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TOXICITY EVALUATION OF *PHYLLANTHUS EMBLICA*, *SIDA RHOMBIFOLIA* AND *LEUCAS INDICA* LEAVES IN WISTAR ALBINO RATS

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Abstract:

Phyllanthus emblica, *Sida rhombifolia* and *Leucas indica* are widely employed in traditional Indian medicine, yet their safety profiles have not been comprehensively characterised. In this study, the acute oral toxicity of leaf powders from these three ethnobotanicals were evaluated in Wistar rats as per Organisation for Economic Co-operation and Development (OECD) guideline 420. After acute oral administration of the test substances at 2000 mg/kg in both sighting and main study, no mortality or treatment-related adverse signs were observed and the key physiological parameters including respiratory rate, rectal temperature, locomotor activity and body weight gain remained normal throughout 14 days of experiment. Gross necropsy did not reveal any pathological changes and the organ-to-body weight ratios were maintained within normal ranges, enabling these tested plant species to be classified as GHS Category 5 (No Observed Adverse Effect Level (NOAEL) ≥ 2,000 mg/kg). Therefore, the present findings were indicative of the preliminary safety profile of these botanicals and provide a solid foundation for subsequent subacute and chronic toxicity evaluations and pharmacodynamic investigations.

Keywords: *Phyllanthus emblica*, *Sida rhombifolia*, *Leucas indica,* Oral Toxicity, OECD guideline 420

1. Introduction

The global interest in herbal medicine has grown considerably in recent years, largely due to widespread concerns about the side effects, rising expenses and increasing resistance problems associated with synthetic pharmaceutical products. This shift also reflects a renewed interest in traditional medicinal practices that

have long formed the basis of indigenous healthcare systems (WHO, 2019). Herbal formulations are typically composed of diverse phytochemicals such as plant-derived secondary metabolites that exert therapeutic effects through multiple mechanisms, including antioxidant, anti-inflammatory, immunomodulatory, antimicrobial and anticancer actions (Atanasov *et al*., 2021).

From an economic perspective, the herbal medicine sector is expanding rapidly, with India's herbal drug market estimated at around USD 1 billion and experiencing an annual growth rate of 11%, driven by increasing global demand and recognition of the safety and efficacy of plant-based remedies (Sharma *et al*., 2008). This expansion reflects increasing consumer preference for natural remedies, greater awareness of the limitations of synthetic drugs and the formal integration of traditional medicine into national healthcare frameworks. Despite their widespread ethnopharmacological use, many medicinal plants remain inadequately studied in terms of safety and efficacy. Scientific validation, particularly in the form of toxicological investigations, is essential to support their rational inclusion in evidence-based medicine.

Currently, less than 15% of herbal plants utilised in traditional healthcare systems have undergone rigorous toxicological evaluation (WHO, 2019). Among the foundational steps in preclinical safety assessment, determination of acute oral toxicity provides critical data on dose-related toxic effects, target organ susceptibility and safe exposure thresholds. These studies are instrumental in estimating the therapeutic index and selecting appropriate doses for subsequent pharmacological trials (Gurib-Fakim, 2006).

Keeping these facts under consideration, the present study envisages preliminary safety assessment of three ethnomedicinal plants utilised in Indian traditional medicine: *Phyllanthus emblica* (Indian gooseberry), *Sida rhombifolia* (Jungle Roselle) and *Leucas indica* (Thumbai) by acute oral toxicity evaluation. *P. emblica* is extensively documented for its antioxidant potential and its traditional use in managing conditions related to oxidative stress, such as diabetes, hepatic disorders and immune dysfunctions (Suja *et al*., 2009; Ahmad *et al*., 2021). *S. rhombifolia* is traditionally employed in treating respiratory ailments, wounds and inflammation, owing to its bioactive constituents including flavonoids, ecdysteroids and alkaloid derivatives (Ahirrao, 2024). *L. indica*, an aromatic herb with recognised antipyretic and

antimicrobial properties, contains notable levels of monoterpenes and phenolic acids (Babu *et al*., 2018).

Despite their prominence in ethnomedicinal practices, comprehensive data regarding their toxicological safety profiles remain scarce. Hence, the objective of this study was to evaluate the acute oral toxicity of *P. emblica*, *S. rhombifolia* and *L. indica* leaves in Wistar rats. The findings are expected to contribute valuable insights into their safety profiles, thereby supporting their advancement as scientifically validated phytotherapeutic agents.

