Research Paper

Exploring The Safety and Phytochemical Composition of Nanoherbal Formulations from *Phyllanthus Emblica* L. Fruit

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ABSTRACT

Background: *Phyllanthus emblica* L. exhibits notable antioxidant and therapeutic activities attributed to phenolic and flavonoid compounds. This study aimed to identify the phytochemical constituents of *P. emblica* fruit nanoherbs and evaluate their acute oral toxicity.

Methods: Both in silico and in vivo approaches were employed. Toxicity tests were conducted using a fixed-dose method, followed by clinical, macroscopic, and histopathological assessments of the liver and kidneys in Wistar rats.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. In silico predictions using SwissADME, Pro-Tox II, and pkCSM showed LD_{50} values ranging from 159 to 2500 mg/kg. Quercetin and kojic acid were classified as moderately toxic (class 3), while nicotinamide was categorized as practically non-toxic (class 5) with a potential hepatotoxic risk. In vivo studies demonstrated no significant changes in body weight, organ weight, or mortality up to 5000 mg/kg, and histopathological examinations confirmed preserved hepatic and renal structures, with only mild alterations at the highest dose.

Conclusion: Overall, nanoherbs derived from *P. emblica* fruit were found to be safe in acute and subchronic exposure, supporting their potential application in pharmaceutical development.

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Introduction

The global use of herbal medicines continues to rise as people seek affordable and natural therapies for chronic diseases. According to the World Health Organization (WHO), approximately 80% of the global population still relies on traditional medicine, particularly plant-based remedies [1]. *Phyllanthus emblica* L. (Indian gooseberry, amla) is a widely known medicinal plant with antioxidant, antimicrobial, anticancer, and anti-aging properties, primarily attributed to its tannins, flavonoids, and phenolic compounds [2–4]. However, its potential remains underutilized in Indonesia, including in Bali, emphasizing the need for scientific validation.

Phytochemical screening is a key step to identify active secondary metabolites that underpin therapeutic effects, while safety must be confirmed through toxicity testing. Acute oral toxicity studies provide essential data on safe dosage and possible adverse effects [5]. Nanoparticle technology offers an advantage by improving solubility, absorption, and efficacy of herbal compounds with poor bioavailability [6, 7].

Conventional in vivo toxicity studies, however, are resource-intensive and raise ethical issues. Thus, computational in silico prediction methods are increasingly used to complement animal testing, thereby enabling efficient toxicity assessment pipelines [5].

This study aimed to identify phytochemical constituents and evaluate the acute toxicity of *P. emblica* fruit nanoherbal formulations in Wistar rats. The safety assessment included clinical observations, body weight, food and water intake, organ weights, and histopathological examination of the liver and kidneys.

Materials and Methods

Materials and Animals

Phyllanthus emblica fruits were collected from Bali, Indonesia, and authenticated at the Herbarium Medanense, Universitas Sumatera Utara (Voucher No. 5580/MEDA/2021). The dried fruits were milled into nanoparticles (731.4 ± 168.2 nm). Twenty-five male (20–30 g) and twenty female (110–200 g) Wistar rats (3 months old) were maintained under controlled conditions with free access to food and water. Ethical approval was granted by the Ethics Committee of

Table 1. Preliminary Test Experimental Design.

Number of Rats	Treatment
1	Provided a 0.5% sodium carboxymethyl cellulose (CMC Na) suspension
1	Given a *P. emblica* fruit nanoherbal dose of 5 mg/kg BW
1	Given a *P. emblica* fruit nanoherbal dose of 50 mg/kg BW
1	Given a *P. emblica* fruit nanoherbal dose of 300 mg/kg BW
1	Given a *P. emblica* fruit nanoherbal dose of 2000 mg/kg BW

CMC: **Carboxymethyl cellulose,** *P. emblica:* ***Phyllanthus emblica***

Table 2. Experimental Design for Primary Testing.

Number of Rats	Treatment	Description
5	Provided a 0.5% sodium carboxymethyl cellulose (CMC Na) suspension	Control Group
5	Given a *P. emblica* fruit nanoherbal dose of 2000 mg/kg BW	Testing Group
5	Given a *P. emblica* fruit nanoherbal dose of 5000 mg/kg BW	Testing Group

Table 3. Subchronic Toxicity Test Experimental Design.

Number of Rats	Treatment
1	Provided a 0.5% sodium carboxymethyl cellulose (CMC Na) suspension
1	Given a *P. emblica* fruit nanoherbal dose of 100 mg/kg BW
1	Given a *P. emblica* fruit nanoherbal dose of 500 mg/kg BW
1	Given a *P. emblica* fruit nanoherbal dose of 1000 mg/kg BW
1	Satellite group: given a *P. emblica* fruit nanoherbal dose of 1000 mg/kg BW
1	Satellite group: provided a 0.5% sodium carboxymethyl cellulose (CMC Na) suspension

CMC: Carboxymethyl cellulose, ***P. emblica:*** *Phyllanthus emblica*

Universitas Sumatera Utara (No. 00468/KEPH FMIPA/2021).

Phytochemical Analysis of P. emblica Fruit Nanoherbal

Qualitative screening was performed to detect alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids using standard chemical tests [5].

In Silico Toxicity Prediction

Selected phytochemicals (quercetin, kojic acid, and nicotinamide) were analyzed using SwissADME, Pro-Tox II, and pkCSM. Parameters included ADMET profiles (absorption, distribution, metabolism, excretion, toxicity), LD50, hepatotoxicity, carcinogenicity, mutagenicity, and interactions with biological targets [4, 8].

