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RESEARCH ARTICLE

TOXICITY OF *PHYLLANTHUS NIRURI* CRUDE EXTRACT: HEMATOLOGICAL CHANGES IN MICE AFTER 14 DAYS OF ORAL ADMINISTRATION

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ABSTRACT

Phyllanthus niruri, a widely used herbal remedy, has demonstrated various therapeutic properties. However, limited toxicological data exist regarding its safety profile. This study aimed to investigate the sub-acute toxicity manifested as hematological changes in a mice model following repeated oral administration over 14 days. A controlled randomized design was used with 24 mice, divided into four groups of six animals each. One control group was administered distilled water, while three treatment groups received aqueous extracts at low (1000 mg/kg), intermediate (2000 mg/kg), and high (4000 mg/kg) doses. No mortality was observed, and clinical signs of acute toxicity were absent. Behavioral changes such as mild lethargy and hypokinesia were transient and reversible. Body weight measurements indicated a dose-dependent reduction, with the high-dose group showing a significant decrease by day 14. Hematological analysis revealed a significant increase in neutrophils, monocytes, and eosinophils at higher doses, indicating an inflammatory or immune response. Additionally, a significant decrease in RBC count was observed in the high-dose group, suggesting potential hematological effects, including anemia. These findings suggest that while Phyllanthus niruri extract is generally nontoxic at lower doses, prolonged use or higher doses may lead to immune-related pathology and anemia.

KEYWORDS

anemia, hematology, Phyllanthus niruri, sub-acute toxicity

1. INTRODUCTION

It is estimated that up to 80% of the world's population still relies on traditional medicine for their primary healthcare needs. In 2012, the U.S. market for herbal supplements was estimated to be worth $34 billion and the demand continues to grow globally in both developed and developing countries due to their effectiveness in prevention and treatment of different conditions (Aziz et al., 2018; Msomi et al., 2018). This has led to their availability without prescriptions and a lack of recognition of potential hazards in inferior products (Petejova et al., 2019; Walum, 1998). Although herbal medicines are generally considered safer, their indiscriminate use without proper dosing and a lack of research on drug-drug interactions raise serious concerns for the global healthcare system. Additionally, the potential presence of toxic compounds in some plants, their increased toxicity at high doses or prolonged usage, and the risk of contamination with heavy metals or pesticides have highlighted the broader issue of herbal medicine and a subject of intensive research (Dang and Van Damme, 2015; Karturi et al., 2016).

Phyllanthus niruri, an herbaceous plant commonly referred to as Bhuiamala, is prevalent as a weed in both cultivated and wastelands throughout Nepal. Its ethnoveterinary uses have been well documented across the world. Primarily it is used in management of Kidney stone and as hepatoprotective agent. Proven pharmacological properties, including anti-inflammatory, anti-viral, anti-bacterial, anti-cancers, antinociceptive, analgesic activity have been already documented (Ak et al., 2003; Adedapo et al., 2015). Phytochemical analysis revels a variety of bioactive compounds, such as phyllanthin, hypophyllanthin, niranthin, phyllanthocin D, geraniin, ellagitannins, quercetin, flavonoids, alkaloids, lignans, tannins, responsible for its therapeutic effects (Ghosh et al., 2022; Kumar et al., 2015; Wu et al., 2019). Limited information is available on toxicological property of *Phyllanthus niruri* till date. In mice model, the current study attempts to correlated the sub-acute toxidromes with blood after repeated dose administration. We aim to provide baseline information to help healthcare professionals and general public about potential toxicity for Promoting public health as a whole.

