ASIAN J NAT PROD BIOCHEM
Volume 20, Number 1, June 2022
Pages: 6-9
E-ISSN: 2580-2550
DOI: 10.13057/biofar/f200102

Kidney function status of Wistar rat treated with ethanolic extract of *Phyllanthus amarus* leaves owing to diethylnitrosamine intoxication

OJOCHENEMI EJEH YAKUBU[1,♥], DAVID CHRISTOPHER BANDO[1,2], MOSES ZEPHANIAH[1], ISAAC JOHN UMARU[1], MICHAEL SUNDAY ABU[1]

[1]Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari. PMB 1020 Katsina-Ala Road, Wukari, Taraba State, Nigeria. Tel./fax. +23-48-030000000, ♥email: oj4real_2007@yahoo.co.uk

[2]Bioresources Development Centre, National Biotechnology Development Agency. Jalingo, Taraba State, Nigeria.

Manuscript received: 6 January 2022. Revision accepted: 25 March 2022.

Abstract. *Yakubu OE, Bando DC, Zephaniah M, Umaru IJ, Abu MS. 2021. Kidney function status of Wistar rat treated with ethanolic extract of* Phyllanthus amarus *leaves owing to diethylnitrosamine intoxication. Asian J Trop Biotechnol 19: 6-9.* The kidney plays a central role in detoxification and excretion of harmful metabolites and therefore is susceptible to toxicity by xenobiotics. This research investigated the possible modulatory effect of *Phyllanthus amarus* Schumach & Thonn on diethylnitrosamine (DEN) induced nephrotoxicity in rats. Wistar rats received a single dose intraperitoneal injection of DEN. Twenty (20) rats were randomly allocated into four groups of five (5) rats each, and toxicity was induced using a single dose of DEN at 200 mg/kg intraperitoneally. Treatment was carried out using P. amarus extract at 200 mg/kg for 14 days, while silymarin was used as a standard drug orally at 5mg/kg. Kidney function parameters (Urea, Creatinine, sodium (Na^+)), Potassium (K^+), and Chloride (Cl^-) were determined. After 2 weeks, DEN-induced rats showed renal injury evidenced by the significant increase in circulating kidney function markers. Results showed that concurrent supplementation of *P. amarus* significantly ($p<0.05$) modulated kidney function markers and prevented renal tissue damage induced by DEN. In conclusion, *P. amarus* prevents DEN-induced nephrotoxicity via attenuation of oxidative stress and alleviation of the antioxidant defense system.

Keywords: Diethylnitrosamine, kidney, nephrotoxicity, *Phyllanthus amarus*, xenobiotics

INTRODUCTION

The genus *Phyllanthus*, which belongs to the Euphorbiaceae family, is one of the largest genera of flowering plants, with about 800 species found throughout the tropical and subtropical regions of both hemispheres, exhibiting a relatively wider range of habits such as annual or biennial herbs, shrubs, and trees.

Phyllanthus amarus Schumach. & Thonn. (Family: Euphorbiaceae) is widely found in all tropical and subtropical regions of the planet (Edeoga et al. 2006). The *P. amarus* is one of the most important herbs discovered recently in Nigeria and Akwa Ibom State, in particular. It is known among Ibibios and Efik's as ‘‘oyomokisoamankeedem,’’ Yoruba as “eyinolobe,” Hausa as “geeron tsutsaayee,” and Igbo as “Iteknwonwanazu” and in English as “leaf flower” or “chamber bitter” (Jagtap et al. 2016).

In several countries, the aerial section of *P. amarus* is highly prized in traditional and indigenous medicine for its healing powers, according to Foo and Wong (1992). This herb has long been used to cure liver problems and kidney stones worldwide. 'Chanca Piedra' is a Spanish word that means "stone breaker or shatter stone." 'Chanca piedra' has been utilized in South America to treat gall bladder and kidney stones and gall bladder infections. Antifungal, antibacterial, and antiviral properties have been discovered in *P. amarus* (Mirunalini and Krishnaveni 2010). Plant extracts of *P. amarus* can be utilized as blood purifiers for light malaria fevers and anemia, according to Heyde (1990). In combination with other herbs, *P. amarus* aids in the release of phlegm (Oudhia and Tripthi 2002); when the plant's leaves are boiled, it acts as a diuretic and can be used to treat diabetes, diarrhea, hepatitis, menstrual abnormalities, and skin conditions (Oudhia and Tripthi 2002).