Acute and Subchronic Toxicity

For acute toxicity, rats received oral doses of 5, 50, 300, and 2000 mg/kg body weight [BW] (preliminary) and 2000 or 5000 mg/kg BW (primary) of P. emblica nanoherbal suspension. Control animals received 0.5% Na-CMC. Animals were observed for 14 days for clinical signs, body weight, and mortality.

For subchronic toxicity, nanoherbal suspensions (100, 500, and 1000 mg/kg BW) were administered orally for 28 days, with daily clinical observations and body weight monitoring. A satellite group was observed for an additional 14 days. At study end, animals were euthanized, and liver and kidney samples were collected for macroscopic, relative organ weight, and histopathological examinations [5, 9].

Based on the preliminary findings, a primary acute toxicity test was conducted at doses of 2000 and 5000 mg/kg BW. Rats were divided into control and test groups (n = 5 each). The control received 0.5% Na-CMC, while the test groups received P. emblica suspension orally after fasting. The 5000 mg/kg BW dose was administered only after confirming no toxicity at 2000 mg/kg BW during the first 2 days. Animals were observed intensively for the first 4 hours and then daily for 14 days. Body weight was monitored every two days, and mortality was recorded to estimate LD_{50} (Table 2).

The subchronic study was conducted for 28 days in accordance with non-clinical toxicity guidelines. P. emblica nanoherbal was administered orally once daily (≥5 days/week). Body weight was monitored daily to adjust dosing, and changes were analyzed twice weekly. At day 28, animals were euthanized, and organs were collected for analysis. A satellite group was observed for 14 additional days to assess recovery. The study design is presented in Table 3.

Organ and Histopathological Examination in Acute Toxicity

At the end of the acute phase, surviving rats were weighed, euthanized, and their liver and kidneys collected. Organs were examined macroscopically (color, surface, consistency) and weighed to calculate relative organ weight. Tissues were fixed in 10% formalin and processed for histopathological evaluation at the Faculty of Medicine, Universitas Sumatera Utara, to detect cellular changes associated with toxicity [5, 9].

Mortality and Organ Analysis

During the subchronic phase, mortality was monitored daily. Deceased animals were necropsied, and the kidneys were collected. At study completion, surviving rats were euthanized, and their kidneys were examined macroscopically and histologically [5, 9].

Kidney Analysis: Weighing, Macroscopic, and Histopathology

In both the acute and subchronic phases, kidneys were weighed to calculate relative organ weight and examined macroscopically for color, surface appearance, and consistency. Tissues were fixed in 10% formalin and processed for histopathological evaluation at Adam Malik Hospital to detect structural alterations [5, 9].

Data Analysis

Body weight, food and water intake, and organ weights were analyzed using one-way ANOVA with Tukey's post hoc test (SPSS). Significance was set at $p < 0.05$.

Results

Phytochemical Screening

Phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids in *P. emblica* nanoherbal (Table 4).

Toxicity Prediction

Based on Masfria (2024), *P. emblica* nanoherbal contains flavonoids (quercetin, myricetin), phenolics (kojic acid, ellagic acid, coumaric acid), vitamins (nicotinamide, nicotinic acid, choline), and alkaloids

Table 4. Phytochemical Screening.

No	Secondary Metabolites	Nanoherbal	Description
1	Alkaloids	+	Bouchardat: blackish precipitate; Dragendorff: orange yellow
2	Flavonoids	+	Amyl alcohol layer: yellow–orange
3	Glycosides	+	$FeCl_3$: blue
4	Saponins	+	Foam does not disappear with addition of HCl 2N
5	Tannins	+	LB reagent: green
6	Steroids/triterpenoids	+	With H_2SO_4 (p): purple ring

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Table 5. Profile ADME of Quercetin, Kojic Acid, and Nicotinamide.

Property	Model Name	Predicted Value of Compounds			Unit
		Quercetin	Kojic Acid	Nicotinamide	
Absorption	Water Solubility	-2.925	-1.752	-0.719	Numeric (log mol/L)
Distribution	VDss (human)	1.559	-0.086	-0.224	Numeric (log L/kg)
Metabolism	CYP2D6 Substrate	No	No	No	Categorical (Yes/No)
Excretion	Total Clearance	0.407	0.638	0.608	Numeric (log ml/min/kg)

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Table 6. Toxicity Prediction of Quercetin, Kojic Acid, and Nicotinamide (pKCSM).

Property	Model Name	Predicted Value of Compounds			Unit
		Quercetin	Kojic Acid	Nicotinamide	
Toxicity	AMES toxicity	No	No	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.499	0.748	1.15	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	No	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	No	No	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD_{50})	2.471	2.037	2.116	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.612	1.613	2.616	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	No	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	No	No	Categorical (Yes/No)
Toxicity	*T. Pyriformis* toxicity	0.288	-0.219	-0.585	Numeric (log µg/L)
Toxicity	Minnow toxicity	3.721	3.178	2.441	Numeric (log mM)

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(trigonelline) [4]. In this study, quercetin, kojic acid, and nicotinamide were selected for in silico ADMET prediction using pkCSM, ProTox-II, and ChemDraw.

The ADME profiles (Table 5) showed low water solubility for all three compounds, suggesting limited oral absorption. Predicted VDss values indicated restricted tissue distribution, and none were substrates of CYP2D6, implying low risk of metabolism-related interactions. Total clearance was moderate (0.407–0.638 log ml/min/kg) [10, 11].

pkCSM results showed no AMES toxicity, hERG inhibition, hepatotoxicity, or skin sensitization.

Table 7. Quercetin, Kojic Acid, and Nicotinamide Toxicity Class (Protox Online).