2. MATERIALS AND METHODS

The whole plant was collected (GPS 27.705740626537246, 84.4126859371659) identified by its vernacular names by the farmers and authenticated at The National Herbarium and Plant Laboratories (NHPL), (https://kath.gov.np/) Godawari Kathmandu with Ref. No.-080/81 57. The collected plant was washed, shed dried for 48 hours and processed into fine powder. 100 grams powder was mixed with 500 ml distilled water and agitated with reciprocating shaker (150 rev/ min, 2 hrs.). The extract was filtered with Whatman No. 1 filter paper. Concentrated using a rotary vacuum evaporator at a temperature of 50°C. A stock solution of 100 mg/ml concentration was prepared by adding 100 mg of crude extract with 1 ml of distilled water.

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Table 1: The yield of aqueous extract of *Phyllanthus niruri*

Batch	Solvent (Distilled water)	Wt. of plant powder (g)	Wt. of crude extract (g)	Yield
B1	500 ml	100	8.6	8.6%
B2	500 ml	100	8.1	8.1%
B3	500 ml	100	8.4	8.4%
	Average	**300**	**31.2**	**8.3%**

Adult *Mus musculus* (Albino lab mice) of either sex was maintained at the facility in the Veterinary Pharmacology laboratory, Agriculture and Forestry University, Nepal adhering the hosing and feeding guidelines by National Institutes of Health (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Approval for animal use for research was obtained from the Ethical Committee of Nepal Veterinary Council Ref No. Ethical 328(ka)2079/80.

CRD trial with three treatment groups and one control group was used.

Each group had 3 male and 3 female mice. 1 ml fluid was administered the extract through forced feeding at the same time of day for 14 consecutive days. The trial employed the repeated dose toxicity testing protocol as recommended by (Das et al., 2015). During the observation period clinical signs of toxidromes as well as mortality were monitored as outlined in the study by (Silva et al., 2021). Body weights were measured before dosing on the day of administration and continued to be measured every week thereafter.

Table 2: The research design for acute toxicity study of aqueous extract of *Phyllanthus niruri*

	Control Group (PC)	Treatment		
		Low dose (LD)	Intermediate dose (ID)	High dose (HD)
Replication (n)	6	6	6	6
Dose (mg/kg b.wt.)		1000	2000	4000

On the 15th day hematological examinations were carried using the DP-H10 Automatic Hematology Analyzer, manufactured by Shenzhen Dymind Biotechnology Co., Ltd. (https://www.dymind.com/en-US/products/95). The statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 27.0. All data were expressed as Mean ± SEM. Statistical tests were conducted at 95% level of confidence. All graphical representation was done in Graph pad prism version 9.

3. Results and Discussion

3.1 Cage Side Observation

Following repeated dose administration, no clear signs of intoxication were observed in either sex across the tested dose levels within the first 24 hours. Normal behavioral changes such as lethargy, repeated head flicking, mouth scratching, and transient hypokinesia were observed within the first hour after administration and were reversible.

Table 3: Cage-side observations following repeated dose administration of the plant extract

FOB Parameters	Acute Toxicity (x/n)			
	Control	LD	ID	HD
Piloerection	0/6	0/6	0/6	0/6
Abnormal reaction to handling	0/6	0/6	0/6	0/6
Palpebral closure	0/6	0/6	0/6	0/6
Lacrimation	0/6	0/6	0/6	0/6
Abnormal gait	0/6	0/6	0/6	0/6
Tremor	0/6	0/6	0/6	0/6
Convulsion	0/6	0/6	0/6	0/6
Defecation	0/6	0/6	0/6	0/6
Vocalization	0/6	0/6	0/6	0/6
Loss of righting reflex	0/6	0/6	0/6	0/6
Oculonasal discharge	0/6	0/6	0/6	0/6
Hypokinesia	0/6	0/6	0/6	0/6
Tail elevation	0/6	0/6	0/6	0/6
Fear	0/6	0/6	0/6	0/6
Death	0/6	0/6	0/6	0/6

x/n denotes fraction of animal affected; x=number of animals affected; n (sample size) = 6

