

Biosafety evaluation of ethanolic extract of *Phyllanthus amarus* leaves on liver and kidney of wistar rats

OJOCHENEMI EJEH YAKUBU, MICHAEL SUNDAY ABU*, OKONKWO CHIBUIKE INNOCENT,
JOB ITANYI ONUCHE

Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari. PMB 1020 Katsina-Ala Road, Wukari, Taraba State, Nigeria. Tel./fax.: +23-480-000-0000, *email: abulex77@yahoo.com

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Abstract. Yakubu OE, Abu MS, Innocent OC, Onuche JI. 2022. Biosafety evaluation of ethanolic extract of *Phyllanthus amarus* leaves on liver and kidney of wistar rats. *Asian J Nat Prod Biochem* 20: 1-5. Medicinal plants such as *Phyllanthus amarus* Schumach. & Thonn. have played a significant role in maintaining human health since ancient times. Documented evidence shows that the plant has been used to treat various ailments. Still, its biosafety on vital organs such as the liver and kidney has not been specifically studied. The study was designed to ascertain the biosafety of ethanolic extract of *P. amarus* leaves on the liver and kidney of wistar rats. A total of twenty (20) wistar rats were used, divided into 4 groups of 5 animals each (Group A, B, C, D). Group B, C, D received 100 mg/kg, 200 mg/kg, 400 mg/kg of the extract, respectively. Group A (Control) received only distilled water. The treatment was carried out for two weeks. At the end of the experiments, the animals were sacrificed. Afterward, liver function and renal function evaluations were conducted. The indicators of liver function include Total protein (TP), aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin (ALB), direct bilirubin (DB), and total bilirubin (TB) were determined. The indicators of renal function include sodium ion (Na⁺), potassium ion (K⁺), chlorine ion (Cl⁻), urea, and creatinine. The results showed that the parameters of the liver function of treated animals were no significant ($p > 0.05$) difference compared to the normal rats (control). Concentrations of sodium ions (Na⁺), potassium ions (K⁺), chlorine ions (Cl⁻), urea, and creatinine in the treated animals were statistically comparable to the normal rats at ($p < 0.05$). This research showed that *P. amarus* did not induce hepatotoxicity or nephrotoxicity at the dose of 100-400 mg/kg BW. Furthermore, this finding has validated the use of *P. amarus* as a traditional herb for treating and managing several diseases, including kidney and liver diseases, without significant organ damage.

Keywords: Biosafety, ethanolic extract, kidney, liver, *Phyllanthus amarus*, wistar rats

INTRODUCTION

Medicinal plants have continued to play a dominant role in maintaining human health since ancient times. Most modern medicinal drugs are of plant origin (Tuhinadri and Sami 2015) and are essential in drug development programs of the pharmaceutical industry. The knowledge generated over decades has revealed medicinal plants such as *Phyllanthus amarus* Schumach. & Thonn. have been effective in the traditional management of various diseases. Based on these conditions, about 80% of the world population depends solely on traditional (Emeka et al. 2013) or herbal medicine to treat various diseases, especially in Africa and other developing nations (Ilonga et al. 2018; Yakubu et al. 2020a). However, the historical role of medicinal herbs in treating and preventing disease does not assure their safety for uncontrolled usage by an uninformed public (Matthews et al. 1999). Most medicinal plants have relatively non-toxic potential or adverse effects when used by humans; however, some medicinal plants have a high level of toxicity. In addition, they have the possibility of damaging organs. For this reason, caution in using medicinal plants is presently on the increase due to easy availability, affordability, accessibility, and promising efficacy compared to the often-high cost and adverse effects of the standard synthetic drug (Yakubu et al. 2020c).

Phyllanthus amarus is a broad-spectrum medicinal plant that has received worldwide recognition (Srividya and Perival 1995). They are widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver diseases (Liu et al., 2003). *P. amarus* is generally employed to reduce pain, expel intestinal gas, stimulate and promote digestion, as anti-helminths to expel intestinal worms, and act as a mild laxative. *P. amarus* also has antiseptic, diuretic, antiviral, antidiabetic, hypotensive, and antipyretic, and it is also used to treat jaundice, diarrhea, dysentery, wound ulcers, and urogenital diseases (Calixto et al. 1998). The plant extracts have been found to contain high levels of saponins, tannins, flavonoids, and alkaloids (Fernand 1998; Naaz et al. 2007; Krithika and Verma 2009). *P. amarus* has been classified as having a low potential for toxicity, with an LD₅₀ averaging 2000 mg/kg/day (Krithika and Verma 2009). However, the toxicity of this plant on vital organs such as the liver and kidney needs to be revalidated, considering its increased demand for several herbal medicine preparations. Therefore, this study was designed to ascertain the biosafety of ethanolic extract of *P. amarus* leaves on the liver and kidney of wistar rats.