No.	Parameters	Quercetin	Kojic Acid	Nicotinamide
1	Predicted LD_{50}	159 mg/kg	550 mg/kg	2500 mg/kg
2	Predicted toxicity class	Class 3	Class 3	Class 5
3	Average similarity	100%	72.28%	100%
4	Prediction accuracy	100%	69.26%	100%

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Table 8. Toxic Symptoms of Preliminary Test.

Observation	CMC Na 0.5%	P. emblica 5 mg/kg BW	P. emblica 50 mg/kg BW	P. emblica 300 mg/kg BW	P. emblica 2000 mg/kg BW
Shaking	–	–	–	–	–
Diarrhea	–	–	–	–	–
Convulsions	–	–	–	–	–
Weak	–	–	–	–	–
Eye	–	–	–	–	–
Skin and Fur	–	–	–	–	–
Walk on the Belly	–	–	–	–	–
Walk Backwards	–	–	–	–	–

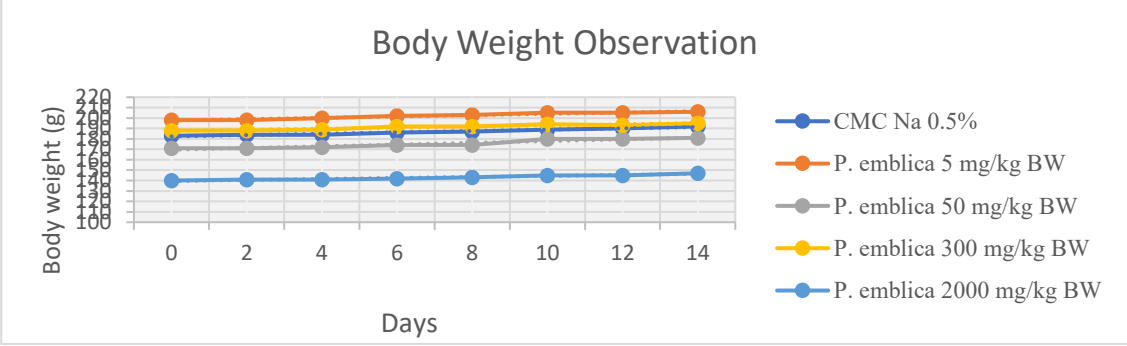

Figure 1. Body Weight Observation.

Table 9. Animal Death Observation.

Number of Rats	Treatment	Number of Deaths
1	CMC Na 0.5%	0
1	*P. emblica* 5 mg/kg BW	0
1	*P. emblica* 50 mg/kg BW	0
1	*P. emblica* 300 mg/kg BW	0
1	*P. emblica* 2000 mg/kg BW	0

Table 10. Toxic Symptoms of Primary Test.

Observation	CMC Na 0.5%	P. emblica 2000 mg/kg BW	P. emblica 5000 mg/kg BW
Tremors	–	–	–
Diarrhea	–	–	–
Convulsions	–	–	–
Weak	–	–	–
Eye	–	–	–
Skin and Fur	–	–	–
Walk on the Belly	–	–	–
Walk Backwards	–	–	–

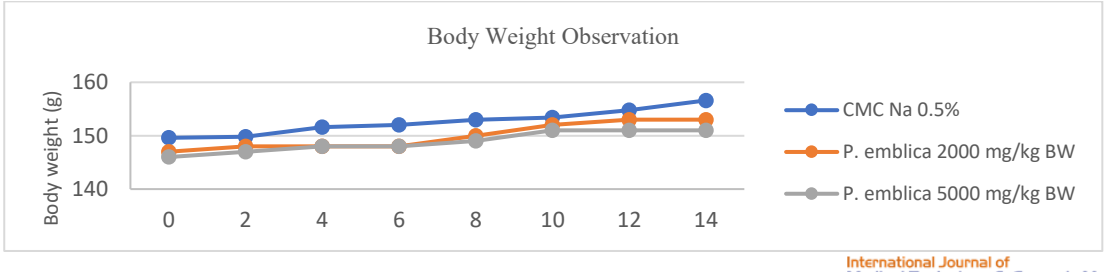

Figure 2. Body Weight Observation.

Predicted LD_{50} values indicated moderate toxicity for quercetin and kojic acid, while nicotinamide was

Table 11. Animal Death Observation.

Number of Rats	Treatment	Number of Deaths
5	CMC Na 0.5%	0
5	*P. emblica* 2000 mg/kg BW	0
5	*P. emblica* 5000 mg/kg BW	0

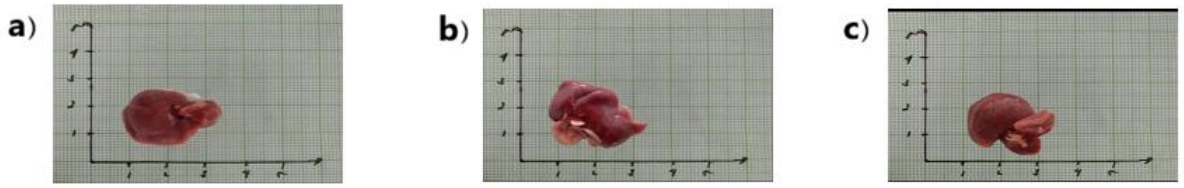

Figure 3. Liver macroscopy (a) Control; (b) 2000 mg/kg BW; (c) 5000 mg/kg BW.

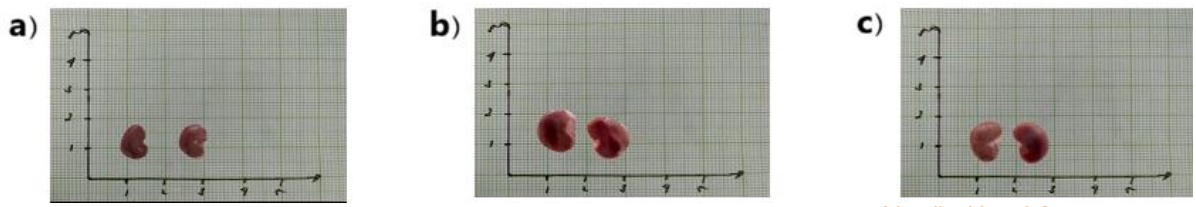

Figure 4. Kidney macroscopy (a) Control; (b) 2000 mg/kg BW; (c) 5000 mg/kg BW.