Cage side observation in toxicological studies helps in identifying early signs of toxicity (Ifeoma et al., 2013) (Lee et al., 2023) where each signs has its own significance. Piloerection, the raised fur or hair, can indicate stress, discomfort, or autonomic nervous system response. Abnormal reaction to handling suggests elevated sensitivity, pain, or anxiety related to the toxic effects. Palpebral closure, the closing of eyelids, may signify eye irritation or discomfort. Lacrimation, and excessive tearing, can be a response to eye irritation or inflammation. Abnormal gait indicates impaired motor function or coordination. Tremors, convulsion, Loss of righting reflex may indicate neurological dysfunction or muscular abnormalities. Defecation can be influenced by stress, gastrointestinal irritation, or changes in gut motility. Vocalization, tail elevatiom may indicate pain, distress, or discomfort. Oculonasal discharge suggests irritation, inflammation, or respiratory tract involvement. Hypokinesia refers to reduced voluntary movement or activity. Fear reflects anxiety, apprehension, or avoidance behavior. Finally, death represents the outcome of severe toxicity or adverse effects caused by the substance, indicating the inability to survive.

The absence of adverse cage side observation indicates lack of acute visible toxicity at administered dose. This is consistent with the results of other studies of the acute toxicity of Phyllanthus species, which have also found that these plants are generally non-toxic. For example, a study by (Pingale and Shewale, n.d.) found that oral administration of aqueous

extract of *Pacidus* at doses up to 1000 mg/kg b.wt. did not cause any signs of toxicity in Pregnant Wistar Rats. Similarly, a study by found that oral administration of aqueous extract of *P. niruri* at doses up to 800 mg/kg b.wt. did not cause any signs of toxicity in Wistar rats (Singh et al., 2016).

3.2 Body Weight Measurement

HD group showed significant decrease in body wt. after 14 days. Other changes were statistically non-significant. Overall, the results suggest a dose-dependent effect of the extract on body weight in mice.

Table 4: B.wt. (in gm) changes following repeated dose administration of the plant extract

		Control	Treatment		
			LD	ID	HD
b.wt. (gm)	Day 0	32.75 ± 0.61	32.63 ± 0.71	31.96 ± 0.44	32.70 ± 0.84
	Day 7	33.36 ± 1.1	32.98 ± 0.57	32.63 ± 0.68	31.81 ± 1.85
	Day 14	33.41 ± 0.80	33.67 ± 0.67	32.65 ± 0.55	31.23 ± 0.61*
p-value		0.21	0.69	0.18	0.04

Values are expressed as mean ± SEM, n=6 *Significantly different from control, $p < 0.05$

When an animal is exposed to a toxic substance, a decrease in body weight is generally observed (Wang et al., 2012). The reduction in body weight in the high-dose group may be due to the presence of high levels of polyphenols, which could be above the tolerable dose (Wu et al., 2022). Polyphenols are known to induce weight loss through mechanisms such as appetite suppression, increased metabolic rate, and inhibition of fat absorption, all of which contribute to weight reduction (Aloo et al., 2023). However, the normal feed and water intake throughout the study period suggests that appetite suppression was not a factor.

In contrary different studies suggest that Phyllanthus species do not significant changes in b. wt. in animal models. (Lawson-Evi et al., n.d.) reported that mice treated with *P. amarus* at doses of 2, 4, 6, or 8 g/kg b.wt. did not show significant alterations in b.wt. compared to the control group. Similarly, a group researchers found no significant changes in b.wt. in rats treated with an ethanol extract of *P. emblica* at doses of 200, 400, 800, or 1600 mg/kg b.wt. (Anto et al., 2022). As reported by a group researcher *P. niruri* at doses of 22.5, 45, or 90 mg/kg b.wt. did not induce any significant changes in b.wt. of rats after 90 days of treatment (Jantan et al., 2019). In line with these findings, a group researchers conducted another study using *P. amarus* on mice at doses of 1 & 3 g/kg b.wt. for 28 days did not result in significant alterations in b.wt. some researchers reported *P. tenellus* no significant changes in b.wt. at doses of 200, 1000, or 3000 mg/kg b.wt. for 28 on mice (Lawson-Evi et al., n.d.; Yeap et al., 2021).