MATERIALS AND METHODS

Collection of plant

The fresh leaves of *P. amarus* were collected in December 2019 within the Biological garden of Federal University Wukari Taraba State, Nigeria. The plant was identified and authenticated in the herbarium of the Biological Science Department, Federal University Wukari, Nigeria. The fresh leaves were air-dried for three (3) days and pounded into a fine powder using a mortar and pestle.

Extraction

The dried material (500 g) was macerated in 2000 mL of ethanol (1:4 w/v) for 48 hours at room temperature, according to Yakubu et al. (2014). It was continually stirred every 5 hours. After finishing maceration, the filtrate was sieved with clean white mesh before filtering using Whitman No 1 filter paper. Next, the filtrate was poured into a beaker and air-dried. Finally, the resultant extract (18 g) was placed into the incubator for further studies.

Animals management

Twenty healthy adult wistar rats of both sexes were obtained and maintained in the animal house of the Biochemistry Department, Faculty of Pure and Applied Science, Federal University Wukari, Taraba State, Nigeria. They were acclimatized for two weeks and fed with growers' mash and water ad libitum.

Experimental design

The wistar rats with an average weight of 177 g were randomly assigned into four (4) groups: A, B, C, and D of five (5) animals each. Group B, C, and D served as treatment groups, while group A served as the control. The rats in the treatment groups (B, C, and D) received 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight of ethanolic extracts of *P. amarus* orally through an or gastric tube daily, respectively. The control group received an equal volume of distilled water without the *P. amarus* extract. After the experimental period, animals were sacrificed under chloroform anesthesia, venous blood was collected by cardiac puncture, and the liver was harvested. Blood samples were collected, and serum was obtained by centrifuging at 3000 rpm for 5 min.

Group A: Normal control received distilled water

Group B: 100 mg/kg/p.o/day of extract

Group C: 200 mg/kg/p.o/day of extract

Group D: 400 mg/kg/p.o/day of extract

Determination of biochemical parameters

Analysis of Aspartate Aminotransferase (AST) activity

AST activity was determined by the method described by Amador and Wacker (1962).

Principle: L-Aspartate and α -Ketoglutarate react in the presence of AST in the sample to yield oxaloacetate and L-glutamate. The oxaloacetate is reduced by malate dehydrogenase to yield L-malate with NADH oxidation to NAD^+ . The reaction is monitored by measuring the decrease in NADH absorbance at 340 nm. The reduction rate in absorbance is proportional to AST activity in the

sample.

Procedure: To 1 mL of reagent was added to all required test tubes, 0.05 mL of the sample was added to the sample test tube, and none to the blank. Next, it was incubated at room temperature for 20 minutes and mixed immediately, and the first absorbance of the test was read exactly at 1 minute and then at 30, 60, 90, and 120 seconds at 340 nm. Finally, the mean change in absorbance per minute was determined, and the test results were calculated as follows :

$$\text{Serum AST activity (IU/L)} = \text{Change in A/min} \times 3376.$$

Assessment of Alanine Aminotransferase (ALT) activity

ALT activity was determined by the method described by Amador and Wacker (1962).

Principle: L-alanine and α -ketoglutarate react in the presence of ALT in the sample to yield Pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with oxidation of NADH to NAD. The reaction is monitored by measuring the decrease in absorbance at 340 nm. The rate of reduction is proportional to ALT activity in the sample.

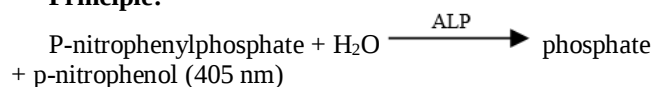
Procedure: To 1 mL of reagent was added to all required test tubes, 0.05 mL of the sample was added to the test sample tube, and none to the blank. Next, it was incubated at room temperature for 20 min and mixed immediately, and the first absorbance was read at precisely 1 minute and then at 30, 60, 90, and 120 seconds at 340 nm. Finally, the mean change in absorbance per minute was determined, and test results were calculated as follows:

$$\text{Serum ALT activity (IU/L)} = \Delta A/\text{min} \times 3376.$$

Assessment of Alkaline Phosphatase (ALP) activity

The serum activity of Alkaline Phosphatase (ALP) was determined by the method Haussament (1977) described.

Principle:



Alkaline phosphatase in a sample hydrolyses para nitrophenyl phosphate into para nitrophenol and phosphate in the presence of magnesium ions. The absorbance increase rate of the reaction mixture at 405 nm and 37°C due to the liberation of para nitrophenol is proportional to the alkaline phosphatase activity.