Table 12. Results of Liver–Kidney Macropathology.

Group	Color	Surface	Consistency
CMC Na 0.5%	reddish-brown	slippery	springy
P. emblica 2000 mg/kg BW	reddish-brown	slippery	springy
P. emblica 5000 mg/kg BW	reddish-brown	slippery	springy

Table 13. Relative Organ Weight per 100 g Body Weight of Female Rats.

Organ	CMC Na 0.5%	P. emblica 2000 mg/kg BW (mean ± SD)	P	P. emblica 5000 mg/kg BW (mean ± SD)	P
Right kidney	0.41 ± 0.06	0.35 ± 0.02	0.23	0.36 ± 0.05	0.35
Left kidney	0.43 ± 0.05	0.36 ± 0.04	0.06	0.37 ± 0.05	0.11
Liver	4.06 ± 0.57	4.18 ± 0.25	0.88	3.90 ± 0.26	0.80

classified as practically non-toxic (Tables 6 and 7) [10, 11]. Chronic toxicity values (LOAEL) remained within

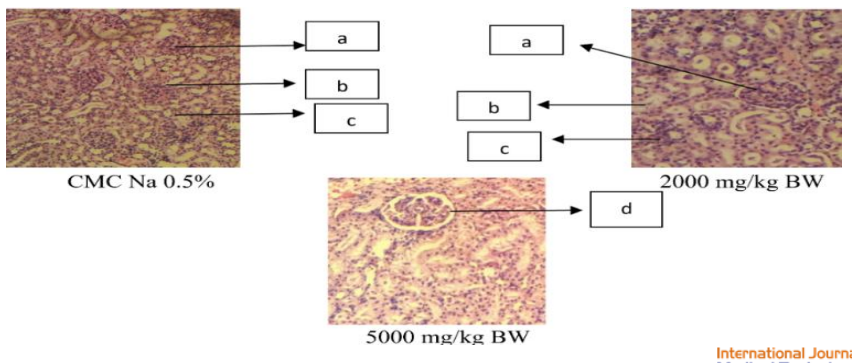

Figure 5. Kidney Histopathology (a) glomerulus (b) proximal tubule (c) distal tubule (d) Bowman's space dilation.

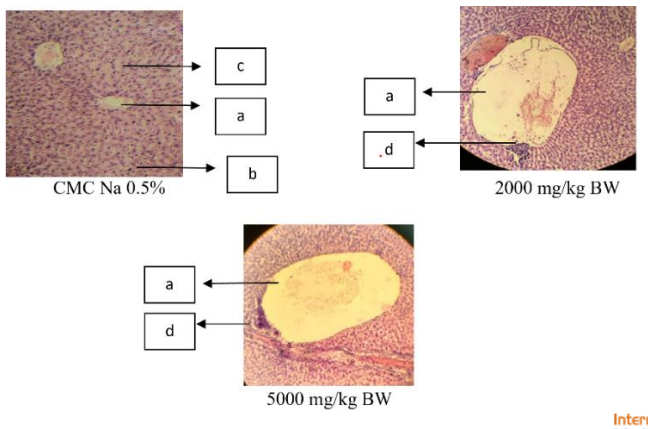

Figure 6. Liver Histopathology (a) central vein; (b) hepatocytes; (c) sinusoid; (d) hepatocyte necrosis.

Table 14. Toxic Symptoms of Subchronic Toxicity Test in Male and Female Rats.

Observation	CMC Na 0.5%		P. emblica 100 mg/kg BW		P. emblica 500 mg/kg BW		P. emblica 1000 mg/kg BW	
	Male	Female	Male	Female	Male	Female	Male	
Shaking	–	–	–	–	–	–	–	
Diarrhea	–	–	–	–	–	–	–	
Convulsions	–	–	–	–	–	+	–	
Weak	–	–	–	–	–	–	–	
Eye	–	–	–	–	–	–	–	
Skin and Fur	–	–	–	–	–	–	–	
Walk on the Belly	–	–	–	–	–	–	–	
Walk Backwards	–	–	–	–	–	–	+	

Description: (+) = occur; (−) = not occur

Table 15. Animal Death Observation.

Groups	CMC Na 0.5%	P. emblica 5 mg/kg BW	P. emblica 50 mg/kg BW	P. emblica 300 mg/kg BW	P. emblica 2000 mg/kg BW
Number of Animals	**Male**	10	10	10	10
	Female	10	10	10	10
Number of Mortality	**Male**	0	0	0	0
	Female	0	0	0	0
Week of Mortality	**Male**	–	–	–	–
	Female	–	–	–	–

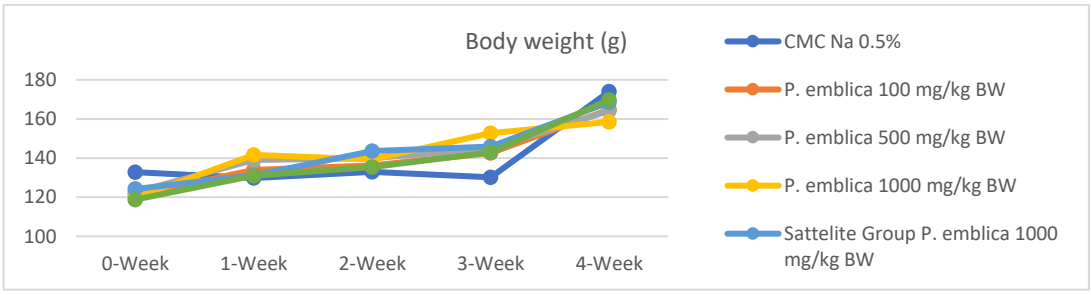

Figure 7. Body weight measurements in Female Rats.