In Suriname, a decoction of *P. amarus* is consumed with other plants to relieve stomachache, and constipation can also be treated with this plant (Oudhia and Tripthi 2002). Antioxidant properties of *P. amarus* extract to aid in eliminating free radicals from the human body (Nwanjo et al., 2007; Yakubu et al., 2019; Yakubu et al., 2021). Alkaloids, flavonoids, hydrolyzable tannins, major lignans, and polyphenols are secondary metabolites found in *P. amarus*.

Phyllanthus amarus has an antiurolithic action on the excretory system and treats kidney/gallstones, other kidney-related issues, appendix inflammation, and prostate problems (Bjelakovic et al., 2012). It is used in treating dyspepsia, colic, diarrhea, constipation, and dysentery due to its efficacy in gastrointestinal illnesses. The herb has been used to treat leucorrhoea, menorrhagia, and breast abscess in women, and it can also work as a galactagogue. For the treatment of chronic dysentery, the young branches of the plant are given as an infusion. Fresh leaf paste can heal wounds and treat white spots on the skin and jaundice. The stem juice can also be utilized to treat wounds. Urinary difficulties and liver edema are treated with the whole plant extract. Stomach pain is treated with the root extract. The plant's floral paste is administered externally as a snake bite remedy (Chandewar and Dhongade 2013).

Over the years, there has been rising concern over the side effect of synthetic drugs on humans to treat various illnesses and diseases. There is also a lack of information on the health benefit of some plants, such as *P. amarus*. This research work is aimed at investigating the effect of ethanol extract of *P. amarus* leaves owing to diethylnitrosamine (DEN) intoxication on kidneys.

MATERIALS AND METHODS

Collection, identification, and preparation of plant (sample)

Fresh and well-grown leaves of *P. amarus* were harvested at the University Environment, Federal University Wukari, Taraba State, Nigeria. The leaves were identified at the herbarium of Biological Science, Federal University Wukari. The plant leaves were thoroughly washed and air-dried for two days, pounded to a fine powder using mortar and pestle, then stored and labeled in a dried container.

Plant ethanolic extraction

The fine powder was soaked in an adequate volume of ethanol (95%) measured at 1,300 mL, and the sample was stirred and allowed to stand for 48 hours before filtration. After that, the suspension was first filtered using a clean white sieving mesh and then filter paper (Whatman No.1) (Yakubu et al. 2016). The filtrate was then subjected to dryness to evaporate the ethanolic content at 45ºC under reduced pressure on a rotary evaporator to obtain an oily gel-like extract which was weighed with the use of analytical weighing balance (AHUS); the extract was stored in an air-tight container, corked and preserved for use. The aliquots portion of the crude plant extract was weighed and used for phytochemical screening, and also, a portion was given to the Wistar rat of the labeled and selected group (Yakubu et al., 2016).

Animal procurement and treatment

Twenty (20) albino rats (Wistar rats) of both sexes weighing 150-350g were purchased from a commercial breeder in the Obudu area in Cross River State, Nigeria. The rats were acclimatized for 14 days after randomization under standard laboratory conditions at (25±2ºC) and relative humidity of 60±5% and 12 hours of light/dark cycle before and during the experimental period. The rats were maintained on commercial poultry feed (growers mash) and water *ad libitum.*

Animal grouping and administration

Twenty (20) albino Wistar rats weighing 150-350 g of both sexes were divided into 4 groups (n=5 rats) and kept in aluminum cages. A single dose (200 mg/kg) of DEN was used to induce nephrotoxicity, while 200 mg/kg and 5 mg/kg of the extract and silymarin were used as treatment drugs daily for 14 days (Yakubu et al. 2016). (i) Group 1 (Normal control) received distilled water and fed only. (ii) Group 2 (DEN control) 200 mg/kg/ip. (iii) Group 3 (DEN and extract) 200mkg/kg/po. (iv) Group 4 (DEN and silymarin) 5 mg/kg/po.