3.3 Hematological Changes

Table 5: Hematological changes following repeated dose administration of the plant extract

	Parameters	Unit	Range	Control	Treatment			p-value
					LD	ID	HD	
WBC	WBC Count	(* 10^3/uL)	9.1-28.7	14.07 ± 0.28^{a}	14.08 ± 1.43^{a}	14.21 ± 0.27^{a}	14.14 ± 0.27^{a}	0.99
	Neutrophils	(* 10^3/uL)	1.9-11.5	2.96 ± 0.13^{a}	2.99 ± 0.3^{a}	3.52 ± 0.17^{ab}	3.80 ±0.16^{b}	0.022
	Lymphocytes	(* 10^3/uL)	6.7-15.7	10.02 ± 0.42^{a}	8.99 ± 0.86^{a}	10.58 ± 0.59^{a}	10.42 ± 0.45^{a}	0.272
	Monocytes	(* 10^3/uL)	0.3-1.4	1.28 ± 0.05^{a}	1.12 ± 0.12^{a}	1.39 ± 0.08^{ab}	1.45 ± 0.06^{b}	0.041
	Eosinophils	(* 10^3/uL)	0.05-0.51	0.54 ± 0.02^{a}	0.48 ± 0.05^{a}	0.65 ± 0.04^{ab}	0.78± 0.03^{b}	<0.01
RBC	RBC Count	(* 10^6/uL)	8.48-15.15	9.54 ± 0.15^{a}	9.38 ± 0.25^{a}	9.25 ± 0.25^{ab}	8.74 ± 0.15^{b}	<0.01
	Hb Concn	g/dL	14.8-18.3	10.70 ± 0.33^{a}	11.03 ± 0.38^{a}	11.33 ± 0.52^{a}	12.23 ± 3.35^{a}	0.930
	Mean corpuscular volume	fL	47.4-54.4	50.83 ± 1.33^{a}	51.45 ± 0.67^{a}	50.03 ± 1.29^{a}	51.17 ± 1.86^{a}	0.892
	Hematocrit	%	41.16-1.11	42.25 ± 1.48^{a}	40.55 ± 0.80^{a}	43.95 ± 1.74^{a}	46.48 ± 1.82^{a}	0.067
Platelet	Platelet Count	(* 10^3/uL)	668-1543	707.83 ± 6.07^{a}	682.17 ± 17.57^{a}	668.83 ± 83.29^{a}	669.33 ± 2.85^{a}	0.923

Values are expressed as mean ± SEM, n=6

Treatment means superscripted with the same letter of the same column are not statistically significant

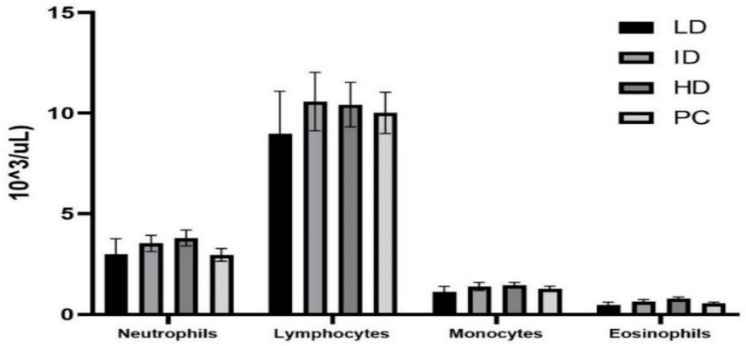

Figure 1: Change in WBC components following repeated dose administration of the plant extract

Changes in the WBC count indicate alterations in immune response, inflammation, infection, and immunosuppression caused by toxicants (Carter, 2018). The treatment groups did not affect the WBC count significantly however an elevation in neutrophil, monocyte, and eosinophil counts indicate triggered inflammatory or immune response (Shakeri et al., 2017). Toxicants or irritants can stimulate the release of pro-inflammatory molecules, such as cytokines and chemokines. These molecules attract neutrophils to the site of exposure, resulting in an accumulation of neutrophils and an inflammatory response (Chen et al., 2017). The increased monocytes reflect an initiated immune response to remove the harmful plant extract components.