acceptable ranges. Overall, the in silico evaluation suggested that these compounds have favorable pharmacokinetic profiles and low risks of mutagenicity, cardiotoxicity, and hepatotoxicity,

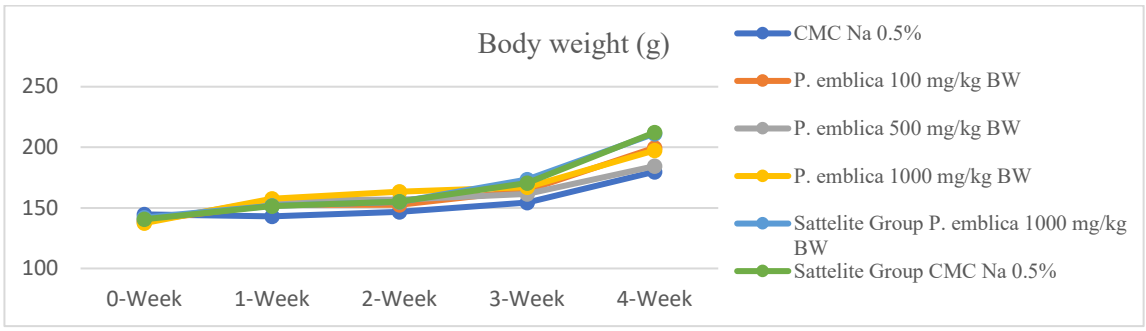

Figure 8. Body weight measurements in Male Rats.

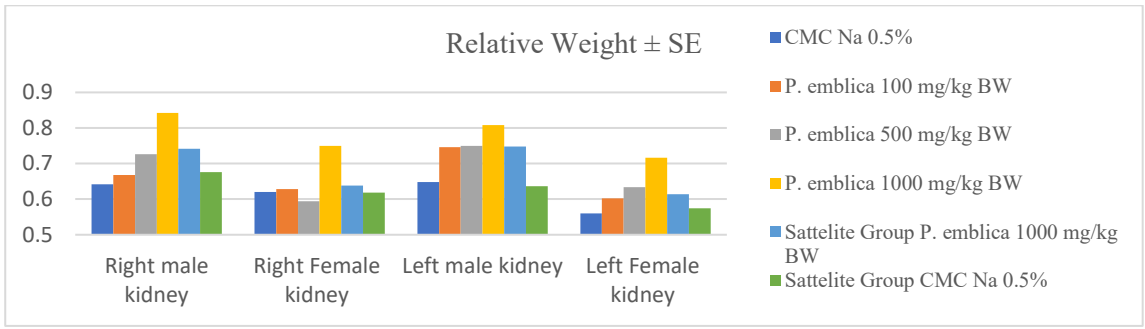

Figure 9. Relative Weight of Right and Left Kidney.

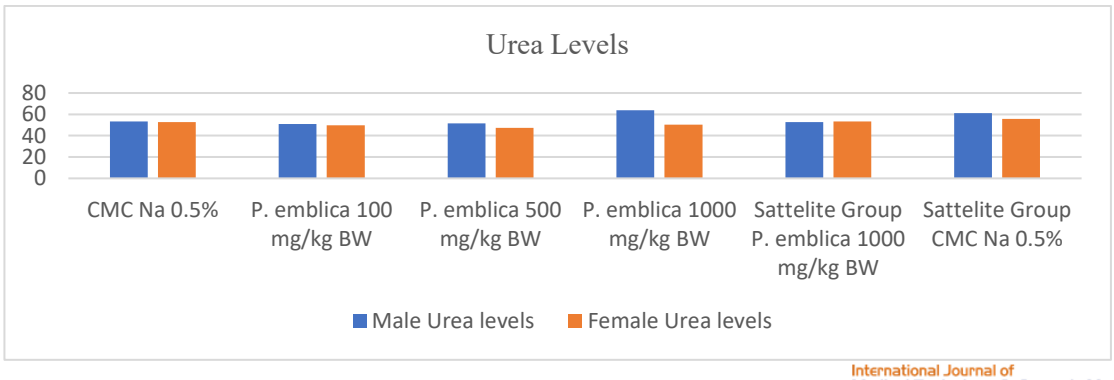

Figure 10. Urea Levels of Rats.

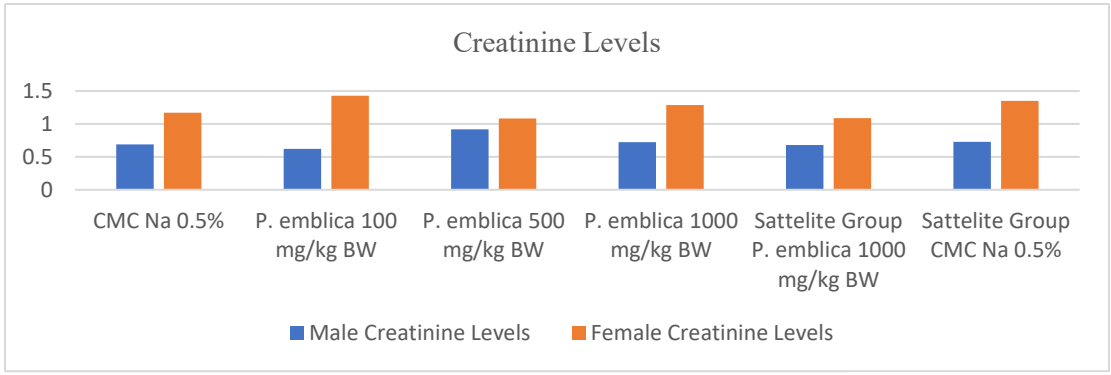

Figure 11. Creatinine Levels of Rats.

supporting their use in nanoherbal formulations.

