Kwaghe International
Journal of Sciences and Technology

ISSN: 1595-7977

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/KIJST.v2i2.6145

Toxicological Evaluation of Aqueous Leaf Extract of *Phyllanthus niruri* in Wistar Rats

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Apr 22, 2025	May 20, 2025	Jun 2, 2025	Jun 7, 2025

Abstract

Natural products have long been used as therapeutic agents; however, many lack comprehensive scientific evaluation of their toxicity. This study investigates the potential toxicity of *Phyllanthus niruri* (P. niruri) aqueous leaf extract in Wistar rats. Twenty-five male albino rats were randomly divided into five groups of five animals each. Groups 2 through 5 received daily oral doses of 100, 200, 400, and 800 mg/kg body weight of the extract, respectively, while Group 1 served as the control and received only grower mash and distilled water for 28 days. Toxicological assessment was conducted through hematological profiling and evaluation of liver and kidney function using standard biochemical methods. The results indicated a significant, dose-dependent increase ($p<0.05$) in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), suggesting hepatic stress. In contrast, albumin, creatinine, and urea levels did not differ significantly ($p<0.05$) from the control group, indicating no observable impairment in renal function. Hematological analysis revealed a significant decrease ($p<0.05$) in packed cell volume (PCV) and red blood cell (RBC)

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count, while white blood cell (WBC) count, hemoglobin (Hb) concentration, and mean corpuscular volume (MCV) significantly increased with higher extract doses. These findings suggest that while *P. niruri* is traditionally regarded as a natural remedy, its aqueous leaf extract may induce hematological and hepatic alterations at elevated doses. Caution is therefore advised in its use, particularly in unregulated or prolonged applications.

Keywords: *P. niruri*; Liver; Kidney; White Blood Cell; Packed Cell Volume

INTRODUCTION

Medicinal plants represent the oldest form of medication, used for thousands of years in traditional medicine across many countries worldwide. The empirical knowledge regarding their beneficial effects has been passed down through generations within human communities (Khan, 2014). Natural products play a crucial role as a source of drug compounds, and many modern drugs derived from traditional herbal medicine are now utilized in contemporary pharmacotherapy (Patwardhan et al., 2008). Consequently, medicinal plants hold great importance because of their unique characteristics as a vast source of therapeutic phytochemicals, which may facilitate the discovery and development of novel drugs (Azwanida, 2015). However, most of these traditional herbal drugs are produced and obtained without having been scientifically assessed for their safety.

Phyllanthus niruri is an annual shrub that can grow between 30 and 100 cm tall. Its trunk consists of a large, round, smooth, hairless stem, approximately 3 mm in diameter and green in color. The leaves are compound and arranged alternately (Obat and Indonesia, 2008; Triyono, 2016). Each leaf features 15 to 24 ovate leaflets with blunt tips and rounded bases. The leaves are about 1.5 cm long, 7 mm wide, flat-edged, and green (Triyono, 2016; Ezzat *et al.*, 2020). Flowers appear as single blooms located near the petiole, hanging down, and are white in color (Meilani *et al.*, 2020). The petals are star-shaped, while stamens and pistils are not visible. The small crown is white, and the fruit is round and flat, approximately 2 mm in diameter and purplish-green. The seeds are small, hard, kidney-shaped, and brown (Meilani *et al.*, 2020). The Phyllanthus genus contains over 600 species distributed throughout the tropical and subtropical regions of the world (Ezzat *et al.*, 2020). Plants in the Phyllanthus genus have long been used to treat liver diseases (Calixto *et al.*, 1998). The P. niruri plant has been reported to possess anti-inflammatory, hypoglycemic,

antiviral, antioxidant, hepatoprotective, and inhibitory effects on renal stone formation (Shilpa *et al*., 2018). The P. niruri plant is widely used by the people of Central Sulawesi as a remedy for diarrhea, fever, and cough (Ifandi *et al*., 2016). The leaves are effective in accelerating wound healing as an anti-inflammatory agent (Couto *et al*., 2013). P. niruri has also been traditionally used to treat several ailments such as jaundice, kidney stones, flu, fever, and diabetes (Suraya *et al*., 2021). Several researchers have discovered that P. niruri possesses anti-tumor, antioxidant, anti-carcinogenic, and hepatoprotective properties (Chatterjee and Sil, 2006; Harish and Shivanandappa, 2006). This study evaluated the toxic effects of P. niruri aqueous leaf extract on the liver, kidney, and hematological parameters of albino rats.

MATERIALS AND METHODS

Plant Material:

Fresh leaves of *Phyllanthus niruri* were collected from Mubi North Local Government, Adamawa State. The plant material was taxonomically identified by a Botanist in Botany Department of Adamawa State University, Mubi, Nigeria where the voucher specimen was deposited.