Procedure: Reagent (1 mL) containing diethanolamine buffer, magnesium chloride, and substrate (P-nitrophenyl phosphate) was added into a clean test tube and incubated at 37°C, followed by the addition of 0.02 mL of sample. The mixture was mixed thoroughly, and the immediate absorbance of the sample was read precisely at 30, 60, 90, and 120 seconds at 405 nm against the reference blank (distilled water). The mean change in absorbance per minute was determined, and the test results were calculated.

The ALP activity was calculated using the following formulae:

Serum alkaline phosphatase activity (IU/L) = $\Delta A/\text{min} \times 2713$.

Serum creatinine and urea

Serum creatinine and urea were determined using an auto-analyzer: Selectra ProM, while the determination of Serum Electrolytes was carried out using the Ion-Selective Electrode (ISE) method (Burnett et al. 2000)

Statistical analysis

The mean $\pm$ SD of all values was calculated, and the change observed between the treatment groups and control was subjected to analysis of variance (ANOVA) using SPSS version 23. Differences between groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of *Phyllanthus amarus* leaves extract on serum electrolytes of wistar rat

The effect of the *P. amarus* leaves extract on some serum electrolyte levels is depicted in Table 1. There was no significant ($p < 0.05$) difference between the rats administered with extract and the normal/control rats. However, a significant ($p < 0.05$) decrease in the Na^+ level of animals that received 100 and 200 mg/kg of the extract compared to the control animals. Similarly, a significant

($p < 0.05$) reduction was observed in the K^+ level of the group that received 100 mg/kg compared to the other extract groups, including the control group.

Effect of *Phyllanthus amarus* leaves extract on urea and creatinine level in wistar rat

All the groups, including the extract administered and the control groups, showed no significant ($p > 0.05$) differences in the urea and creatinine levels, as represented in Table 2.

Effect of *Phyllanthus amarus* leaves extraction liver enzyme of wistar rat

Table 3 showed that the liver enzymes' activity was significantly ($p < 0.05$) high or low in the group that received 200 mg/kg compared to the control group. However, the other extract-administered groups showed no consistent difference in enzyme activity compared with the control.

Effect of *Phyllanthus amarus* leaves extract on some liver function biomarkers of wistar rat

Rats treated with the extract at different doses did not show significant ($p > 0.05$) differences in the total protein levels, total bilirubin, direct bilirubin, and albumin, as represented in Table 4 when compared to the control rats.

Table 1. Effect of *Phyllanthus amarus* leaves extract on serum electrolytes of wistar rat

Treatment	Na^+ (mmol/L)	K^+ (mmol/L)	CL (mmol/L)
Control	238.6 $\pm$ 1.34 ^b	38.7 $\pm$ 5.55 ^b	145.0 $\pm$ 25.14 ^a
Extract 100 mg	171.0 $\pm$ 06.74 ^a	36.18 $\pm$ 7.29 ^b	136.4 $\pm$ 19.06 ^a
Extract 200 mg	185.6 $\pm$ 05.85 ^a	19.5 $\pm$ 5.99 ^a	145.6 $\pm$ 19.96 ^a
Extract 400 mg	223.6 $\pm$ 26.15 ^b	39.7 $\pm$ 6.08 ^b	149.4 $\pm$ 19.93 ^a

Note: Results represent mean $\pm$ standard deviation of group results (n=5). Values having different superscripts along the column are considered statistically significant ($p < 0.05$)

Table 2. Effect of *Phyllanthus amarus* leaves extract on urea and creatinine level in wistar rat

Treatment	Urea (mmol/L)	Creatinine (mmol/L)
Control	10.44 $\pm$ 5.01 ^a	5.12 $\pm$ 3.21 ^a
Extract 100 mg	0.784 $\pm$ 1.65 ^a	4.96 $\pm$ 3.68 ^a
Extract 200 mg	13.52 $\pm$ 5.67 ^a	3.12 $\pm$ 3.64 ^a
Extract 400 mg	12.68 $\pm$ 9.29 ^a	1.92 $\pm$ 2.10 ^a

Note: Results represent mean $\pm$ standard deviation of group results (n=5). Values having different superscripts along the column are considered statistically significant ($p < 0.05$)

Table 3. Effect of *Phyllanthus amarus* leaves extraction liver enzyme of wistar rat