2 Materials and methods

2.1 Plant Materials

Mature leaves of *Phyllanthus emblica* (Phyllanthaceae), *Sida rhombifolia* (Malvaceae) and *Leucas indica* (Lamiaceae) were collected from wild populations in Wayanad district, Kerala, India, during the post-monsoon season to maximise phytochemical content. Voucher specimens were authenticated by University of Calicut Herbarium and subsequently deposited in the herbarium repository of the College of Veterinary and Animal Sciences, Pookode, following standard herbarium protocol (Smith and Chinnappa, 2015). Leaves were sequentially rinsed with deionised water, shade-dried at 25 ± 2 °C and 40-50 % relative humidity to minimise thermal degradation, pulverised and sieved with 250 µm mesh and stored in amber glass bottles at 4 °C.

2.2 Experimental Animals

Fifteen healthy adult female Wistar rats (*Rattus norvegicus*; 180 ± 20 g, 6-8 weeks old) were procured from a Committee for Control and Supervision of Experimentation on Animals (CCSEA) approved breeding facility and housed in polypropylene cages with autoclaved paddy husk bedding, replaced every 48 hours, under controlled environmental conditions (25 ± 2°C; 50 ± 10% relative humidity; 12:12-hour light-dark cycle) (CPCSEA, 2003). Animals received certified pelleted diet with *ad libitum* water, acclimatised for 14 days and underwent daily health monitoring by veterinary staff.

2.3 Acute Oral Toxicity Assessment

The acute oral toxicity of *P. emblica*, *S. rhombifolia* and *L. indica* leaf powders was evaluated following OECD Guideline 420 (Fixed Dose Procedure). An initial sighting study was conducted by administering aqueous suspensions of each test material via oral gavage initially to a single animal at limit dose level of 2000 mg/kg body weight. After a 48-hour observation period for assessing mortality and clinical signs of toxicity, a 14-day main study was conducted using four additional

rats per group, administered the same dose level in accordance with OECD guidelines (OECD, 2002). The dosing volume was standardized at 10 mL/kg body weight, and the test suspensions were freshly homogenized prior to administration to maintain consistency and uniformity.

2.4 Clinical Monitoring and Necropsy

Animals were closely monitored for signs of toxicity, including neurobehavioural (e.g., righting reflex, tremors, convulsions), autonomic (e.g., lacrimation, salivation, pupillary response) and physiological alterations (e.g., respiratory patterns, urination, defecation). Detailed observations were recorded at 10, 30, 60, 120 and 240 minutes post-dosing, followed by twice-daily assessments for 14 days. Body weight measurements were taken weekly to evaluate potential metabolic disturbances. On day 15, surviving animals were humanely euthanised by approved method and a complete gross necropsy was performed. Key organs, including the liver, kidneys, heart, lungs, spleen and brain, were examined for any abnormalities. Relative organ-to-body weight ratios were calculated to assess organ-specific toxicity. All experimental procedures were conducted in strict compliance with institutional ethical guidelines and were approved by the Institutional Animal Ethics Committee (IAEC Approval No. IAEC/COVAS/PKD/05/09.2016).

3. Results and Discussion

Acute oral administration of *L. indica*, *P. emblica* and *S. rhombifolia* leaf powder suspension at 2,000 mg/kg body weight produced no mortality or treatment-related clinical signs in Wistar rats during the 14-day observation period (Tables 1a, 1b). All survived animals exhibited normal physiological parameters. Although, transient behavioural changes (e.g., mild hypoactivity and piloerection) were observed in some animals, it was resolved spontaneously within 1-2 hours, consistent with OECD Guideline 420 criteria (OECD, 2002). No adverse clinical signs, such as alterations in fur texture, skin condition or mucous membranes, were detected, signifying absence of systemic toxicity. These findings are in concordance with

Table 1a. Sighting study observations (n=1 for each treatment group)

Plant	Dose (mg/kg)	Mortality	Behavioural observations	Onset	Recovery
L. indica	2000	0/1	Mild hypoactivity, piloerection	30 min	1 h
P. emblica	2000	0/1	Slight piloerection	45 min	1.5 h
S. rhombifolia	2000	0/1	Mild hypoactivity	20 min	1 h

Table 1b. Main study observations (n=4 for each treatment group)

Plant	Dose (mg/kg)	Mortality	Behavioural observations	Onset	Recovery
L. indica	2000	0/4	Transient hypoactivity (2/4), piloerection (1/4)	20-40 min	1-2 h
P. emblica	2000	0/4	Mild hypoactivity (1/4), piloerection (2/4)	30-50 min	1-1.5 h
S. rhombifolia	2000	0/4	Transient hypoactivity (2/4)	25-45 min	1-1.5 h

previous studies involving related species, including *Leucas martinicensis* (Eze *et al*., 2013), *Phyllanthus niruri* and *Sida acuta* (Alognon *et al*., 2024), which similarly reported absence of toxic effects at equivalent or higher doses, reinforcing the safety profile of the tested botanicals.

