 10.91	143.00 ± 6.46	0.788
HDL (mg/dL)	44.52 ± 4.01	43.03 ± 2.96	44.31 ± 4.83	51.55 ± 5.76	0.167
LDL (mg/dL)	43.78 ± 4.36	47.95 ± 0.93	45.56 ± 6.94	46.21 ± 11.44	0.907

(Note: Data is represented as Mean ± Standard Deviation. * Significantly different from the control at $p < 0.05$; CHOL= Cholesterol; TG= Triglycerides; HDL= High-Density Lipoprotein; LDL= Low-Density Lipoprotein.)

Table 6 shows the effects of *T. indica* seed extracts on oxidative stress biomarkers in the intestines of albino rats. Localized intestinal oxidative stress evaluations revealed that both extracts maintained comparably low levels of malondialdehyde (MDA), indicating successful protection against cellular lipid peroxidation. However, the ethanolic extract significantly enhanced Superoxide Dismutase (SOD) activity compared to the aqueous extract, establishing it as a more robust driver of endogenous enzymatic antioxidant defense mechanisms within the gastrointestinal tract.

Table 6. The effects of *T. indica* seed extracts on oxidative stress biomarkers in the intestines of albino rats dosed 600 mg/kg of aqueous and ethanolic extracts

Oxidative Stress Biomarker	Aqueous Extract** (Mean ± SD)	Ethanolic Extract** (Mean ± SD)	*p*-value
Malondialdehyde (MDA)	1.49 ± 0.37	1.60 ± 0.48	0.580
Superoxide Dismutase (SOD)	19.19 ± 2.43	22.66 ± 3.51	0.016*
Catalase (CAT)	4.63 ± 0.78	5.23 ± 1.47	0.256
Glutathione (GSH)	3.43 ± 0.37	3.69 ± 0.37	0.115

(Note: Data is represented as Mean ± Standard Deviation. * Significantly different from the control at $p < 0.05$), ** the treatment groups dosed 600 mg/kg of aqueous and the ethanolic extracts respectively.

Discussion

In this study, the higher yield obtained with ethanol indicates that ethanol extracted a greater number of soluble constituents from the seeds than water. This suggests that ethanol is a more efficient solvent for recovering phytochemicals from *T. indica* seeds. This aligns with the principle that ethanol, possessing both polar and non-polar characteristics, is highly efficient at dissolving a broader array of complex phytochemicals compared to water (Bonin et al., 2023). Anthraquinones and cardiac glycosides were detected only in the aqueous extract, demonstrating that solvent polarity influences the phytochemical profile of *Tamarindus indica* seed extracts. Anthraquinones are widely recognized for their potent laxative and gastrointestinal modulating effects (Kumar et al., 2008). Their exclusive presence in the aqueous extract provides a strong biochemical rationale for the historical ethnomedicinal use of aqueous tamarind decoctions in treating digestive disorders. For the acute toxicity (LD_{50}) evaluation, the extracts demonstrated an exceptionally high baseline safety margin, with zero mortality recorded across all administered phases up to the maximum dose of 5000 mg/kg. These findings corroborate previous reports of low acute toxicity profiles for *Tamarindus indica* (Iskandar et al., 2017). Nevertheless, the transient itching and dermal rashes observed at 100 and 1000 mg/kg demonstrate that the extract is capable of producing reversible biological effects despite its low acute lethality. The absence of similar reactions at higher doses may reflect a non-linear dose-response, adaptive physiological mechanisms, or variability in qualitative clinical observations, and therefore warrants further investigation. Importantly, the acute toxicity findings should not be interpreted as evidence of overall safety, as the repeated-dose study revealed significant dose-dependent organ toxicity. These results emphasize that mortality alone is an insufficient indicator of toxicological risk and underscore the importance of comprehensive sub-acute and chronic toxicity evaluations. The ethanol extract of *T. indica* exhibited marked effects on haematological parameters. PCV remained relatively stable across all tested concentrations, with no statistically significant changes observed. This finding aligns with the report by Iskandar et al. (2017), who similarly noted minimal alterations in PCV following administration of tamarind extracts. In contrast, Hb levels showed a significant decline at higher concentrations of the ethanol extract. This decrease may indicate interference with haemoglobin synthesis or stability, corroborating the findings of Mehdi et al. (2019), who reported comparable reductions in Hb levels at elevated doses of tamarind extract. RBC counts remained unaffected across all treatment groups, a trend consistent with the dose-dependent and variable effects noted by Sathiya et al. (2018). WBC counts, however, demonstrated a biphasic pattern, an increase at lower doses and a decrease at higher concentrations. This pattern may reflect potential immune-modulatory effects of the ethanol extract, where lower doses stimulate immune activity, whereas higher doses may suppress it or induce toxicity. Additionally, neutrophil and lymphocyte counts varied across doses, with a significant rise in lymphocyte levels observed at 600 mg/kg, suggesting a possible immune-stimulatory response at higher concentrations.