Treatment	ALT (IU/mL)	AST (IU/mL)	ALP (IU/L)
Control	3.8 $\pm$ 1.30 ^a	5.4 $\pm$ 0.54 ^a	16.0 $\pm$ 4.06 ^c
Extract 100 mg	11.4 $\pm$ 1.34 ^b	5.54 $\pm$ 3.13 ^a	13.2 $\pm$ 5.11 ^{bc}
Extract 200 mg	20.4 $\pm$ 6.46 ^c	10.4 $\pm$ 2.07 ^b	7.8 $\pm$ 1.30 ^a
Extract 400 mg	2.54 $\pm$ 0.95 ^a	4.34 $\pm$ 1.74 ^a	9.2 $\pm$ 3.42 ^b

Note: Results represent mean $\pm$ standard deviation of group results (n=5). Values having different superscripts along the column are considered statistically significant ($p < 0.05$)

Table 4. Effect of *Phyllanthus amarus* leaves extraction on some liver function biomarkers of wistar rat

Treatment	ALB (mg/mL)	TP (mg/mL)	DB (μmol/L)	TB (μmol/L)
Control	2.6±1.25 ^a	8.98±3.07 ^a	30.03±6.34 ^{ab}	30.96±7.70 ^a
Extract 100 mg	2.78±0.38 ^a	8.74±1.21 ^a	30.36±4.58 ^{ab}	34.66±9.89 ^a
Extract 200 mg	3.2±0.72 ^a	9.62±2.82 ^a	33.56±9.11 ^b	36.84±6.50 ^a
Extract 400 mg	3.42±0.61 ^a	11.54±5.82 ^a	23.84±4.33 ^a	56.86±17.63 ^a

Note: Results represent mean ± standard deviation of group results (n=5). Values having different superscripts along the column are considered statistically significant (p<0.05)

Discussion

Kidneys and the liver are vital organs that function for homeostasis, regulating electrolyte balance in the blood, excreting waste products of metabolism, secretion of some enzymes and hormones, metabolism, osmoregulation, and detoxification. However, damage and toxic substances in these essential organs can lead to inefficiency in carrying out their functions.

Electrolytes (sodium, potassium, chloride, and bicarbonate) balance in the blood is a good indicator of kidneys and heart functions. Therefore, adequate information on serum electrolytes can assist in the determination of organ functions. For example, liver function assessment can be done by evaluating liver enzyme (AST, ALT, and ALP) parameters, total protein, albumin, and total bilirubin (Yakubu et al. 2021a).

The result revealed a significant decrease (p>0.05) in sodium ion (Na⁺) concentration between groups administered with doses of extract (100 mg, 200 mg) compared to normal/ control. Still, it showed no significant (p>0.05) difference at 400 mg dosage compared to the normal/ control. Therefore, the results suggested mild to no change of sodium ion concentration in the serum of rats administered with *P. amarus*. Furthermore, the result also revealed no significant (p>0.05) change in the concentration level of the other electrolytes, i.e., chlorine (Cl⁻) and Potassium (K⁺), between animal groups administered with the extract and normal control. These findings imply that the administration of *P. amarus* extract within these experimental doses did not significantly change the electrolyte concentrations in the serum of the wistar rats. This result agrees with the work of Krithika and Verma (2009) that the LD₅₀ value of *P. amarus* was high or categorized as non-toxic. The result also revealed no significant difference in serum urea and creatinine concentration between and within the groups of the administered dose of 100 mg, 200 mg, and 400 mg compared to normal control. Hence, the serum's electrolytes values, urea, and creatinine data indicate that the extract did not exact any deleterious effect on the kidney or negatively affect the heart.

The liver is an essential organ that carries out the biological system's metabolic, secretion, storage, and detoxification functions. Therefore, direct or indirect damage to the hepatocytes will cause an alteration of these functions, thereby affecting the organism's living condition (Yakubu et al. 2020b). The liver parameters showed no significant (p>0.05) difference in the level of ALB, TP, TB, DB, ALT, AST, and ALP of the animal groups

administered with extract compared to the normal control. Based on the parameters observed, it shows that the administration of the extract did not cause damage or toxicity to the liver. *P. amarus* contains alkaloids and a high concentration of antioxidant compounds (Fernand 1998; Naaz et al. 2007; Krithika and Verma 2009), essential for reducing oxidative stress. It may also have been the major factor in stabilizing all the hepatic parameters (Yakubu et al. 2021b). However, inappropriate use of antioxidants can induce oxidative stress (Galati and O'Brien 2004; Atici et al. 2005); hence the use of *P. amarus* extract should be kept at an optimal level.

It can be concluded that administration of *P. amarus* extract up to 400 mg/kg BW did not induce hepatotoxicity or nephrotoxicity. Furthermore, this finding has validated the use of *P. amarus* as a traditional herb for treating and managing several diseases, including kidney and liver diseases, without any significant organ damage.

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