Preliminary and Primary Test

Rats received *P. emblica* nanoherbal at doses of 5–2000 mg/kg BW. Over 14 days, no clinical signs of toxicity or mortality were observed (Tables 8 and 9). Body weight remained stable, with no significant differences compared with controls (Figure 1),

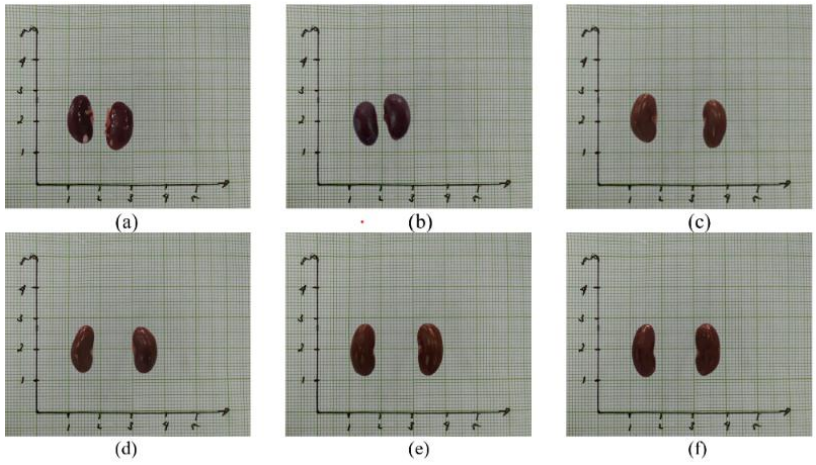

Figure 12. Kidney macroscopy of male rats (a) Control CMC Na 0.5%; (b) *P. emblica* 100 mg/kg BW; (c) *P. emblica* 500 mg/kg BW; (d) *P. emblica* 1000 mg/kg BW; (e) Sattelite group *P. emblica* 1000 mg/kg BW; (f) Sattelite group CMC Na 0.5%.

Table 16. Results of kidney macro pathology .

Group	Observation		
	Color	Surface	Consistency
CMC Na 0.5%	reddish-brown	slippery	springy
P. emblica 100 mg/kg BW	reddish-brown	slippery	springy
P. emblica 500 mg/kg BW	reddish-brown	slippery	springy
P. emblica 1000 mg/kg BW	reddish-brown	slippery	springy
Sattelite group *P. emblica* 1000 mg/kg BW	reddish-brown	slippery	springy
Sattelite group CMC Na 0.5%	reddish-brown	slippery	springy

indicating a favorable safety profile for dose escalation.

At 2000 and 5000 mg/kg BW, no toxic signs or mortality were observed (Tables 10 and 11). All animals remained clinically normal, and body weight increased steadily without significant differences from controls (Figure 2).

According to OECD guidelines [22, 23], the absence of mortality or toxic symptoms at doses up to 5000 mg/kg BW indicates that P. emblica is practically non-toxic (GHS Category 5). These findings suggest that oral administration of P. emblica nanoherbal is safe at doses ≤ 5000 mg/kg BW, with an estimated LD50 exceeding this value.

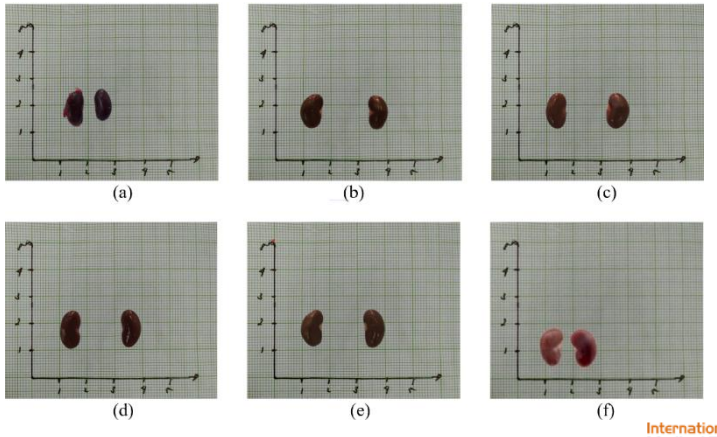

Figure 13. Kidney macroscopy of male rats (a) Control CMC Na 0.5% (b) *P. emblica* 100 mg/kg BW (c) *P. emblica* 500 mg/kg BW (d) *P. emblica* 1000 mg/kg BW (e) Sattelite group *P. emblica* 1000 mg/kg BW (f) Sattelite group CMC Na 0.5%.