In comparison, the aqueous extract of *Tamarindus indica* exhibited a more stable haematological profile. PCV remained consistent across all concentrations, mirroring the findings of Iskandar et al. (2017), who also reported negligible effects on PCV following aqueous tamarind extract administration. Hb levels showed only slight, non-significant reductions at higher concentrations, suggesting that the aqueous extract may exert a less pronounced effect on haemoglobin synthesis than its ethanol counterpart. However, RBC counts decreased significantly with increasing extract concentration, indicating a possible adverse impact on erythropoiesis or red cell survival, as noted by Chacón-Fernández et al. (2019). WBC counts were largely unchanged across all groups, consistent with the observations of Iskandar et al. (2017), who found that aqueous tamarind extracts generally do not alter leukocyte counts significantly. Neutrophil and lymphocyte percentages also remained stable, further supporting the inference that the aqueous extract does not markedly affect these immune cell populations in agreement with findings by Sookying et al. (2022). For the biochemical analysis, no significant changes were observed in AST, ALT, or TP levels for rats administered the aqueous extract of *T. indica*. These findings support the histopathological evidence indicating that the aqueous extract is relatively safe and does not significantly compromise liver function, even at higher doses. The absence of alterations in these markers suggests minimal hepatocellular damage and negligible impact on protein synthesis or hepatic protein metabolism. Conversely, administration of the ethanol extract resulted in significant biochemical changes, particularly at higher concentrations. Elevated ALT and AST levels indicated hepatic stress or injury, as these enzymes are released into circulation following hepatocyte damage. The observed reduction in TP across all ethanol-treated groups further suggests that the extract may impair hepatic protein synthesis at elevated doses. Interestingly, an unexpected increase in TP was noted in the group receiving 350 mg/kg, possibly reflecting a compensatory response or transient protein retention due to moderate liver stress. For the renal parameters, creatinine and urea levels in the ethanol extract-treated groups were significantly decreased compared to the control. This suggests possible alterations in renal processing or function. However, at 600 mg/kg, urea levels increased markedly, indicating potential renal stress or impaired nitrogenous waste excretion. A concurrent rise in creatinine at this dose further supports the likelihood of compromised renal clearance or early nephrotoxicity. These findings diverge from those of Sumithira et al. (2021), who reported nephroprotective effects of *T. indica* extracts. Serum sodium levels remained stable across all groups, indicating that neither extract significantly disrupted sodium homeostasis, an observation consistent with Imo et al. (2019), who reported similar results for other