Experimental Animals

Twenty-five (25) healthy Male albino rats weighing about 100 g to 160 g obtained from the Animal Resource Unit, National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria were used for the study. The animals were housed at room temperature in wired cages for seven (7) days acclimatization under standard environmental conditions before the initiation of the experiment where a 12 h light/dark cycle was maintained. An ethical clearance for conducting the experiment on research animals was secured from the University Ethical Committee prior to the initiation of the experiment.

Extraction of the plant material

P. niruri leaves collected were washed and air dried at room temperature for fourteen (14) days. The samples were then pulverized into fine powder with the aid of mortar and pestle. 200 g of the powdered sample was then soaked into 1000 mL of distilled water and allowed to stand at room temperature where it was stirred every 24 h for a period of 72 hours, after which the mixture was pressed and strained by filtration, using Whatman no. 1 filter paper

and the solvent was evaporated using a water bath and crucible at 400c until the extract was dried.

Phytochemical analysis

Qualitative phytochemical screening of the extract was carried out according to the method described by Nweze et al. (2012).

Sub-acute toxicity assay

Four (4) doses were administered to rats for 21 days for sub-acute toxicity determination. Twenty (25) rats were grouped into 5 groups of 5 rats each. The rats were grouped accordingly were group 1 received grower mash and distilled water only, group 2 received 100 mg/kg b.wt. of the extract, group 3 received 200 mg/kg b.wt. of the extract, group 4 received 400 mg/kg b.wt. of the extract, and group 5 received 800 mg/kg b.wt of the extract.

Sample collection

After 21 days of daily administration, the blood samples were collected by dissecting the rats followed by cardiac puncture after a mild anesthesia with chloroform. The blood samples were analyzed for albumin, ALT, AST, ALP, creatinine, and urea levels based on the methods described by Rietman and Frankel (1957), Wright *et al.* (1972), Penhaker *et al.* (2013), and Rifai (2018). The method described by Dacie and Lewis (2016) was used to determine the levels of hematological parameters.

Statistical analysis

All data collected from the study were statistically recorded as mean ± standard error of mean. Statistical differences among the mean values were determined using Analysis of Variance Test (ANOVA) followed by Duncan's Multiple Range Test. IBM SPSS statistics software version 23 was used to analyze all data collected.

RESULTS

Phytochemical components of aqueous *P. niruri* leaf extract are presented in Table 1. The phytochemicals screened for includes alkaloids, flavonoids, phenols, tannins, and saponins. The result disclosed the presence of all the phytochemicals assayed for.

Table 1. Phytochemical screening of aqueous leaf extract of *Phyllanthus niruri*

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phenols	+

Key: + = present

Table 2 shows the serum levels of liver function parameters, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase of albino rats treated with *P. niruri* leaf extract for 21 days. The results showed that the6 levels of liver function parameters increased with increase in dose ($p<0.05$) when compared with the control group, except for Albumin which showed no significant difference when compared with the control group.

Table 2. Effect of aqueous leaf extract of *P. niruri* on liver function

Group	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	3.70 ± 0.11^{a}	17.00 ± 1.15^{a}	57.66 ± 0.66^{a}	54.33 ± 1.45^{a}
100 mg/kg Extract	3.73 ± 0.06^{a}	21.00 ± 1.15^{a}	65.33 ± 2.02^{b}	59.33 ± 0.88^{ab}
200 mg/kg Extract	4.06 ± 0.06^{a}	27.00 ± 1.15^{a}	77.33 ± 1.20^{c}	63.66 ± 1.76^{b}
400 mg/kg Extract	4.33 ± 0.03^{a}	31.66 ± 0.66^{C}	85.66 ± 1.45^{d}	78.00 ± 1.52^{c}
800 mg/kg Extract	4.33 ± 0.06^{a}	35.33 ± 0.88^{c}	94.00 ± 1.73^{e}	86.00 ± 1.15^{d}

All data are presented as mean ± SEM. Different superscripts down the column indicates that they are significantly different at ($p<0.05$), n=5

Table 3 shows the effects of *P. niruri* aqueous leaf extract on serum creatinine and urea levels, were the results showed no significant difference ($p<0.05$) in all the groups when compared to the control group.

Table 3. Effect of aqueous leaf extract of P. niruri on serum urea and creatinine

Groups	Creatinine (mg/dL)	Urea (mg/dL)
Control	0.53 ± 003^{a}	17.66 ± 0.33^{a}
100 mg/kg Extract	0.63 ± 0.03^{a}	16.33 ± 0.33^{a}
200 mg/kg Extract	0.66 ± 0.03^{a}	15.66 ± 0.88^{a}
400 mg/kg Extract	0.63 ± 0.03^{a}	16.00 ± 0.57^{a}
800 mg/kg Extract	0.73 ± 0.03^{a}	17.00 ± 0.57^{a}

All data are presented as mean ± SEM. Different superscripts down the column indicates that they are significantly different at ($p<0.05$), n=5

The effects of P. niruri aqueous leaf extract on hematological parameters were shown in table 4. There was a significant decrease ($p<0.05$) in the levels of PCV and RBC when treatment groups were compared to the control groups, however, there was no significant difference ($p<0.05$) observed in the groups treated with the extract. WBC, Hb, and MCV showed a significant increase ($p<0.05$) in a dose dependent manner and when compared with the control group.