Liver and Kidney Macroscopic and Histopathology

On day 15, macroscopic examination of the liver and kidneys revealed no abnormalities in the control, 2000 mg/kg BW, or 5000 mg/kg BW groups (Figures 3 and 4; Table 12). Organs displayed standard color, surface, and consistency, with no significant differences in relative organ weights (Table 13),

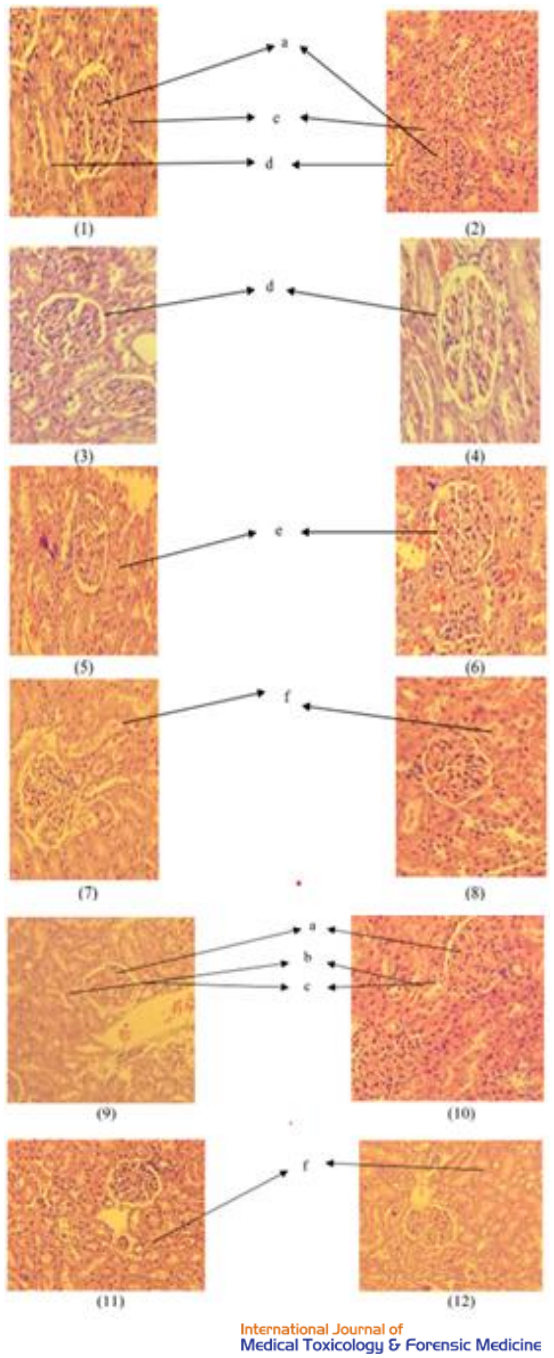

Figure 14. Histopathology of Male Rat Kidneys after P. emblica Administration
Information:
1-2. Control (CMC Na 0.5%)
3-4. P. emblica 100 mg/kg BW
5-6. P. emblica 500 mg/kg BW
7-8. P. emblica 1000 mg/kg BW
9-10. Satellite Control (CMC Na 0.5%)
11-12. Satellite P. emblica 1000 mg/kg BW
Features: (a) Glomerulus; (b) Proximal Tubule; (c) Distal Tubule; (d) Bowman's Space Dilation; (e) Pyknosis; (f) Karyolysis.

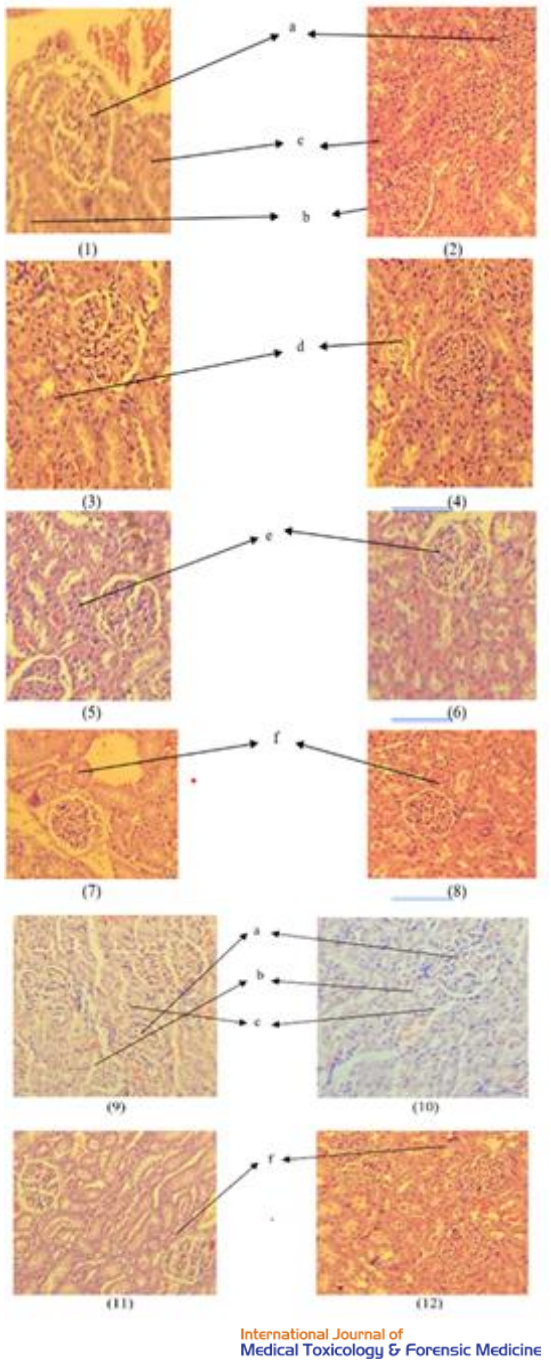

Figure 14. Histopathology of Female Rat Kidneys after P. emblica Administration
Information:
1-2. Control (CMC Na 0.5%)
3-4. P. emblica 100 mg/kg BW
5-6. P. emblica 500 mg/kg BW
7-8. P. emblica 1000 mg/kg BW
9-10. Satellite Control (CMC Na 0.5%)
11-12. Satellite P. emblica 1000 mg/kg BW
Features: (a) Glomerulus (b) Proximal Tubule (c) Distal Tubule (d) Bowman's Space Dilation (e) Pyknosis (f) Karyolysis

indicating no hypertrophy or atrophy.