herbal preparations. For the aqueous extract, significant variability was noted across renal markers. Reduced urea levels in certain groups may suggest enhanced renal excretion or altered protein metabolism, whereas elevated urea in other groups implies possible renal inefficiency or stress at higher doses. Additionally, the consistent rise in creatinine levels among all aqueous extract-treated groups may indicate potential renal burden or impaired glomerular filtration. The extracts exhibited vastly different impacts on lipid metabolism. The ethanolic extract was largely inactive regarding lipid modulation. However, the aqueous extract demonstrated excellent hypolipidemic potential, actively lowering systemic triglycerides and significantly elevating beneficial high-density lipoprotein (HDL) levels. Despite this cardiovascular promise, histological evaluation revealed a profound paradox: both the aqueous and ethanolic extracts induced direct cardiotoxicity, explicitly characterized by myocardial necrosis at the 350 mg/kg and 600 mg/kg doses. This indicates that concentrated bioactive compounds inherent to the tamarind seed exert direct structural damage to the heart muscle at elevated concentrations, highlighting an exceptionally narrow therapeutic window that limits unrefined cardiovascular use. Because the gastrointestinal tract is the primary site of extract absorption, it serves as the frontline for both therapeutic efficacy and localized toxicity. Both extracts (600 mg/kg doses) successfully maintained low levels of MDA, protecting against lipid peroxidation. Notably, the ethanolic extract robustly boosted SOD activity compared to the aqueous extract, acting as a potent cellular protectant that neutralizes highly reactive superoxide radicals (Natukunda et al., 2016). Despite this optimal oxidative stress profile, the physical integrity of the intestine was compromised at peak concentrations. Histological analysis revealed specific, localized morphological damage, namely, the physical sloughing of the intestinal villi, exclusively in subjects administered the maximum 600 mg/kg dose of the ethanolic extract. Histopathological examination showed a remarkably safe profile for the liver and kidney for the aqueous extract. This aligns with the lack of significant changes in liver biochemical markers observed with the aqueous extract. In stark contrast, the ethanol extract resulted in considerable liver damage at higher doses (Mehdi et al.,2019), evidenced by severe hepatic congestion and necrosis of liver tissue at 350 mg/kg and 600 mg/kg. Similarly, the kidney showed oedematous and congested renal tubules with the ethanol extract at higher doses, whereas the aqueous extract had no such effect. These findings suggest that the ethanol extract may contain compounds in higher concentrations or with greater inherent toxicity than the aqueous extract, leading to cellular damage at elevated doses (Escalona-Arranz et al., 2016). The observation of necrosis and congestion highlights potential hepatotoxic and nephrotoxic effects, particularly with higher doses or prolonged exposure (Escalona-Arranz et al., 2016). Unexpectedly, the histopathological examination of the heart showed necrosis at the lowest dose of the aqueous extract (150 mg/kg), in addition to necrosis at higher doses of the ethanol extract. While cardiac glycosides, found uniquely in the aqueous extract, are known to affect heart function, the pattern of necrosis at the lowest dose of aqueous extract needs further investigation and correlation with functional cardiac parameters, which were not assessed in this study. These results emphasize the importance of extraction method (aqueous vs. ethanol) in determining the bioactivity and biochemical impact of *T. indica* seed extracts. Understanding the influence of these extracts on haematological and biochemical parameters is critical for assessing their therapeutic potential and safety profiles. This study contributes to the growing body of evidence on the hepatic, renal, and cardiovascular effects of *T. indica*, providing valuable insights for the use of plant-derived compounds in herbal medicine and pharmacology. Furthermore, through comprehensive biochemical and histopathological analyses, this work advances our understanding of the pharmacological actions and potential toxicities associated with prolonged exposure to *T. indica* seed extracts. These findings may inform safer usage practices in both clinical and experimental settings.

Based on the comprehensive, multi-organ toxicological and biochemical insights garnered from this *in vivo* study, several critical avenues for future research and clinical application have been identified. While *Tamarindus indica* seed extracts demonstrate profound therapeutic potential, their exceptionally narrow safety margins necessitate rigorous subsequent evaluations. To harness the medicinal benefits of *T. indica*, dose-response studies are urgently required. Long-term chronic toxicity studies are strongly recommended spanning 90 to 180 days to evaluate the potential bioaccumulation of toxic metabolites and appropriately assess the long-term structural integrity of highly susceptible organs. Further rigorous investigation is required to thoroughly explore the precise pharmacokinetics and systemic bioavailability of these extracted bioactive compounds.

Conclusion

This study demonstrates that *Tamarindus indica* seed extracts exhibit solvent-dependent biological activities and toxicity profiles following repeated oral administration. While both aqueous and ethanolic extracts showed low acute toxicity ($LD_{50} > 5000$ mg/kg) and contained bioactive phytochemicals with therapeutic potential, the aqueous extract demonstrated a comparatively safer systemic profile, preserving hepatic and renal integrity while improving lipid parameters. In contrast, the ethanolic extract produced dose-dependent haematological alterations and significant hepatic, renal, and intestinal toxicity. Importantly, both extracts induced myocardial necrosis at different doses, identifying the heart as a potential target organ irrespective of the extraction solvent. These findings indicate that the pharmacological benefits of *T. indica* seeds are accompanied by dose-dependent toxicological risks, emphasizing the need for careful dose

optimization and comprehensive safety evaluation before therapeutic application. Further studies are required to isolate the bioactive constituents responsible for both the beneficial and adverse effects and to elucidate the mechanisms underlying the observed cardiotoxicity.

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Author contributions

This work was carried out in collaboration among all authors. Authors SBZ, ILE, AEO, YB, CN, BR, YM, and SK conceived and designed the research and conducted the animal studies. Authors BIJ and MMS performed the data analysis and interpretation. Authors SBZ and ILE contributed to writing the article. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest. The manuscript has not been submitted for publication in another journal.

Ethics approval

This investigation on experimental animals was duly approved by NVRI Animal Use Ethical Committee and the Plateau State Ministry of Livestock Development (Approval No: MANR/SEC/47/S.6.VOL.I) prior to the study.

AI use declaration

Google NotebookLM was used to assist in generating tables and figures and to improve the readability and organization of the manuscript. All outputs were carefully reviewed, verified, and edited by the authors to ensure accuracy, originality, and compliance with journal standards.

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