Table 4. Effect of *P. niruri* on hematological parameters

Group	PCV (%)	WBC (%10^9/L)	RBC (%10^2/L)	Hb (g/dL)	MCV (fl)
Control	41.66 ± 0.66^b	94.66 ± 1.52^a	5.06 ± 0.20^b	9.88 ± 0.19^a	70.44 ± 0.19^a
100 mg/kg Extract	33.66 ± 1.20^a	95.00 ± 0.88^b	4.26 ± 0.15^a	13.44 ± 0.58^b	88.05 ± 1.40^c
200 mg/kg Extract	34.66 ± 0.66^a	101.00 ± 1.15^b	4.16 ± 0.37^a	10.88 ± 0.50^b	71.16 ± 2.65^b
400 mg/kg Extract	37.33 ± 1.85^a	101.66 ± 1.20^c	4.10 ± 0.10^a	11.44 ± 0.19^b	87.43 ± 1.94^c
800 mg/kg Extract	35.00 ± 0.57^a	108.00 ± 1.25^C	4.56 ± 0.15^a	11.77 ± 0.69^b	86.77 ± 1.58^c

All data are presented as mean ± SEM. Different superscripts down the column indicates that they are significantly different at ($p<0.05$), n=5

DISCUSSION

Plants synthesize a wide range of chemical substances, some of which are considered very important in treating several diseases. The extraction of these secondary metabolites relies on their ability to dissolve in various solvents (Edewor and Olajire, 2014). The therapeutic benefits of plants stem from their phytoconstituents, which have a specific physiological action on humans (Owolarafe *et al.*, 2022). Over the years, several studies have demonstrated that these phytoconstituents exhibit biological actions, for instance, antimicrobial (Mada *et al.*, 2013), antifungal (Craig, 1999), antioxidant, anticancer (Jafarian *et al.*, 2014), and hepatoprotective (Chinaka *et al.*, 2011). However, other researchers have reported that these bioactive principles can also have toxic effects such as hepatotoxicity (Salau *et al.*, 2012) and nephrotoxicity (Sunmonu *et al.*, 2014). Some of the active phytochemicals identified in this study include alkaloids, flavonoids, saponins, tannins, and phenols. The liver is an essential organ that performs various biochemical activities,

including synthetic and excretory functions; thus, no single biochemical test can fully assess the general function of the liver (Thapa and Walia, 2007; Ahmed *et al*., 2022). Serum ALT and AST levels reflect hepatocellular injury, while serum ALP indicates impaired bile excretion and bile flow (Ahmed *et al*., 2022). The observed significant increase in the serum levels of the enzymes suggests that the aqueous leaf extract is toxic in a dose-dependent manner, as higher doses result in elevated serum levels. The significant rise in serum ALP levels may indicate hepatic obstruction of bile flow. Albumin is an important protein produced by the liver (Rothschild *et al*., 1997). The serum albumin level in this study neither shows a significant increase nor a decrease; thus, it may suggest that the aqueous leaf extract of *P. niruri* does not impact the metabolic pathways of albumin.

Serum creatinine concentration has been used to assess and evaluate kidney function, rising only when the glomerular filtration rate (GFR) has diminished by one-half. Thereafter, the increase in creatinine is exponential to the decline in GFR (Korhonen, 2015; Ahmed *et al*., 2022). This study observed no significant difference in serum creatinine levels, which may indicate that the aqueous *P. niruri* leaf extract doesn't affect the glomerular filtration rate. Urea, a by-product of protein and amino acid breakdown produced by the liver, is distributed throughout intracellular and extracellular fluid (Gowda *et al*., 2010). It is filtered from the blood by the glomeruli in the kidney and is partially reabsorbed with water. This compound is another common biochemical parameter for estimating kidney function and is useful in the differential diagnosis of acute and pre-renal conditions (Gowda *et al*., 2010).

The decrease in some hematological parameters upon administration of aqueous extracts of *P. niruri* leaf (PCV and RBC), which were not dose-dependently different, however, when compared to the control group, the treatment group showed a significant increase. This may be an indication corroborating toxicity leading to the decrease in these parameters (Zhu *et al*., 2021). Statistically significant increase in WBC, Hb, and MCV concentration gives a suggestion, which may be due to the toxicity of certain phytoconstituents within the which may be affecting an inflammatory response in the biological system (Bello *et al*., 2016).

CONCLUSION

Based on the observed results from this study, the aqueous *P. niruri* leaf extract is not completely safe and a prolonged period of administration of this extract may lead to severe adverse effects on the biological system.

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