Collection of blood samples for analysis

At the end of the experimental period (two weeks), the rats were withdrawn from the cages in each group on day 14 and placed in a desiccator containing cotton wool soaked in light chloroform to anesthetize the rats (Yakubu et al., 2016) partially. Blood samples were collected from the heart via cardiac puncture using a sterile syringe and needle. The blood sample was divided into two fractions: One fraction was put into plain sample tubes while whole blood samples were put in Ethylene diamine tetraacetate (EDTA) treated sample bottles (Yakubu et al., 2016). The serum was collected from the clotted sample in the sample container by letting it stand for 2 hours at room temperature to clot before centrifugation at 3,000 rpm for 20 minutes using an MSE England benchtop centrifuge. Sera obtained from each sample were gently separated using Pasteur pipettes and dispensed into respective dry specimen bottles labeled accordingly. These were kept frozen in a refrigerator until when needed for various biochemical assays. The blood samples collected into the EDTA bottles were corked immediately, shaken gently to allow the blood to mix with the anticoagulant and prevent clotting and cell hemolysis. The hematological analyses were carried out as soon as the blood sample was collected.

Determination of serum urea concentration

This was assessed using the method described by Fawcett and Scout (1960).

Principle: Urease breaks down urea into ammonia and carbon dioxide. In an alkaline medium, ammonia reacts with hypochlorite and salicylate to form dicarboxy indophenol, a colored compound. The reaction is catalyzed by sodium nitroprusside. The intensity of color produced is measured spectrophotometrically at 578 nm.

$$\text{Urea} + H_2O \text{ Urease} \longrightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{salicylate} \longrightarrow \text{dicarboxyindophenols (blue compound)}$$

Procedure: Reagent (1 mL) containing sodium nitroprusside and urease was added into three clean test tubes labeled as a test sample, standard and reagent blank containing 0.01 mL sample, 0.01 mL standard reagent, and 0.01 mL distilled water, respectively. The content in each test tube was mixed and incubated at room temperature (25-30ºC) for 10 minutes. The absorbance of the test sample and standard were read against the reagent blank at 578 nm.

Calculation: The serum urea concentration was calculated using the formula below:

$$\text{Urea Conc. (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \; x \; \text{Concentrationof Standard}$$

BUN concentration (mg/dL) = 0.467 x urea concentration (mg/dL).

Determination of serum creatinine concentration

The colorimetric method was used to determine serum creatinine concentration according to Bertels and Bohme (1973).

Principle: Creatinine in the serum reacts with alkaline picrate to form colored complexes. The rate of formation of colored complexes is directly proportional to creatinine concentration. This reaction rate (intensity of orange color produced) is measured colorimetrically at 510 nm and compared with the standard.

Creatinine + Picric acid ⟶ NaOH Creatinine picrate (510 nm)

(Yellow) (Alkaline medium) (Orange)

Procedure: A working reagent (1 mL) containing picric acid and sodium hydroxide was added into two clean test tubes labeled sample test and standard, containing 0.1 mL of the test sample and 0.1 mL of standard solution. The content in each test tube was mixed, and after 20 seconds, the absorbance of the standard (ST1) and test sample (TS1) was read at 510 nm. Exactly 80 seconds later, absorbance for (ST2) and (TS2) of the standard and sample were read at 510 nm against distilled water (blank).

Calculation: The Concentration of creatinine in serum (mg/dl) was calculated using the formula below:

$$\text{Creatinine Conc. (mg/dl)} = \frac{TS2 - TS1}{ST2 - ST1}\ x \text{ Concentration of Standard}$$

Where: ST: Standard, TS: Test Sample

Estimation of serum sodium, potassium and chloride ions

A flame photometer Model 143, equipped with an automatic diluter Model 144 (ratio of the dilution of 200:1) (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A.) was used. The calibration of the flame photometer was performed with twice distilled water and a standard having a Na^+ concentration of 140 mequiv/L and a K^+ concentration of 5 mequiv/L (Instrumentation Laboratory, Inc., Lexington, Mass., U.S.A). The instrument's stability was checked with the standard solution after each sample measurement.