Kidney histology in the control and 2000 mg/kg BW groups showed normal glomeruli and tubules. At 5000 mg/kg BW, mild Bowman's space dilation was observed, suggesting early glomerular stress, but overall renal architecture remained intact (Figure 5).

Liver sections showed preserved central vein, hepatocyte arrangement, and sinusoidal spaces in all groups (Figure 6). Mild central vein dilation at higher doses did not progress to necrosis, indicating no significant hepatic injury [30, 31]. Despite the dual antioxidant–pro-oxidant potential of flavonoids, no histological evidence of pro-oxidant damage was observed, confirming the hepatorenal safety of *P. emblica* up to 5000 mg/kg BW.

Subchronic Toxicity Test

During the 28-day study, rats were monitored daily for clinical signs. Minor, sporadic symptoms, such as weakness, diarrhea, and occasional abnormal movements, occurred in the higher-dose groups (500–1000 mg/kg BW). Still, no severe or persistent toxic effects were observed in either sex (Table 14).

Over 28 days, no mortality was observed in any treatment or satellite groups (Table 15). Body weight increased consistently across all groups (Figures 7 and 8) and did not differ significantly from controls ($p > 0.05$), indicating that *P. emblica* did not affect appetite or growth.

Kidney weight analysis (Figure 9) showed a significant increase at 1000 mg/kg BW ($p < 0.05$), suggesting mild adaptive hypertrophy, a physiological response to higher metabolic or excretory demands.

Across 28 days, no mortality or severe toxic signs were observed. Urea and creatinine levels remained within normal ranges, confirming preserved renal function. Transient increases in kidney weight at high doses reflected physiological adaptation rather than toxicity, supporting the safety of P. emblica nanoherbal at up to 1000 mg/kg BW when administered orally.

Kidney Macroscopic and Histopathology

After 28 days of treatment, kidney morphology showed no abnormalities in color, surface, or consistency across all groups (Table 16). Organs appeared reddish-brown, smooth, and elastic, indicating preserved integrity and perfusion. No signs of nephrotoxicity were observed, confirming the absence of gross morphological impact up to 1000 mg/kg BW.

At 1000 mg/kg BW, Bowman's space dilation, pyknosis, karyolysis, and tubular lumen enlargement indicated mild glomerular and tubular injury (Figures 14 and 15, Table 16). These changes suggest early nephrotoxicity, likely due to transient circulatory stress or high-dose accumulation. However, the absence of macroscopic changes and normal biochemical parameters indicates that the effects were mild and subclinical during the study period [5, 9].

Discussion

This study integrates qualitative phytochemical profiling, in silico toxicity screening of representative constituents, and acute and 28-day repeated oral exposure in rats to provide a coherent safety characterization of Phyllanthus emblica fruit nanoherbal. Overall, the evidence indicates a wide acute safety margin and an acceptable subacute tolerability profile within practical dose ranges, while also identifying kidney tissue as the most sensitive organ at the highest repeated dose.

The detected phytochemical classes (notably flavonoids, tannins, and other secondary metabolites) are consistent with the known chemical diversity of P. emblica and can plausibly underpin biological activity. Importantly, polyphenol-rich matrices may exhibit context-dependent effects: antioxidant benefits at moderate exposure but potential tissue stress at excessive dosing, particularly in organs involved in xenobiotic handling and excretion, such as the kidney.

The acute oral findings are aligned with the toxicological interpretation commonly applied under OECD fixed-dose procedures and GHS logic, supporting a “very low acute hazard” profile for the preparation. This provides a robust baseline for further development, but acute tolerance alone is insufficient to exclude liabilities that emerge with repeated exposure.

The in-silico outputs should be interpreted as conservative, single-compound hazard flags rather than direct predictors of whole-preparation toxicity. Differences between predicted hazard and in vivo outcomes are expected in botanical formulations due to matrix effects, limited bioavailability of individual constituents, and metabolic transformation, which collectively reduce effective systemic exposure relative to administered mass. Accordingly, the primary utility of the in-silico component is to guide target-organ vigilance, particularly for hepatorenal monitoring.

During a 28-day administration period, the absence of overt systemic toxicity and preserved functional indices support subacute tolerability at low-to-mid dose

levels. Nevertheless, the renal microscopic alterations observed at the highest repeated dose are biologically plausible as early or mild nephrotoxic stress that can precede changes in conventional serum markers, especially when lesions are focal or partially compensated. In regulatory toxicology terms, this pattern supports defining a conservative exposure ceiling for routine use and treating the highest dose with renal histological changes as a practical signal for dose limitation in subsequent studies.

The nanoherbal format may influence dissolution and absorption kinetics, potentially shifting the exposure window compared with non-nano preparations. Therefore, dose selection should prioritize the minimally effective range rather than maximal dosing, and follow-up work should strengthen renal safety characterization using blinded semi-quantitative histopathology, expanded clinical chemistry/hematology, and more sensitive kidney injury biomarkers, ideally in longer-duration studies with standardized batch characterization.

Conclusion

P. emblica fruit nanoherbal contains alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and triterpenoids. Acute toxicity tests showed no mortality or organ changes at doses up to 5000 mg/kg BW (LD_{50} > 5000 mg/kg; practically non-toxic). In silico analysis classified quercetin and kojic acid as moderately toxic, while nicotinamide was practically non-toxic, with possible hepatotoxicity. Subchronic exposure at 100–500 mg/kg BW caused no renal damage, though mild histological alterations appeared at 1000 mg/kg BW without affecting urea or creatinine. Overall, *P. emblica* nanoherbal is considered safe up to 500 mg/kg BW for subchronic use, with caution advised at higher doses.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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