Previous studies on the effects of Phyllanthus spp. on WBC count have produced mixed results. One study found that *P. amarus* caused a significant decrease in WBC count in rats, in contrast reported significant increase in WBC count (Kumar and Kuttan, 2005; Adedapo et al., 2005). A group researchers found *P. amarus* did not cause any significant changes in WBC count in non-insulin-dependent diabetic patients, but did cause a significant decrease in the number of lymphocytes (Moshi et al., 2001). A group researcher in rats infected with *Salmonella typhi* found that treatment with *P. amarus* leaf extract significantly increase the WBC count, but also caused a significant decrease in the number of lymphocytes (Nwankpa et al., 2014). In a study, in rats showed that the extract significantly increased the WBC count (Lata et al., 2014). The increase was most pronounced in the granulocyte count, accompanied by an increase in the number of lymphocytes.

RBCs are particularly vulnerable to damage from free radicals and inflammation due to their high concentration of polyunsaturated fatty acids, which are easily oxidized (Lobo et al., 2010). Oxidative damage and inflammation can lead to the breakdown of RBCs, releasing hemoglobin into the bloodstream. This can result in anemia characterized by a low number of RBCs. Plant extracts may contain compounds like polyphenols and flavonoids that promote ROS production. Compounds like quercetin and catechins found in plant extracts may hinder the activity of antioxidants (Iacopini et al., 2008).

There is scientific evidence suggesting that Phyllanthus extracts can have significant effects on hemolysis and hemoglobin concentration, although the outcomes are not always consistent across different studies. Studies have reported that *P. amarus* extract induced hemolysis in rats and that the aqueous extract of *P. niruri* induced oxidative stress in rats (Adedapo et al., 2005; Chatterjee and Sil, 2006). On the other hand, some studies have found that Phyllanthus extracts can increase hemoglobin concentration in animal models. For example, the aqueous extract of *P. niruri* at a lower dose was found to be a potent erythropoietic substance (Muhammad et al., 2021). Another study demonstrated that Phyllanthus extract increased hemoglobin concentration by 20% in rats with iron deficiency anemia, However, there are also contradictory findings; in a study of rats with diabetes, *P. acidus* extract significantly increased hemoglobin levels, demonstrating the complexity of Phyllanthus extracts' effects on hemoglobin levels (Pramyothin et al., 2007; Chaimum-aom et al., 2016).

Platelets primary function is in hemostasis to control bleeding, they also actively contribute to the inflammatory and immune response by releasing inflammatory mediators and cytokines when activated by toxic agents (Iba and Levy, 2018). Phyllanthus extract exhibits a dual effect on platelets, as evidenced by studies. At low concentrations, the extract can enhance platelet aggregation, attributed to its ability to increase the expression of P-selectin on the platelet surface, promoting platelet adhesion to each other and blood vessel walls (Kassuya et al., 2006). On the other hand, at higher concentrations, the extract inhibits platelet aggregation. In a separate study, *P. amarus* extract demonstrated platelet aggregation inhibition at a concentration of 200 µg/mL, likely through its ability to inhibit platelet-activating factor (PAF) activity (Yuandani et al., 2013).

4. Conclusion

The extract did not cause mortality at the tested dose. A dose-dependent decrease in body weight was observed. There was a significant reduction in neutrophils, monocytes, and eosinophils. A decrease in RBC count was also evident. Overall, these findings suggest that the plant extract is nontoxic at lower doses but may cause WBC-related pathology and anemia at higher doses.

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