Statistical analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package version 20. All data were expressed as mean ± SD, and the difference between groups was considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The results of kidney function parameters such as Sodium (Na^+), Chloride (Cl^-), Potassium(K^+), Urea, and Creatinine obtained are presented in SD ± Mean error in Tables 1 and 2. Results showed that the extract could significantly ($p<0.05$) mitigate the harmful effect of the toxicity caused by DEN in the extract-treated group (Group 3) compared with Group 2, which was DEN-induced but not treated.

Phyllanthus amarus is an important medicinal plant used in ayurvedic medicine to treat diseases for over 2,000 years (Adedapo et al. 2005). The plant is also used in traditional medicine for the treatment of diseases. Scientific investigation revealed the therapeutic value of this medicinal plant; it showed that the plant contains several chemical constituents isolated and characterized and was found to be active against some diseases. For example, phyllanthin is a chemical compound isolated from *P. amarus* and reported hepatoprotective activity. This activity is associated with its radical scavenging activity (Krithika et al., 2009). The *P. amarus* has been used for years in different parts of the world for liver problems. The aqueous extract of *P. amarus* has also been used by Brazilians as a traditional medicine to treat stone disease (Barros et al. 2003) and jaundice and hepatitis (Mirunalini and Krishnaveni 2010). Scientific investigation has proven that the plant positively affects the hepatitis B virus and kidney stones.

Table 2. Urea and creatinine of Wistar rats Intoxicated by DEN and treated with ethanolic extract of *Phyllanthus amarus* leaves

Groups	Treatment	Urea	Creatinine
1	Normal	10.44±5.01[a]	5.12±3.21[b]
2	Den control	19.22±6.09[b]	12.56±2.37[b]
3	DEN + extract	8.4±1.37[a]	6.68±1.98[a]
4	DEN + silymarin	11.0±1.21[a]	4.64±3.82[a]

Results represent the mean ± standard deviation of group results obtained (n=5). Mean in the same row, having different letters of the alphabet is statistically significant ($p<0.05$) compared with the normal control (group one). Legend: Urea and creatinine

Table 1. Some electrolytes levels in Wistar rats intoxicated by DEN and treated with ethanolic extract of *Phyllanthus amarus* leaves

Groups	Treatment	Na^+	K^+	CL^-
1	Normal	138.6±12.34[a]	19.5±21.69[a]	145.0±25.14[a]
2	Den control	295.2±11.47[c]	38.42±23.22[b]	219.4±83.58[b]
3	DEN + extract	151.0±7.28[b]	25.58±20.84[a]	159.4±41.04[a]
4	DEN + silymarin	184.0±3.93[c]	21.26±20.58[a]	161.4±81.65[a]

Note: Results represent mean ± standard deviation of group results obtained (n=5). Mean in the same row, having a different letter of the alphabet is statistically significant ($p < 0.05$) compared with the normal control (group one). Legend: Sodium (Na^+), Potassium (K^+), Chloride (Cl^-)

The kidney helps maintain the body's homeostasis by reabsorbing important material and excreting waste products. Creatinine is a breakdown waste product formed in the muscle by creatinine phosphate metabolism. Creatinine is synthesized in the liver, passes into the circulation, and takes up almost entirely by skeletal muscle for energy production. Creatinine retention in the blood is evidence of kidney impairment.

Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of the urea cycle, where ammonia is converted into urea and excreted through urine (David et al., 2014). It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Renal diseases that diminish urea's glomerular filtration rate will lead to its retention in the blood (David et al., 2012).

The kidney also maintains a marginal concentration of electrolytes in the body. Electrolytes are small inorganic ions prevalent in body fluid that are important in normal physiological functions (Palmer 2014). They are mainly sodium ion Na^+, chloride ion Cl^-, potassium ion K^-, bicarbonate ion $HCO_{3,}$ and hydrogen ion H^+. The volume of extracellular fluid (ECF) depends on the body's sodium content because Na^+ and its salt are the major osmotic solute in ECF (Vasudevan et al., 2011). Renal regulation of these ions is controlled by renal sympathetic, atrial natriuretic peptide, and aldosterone actions which may result in reabsorption or excretion of these ions at the distal tubule of the nephrons. However, nephrology defects caused by xenobiotics such as CCl_4 and DEN toxicity may truncate these functions and result in irregular distribution of these ions in the ECF (Showkat et al. 2011).