Body weight analysis demonstrated a steady increase across all the treatment groups over the 14-day observation period, with percentage gains ranging from 21.5% to 21.8%, which illustrates the progression of body weight and percentage gains in all treatment groups (Table 2). Thus, the present findings indicated that oral administration of *L. indica*, *P. emblica* and *S. rhombifolia* leaf extracts did not adversely impact metabolic activity, nutrient assimilation, or food utilisation efficiency.

These results are in concordance with previous studies, wherein administration of *P. emblica* ethanolic extract exhibited no adverse effects on body weight in Sprague-Dawley rats (Srimangkornkaew *et al*., 2018), *S. rhombifolia* extract was well tolerated in Wistar rats (Ramalho *et al*., 2019) and *L. indica* extract did not impair normal growth patterns in Swiss albino mice (Chandrashekar *et al*., 2022). According to the OECD (OECD, 2000) guidelines, a reduction in body weight greater than 25% within a seven-days period, is indicative of systemic toxicity. The absence of such weight loss in the present study, coupled with consistent weight gain, further supports the safety and non-toxic nature of the evaluated plant extracts (Xavier *et al*., 2022).

Table 2. Body weight changes over 14 days of experiment

Group	Day 0 (g)	Day 7 (g)	Day 14 (g)	% Weight gain
L. indica	167.4 ± 3.1	186.0 ± 3.2	203.6 ± 3.0	21.6 ± 2.9
P. emblica	166.8 ± 3.4	185.9 ± 3.5	203.2 ± 2.9	21.8 ± 3.0
S. rhombifolia	167.9 ± 3.2	186.5 ± 3.3	204.0 ± 3.1	21.5 ± 3.0

Values are expressed as Mean ± SEM; n= 5

Post-mortem evaluation revealed the absence of any gross pathological alterations in vital organs among all treated groups. The relative organ weights remained within normal physiological ranges, with liver weights between 3.45% and 3.52%, kidney weights ranging from 0.76% to 0.79% and heart weights from 0.38% to 0.40%, indicating that administration of *L. indica*, *P. emblica* and *S.* leaves extracts did not elicit any evident organ-specific toxicity (Table 3).

According to the OECD, Guideline 420, acute oral toxicity assessments facilitate the classification of substances under the Globally Harmonised System (GHS) of Classification and Labelling of Chemicals. This system categorises substances based on oral median lethal dose (LD□□) values, with Category 1 (LD□□ ≤ 5 mg/kg), Category 2 (5-50 mg/kg), Category 3 (50-300 mg/kg), Category 4 (300-2000 mg/kg) and Category 5 (2000-5000 mg/kg), the latter indicating a relatively low acute toxicity potential. In the present study, the absence of treatment-related clinical signs, behavioural changes, or mortality at limit dose level of 2000 mg/kg supports the classification of all the three test substances into GHS Category 5/ unclassified substances, further affirming their low acute toxicity and favourable safety profile (Table 4).

The collective findings, thus demonstrate that acute oral administration of *L. indica, P. emblica and S. rhombifolia* leaf powders at 2000 mg/kg is well tolerated in Wistar rats, with no evidence of systemic toxicity. The absence of mortality, significant clinical signs, or organ pathology, combined with normal growth patterns, supports their safety profile and potential for further pharmacological investigation. These results validate traditional uses of these plants and provide a foundation for subsequent subacute and chronic toxicity studies.

Table 3. Relative organ weights (per 100g of body weight) after 14 days of experiment

Group	Liver	Kidneys	Heart	Lungs	Spleen
L. indica	3.45 ± 0.10	0.79 ± 0.04	0.38 ± 0.02	0.91 ± 0.03	0.37 ± 0.01
P. emblica	3.52 ± 0.12	0.76 ± 0.03	0.40 ± 0.02	0.93 ± 0.04	0.36 ± 0.02
S. rhombifolia	3.50 ± 0.09	0.77 ± 0.04	0.39 ± 0.01	0.90 ± 0.03	0.38 ± 0.02

Table 4. Acute toxicity classification

Plant material	Tested dose (mg/kg)	GHS category	NOAEL (mg/kg)
L. indica	2000	5	≥2000
P. emblica	2000	5	≥2000
S. rhombifolia	2000	5	≥2000

Conclusion

The present study concludes that oral administration of *Phyllanthus emblica*, *Sida rhombifolia* and *Leucas indica* leaf powder suspensions at limit dose level of 2000 mg/kg body weight did not produce any mortality, clinical signs of toxicity or significant changes in body weight and relative organ weights in Wistar rats over a 14-days of experiment period. These findings indicate that these three plant extracts are safe at the tested dose and fall under GHS Category 5, suggesting low acute oral toxicity and preliminary safety profile in accordance with OECD guideline 420. The results thereby support the traditional medicinal use of these plants and provide a preliminary safety foundation for their future development as therapeutic agents. However, further investigations involving subacute and chronic toxicity and biochemical parameters are necessary to confirm their long-term safety and pharmacological potential.

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