In this research, upon induction of the rats with a toxic xenobiotic agent, DEN, the levels of the various kidney function parameters determined significantly ($p<0.05$) increased. However, the results of kidney parameters obtained showed that concurrent supplementation of *P. amarus* significantly ($p<0.05$) modulated kidney function markers and prevented renal tissue damage induced by DEN. The *P. amarus* may prevent DEN nephrotoxicity via attenuation of oxidative stress and activation of the body's antioxidant defense mechanism, which agrees with Showkat et al. (2011), where aqueous rhizome extract extracts of *Podophyllum hexandrum* Royle was able to reverse kidney and lung functions owing to its antioxidant propensity.

In conclusion, kidney function parameters (urea, creatinine, sodium, potassium, chloride) were significantly ($p<0.05$) reversed after extract treatment which was an indication of amelioration of the harmful effects of the toxicity caused by DEN by the *P. amarus* leaves extract.

REFERENCES

Adedapo AA, Adegbayibi AY, Emikpe BO. 2005. Some clinico-pathological changes associated with the aqueous extract of the leaves of *Phyllanthus amarus* in rats. Phytother Res 19: 971-976. DOI: 10.1002/ptr.1768.

Barros ME, Schor N, Boim MA. 2003. Effects of an aqueous extract from *Phyllanthus niruri* on calcium oxalate crystallization in vitro. Urol Res 30: 374-379. DOI: 10.1007/s00240-002-0285-y.

Bertels H, Bohmer M. 1973. Serum creatinine determination without protein precipitation. Clin Chim Acta 37: 193. DOI: 10.1016/0009-8981(72)90432-9.

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. 2012. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev 3 (3): 71-76. DOI: 10.1002/14651858.CD007176.pub2.

Chandewar AV, Dhongade H. 2013. Pharmacognostical and Phytochemical studies of *Phyllanthus amarus* leaves. Intl J Biomed Adv Res 4: 383. DOI: 10.7439/ijbar.v4i6.405.

David MS, Jeff SC, Nigel B, David WJ, Glenda CG. 2012. Oxidative stress, antioxidant therapies and chronic kidney disease. Nephrology 17: 311-321. DOI: 10.1111/j.1440-1797.2012.01572.x.

David WI, William EM, Jeff MS. 2014. Urea and ammonia metabolism and the control renal nitrogen excretion. Clin J Am Soc Nephrol 10 (8): 1444-1458. DOI: 10.2215/CJN.10311013.

Edeoga HO, Omosun G, Awomukwu DA. 2006. Tannins and calcium oxalate crystals in lamina of some *Phyllanthus* species. Intl J Mol Med Adv Sci 2: 326-329.

Fawcett JK, Scout JE. 1960. A rapid and precise method for the determination of urea. J Clin Pathol 13: 156. DOI: 10.1136/jcp.13.2.156.

Foo LY Wong H. 1992. Phyllanthusiin D, an unusual hydrolysable tannin from *Phyllanthus amarus*. Phytochemistry 31 (2): 711-713. 10.1016/0031-9422(92)90071-W.

Heyde H. 1990. Medicijnplanten in Suriname. (Den dresiwiwiri foe Sranan). Medicinal Plants in Suriname. Uitg. Stichting Gezondheidsplanten Informaite (SGI), Paramaribo, Suriname.

Jagtap S, Khare P, Mangal P, Kondepudi KK, Bishnoi M, Bhutani KK. 2016. Protective effects of phyllanthin, a lignan from *Phyllanthus amarus*, against progression of high fat diet induced metabolic disturbances in mice. RSC Advances 6 (63): 58343-53. DOI: 10.1039/C6RA10774E.

Krithika R, Mohankumar R, Verma RJ, Shrivastav PS, Mohamad IL, Gunasekaran P, Narasimhan S. 2009. Isolation, characterization and antioxidative effect of Phyllanthin against CCl_4-induced toxicity in HepG2 cell line. Chem-Biol Interact 181: 351-358. DOI: 10.1016/j.cbi.2009.06.014.

Mirunalini S, Krishnaveni M. 2010. Therapeutic potential of *Phyllanthus emblica* (amla): The ayurvedic wonder. J Basic Clin Physiol Pharmacol 21 (1): 93-105. DOI: 10.1515/JBCPP.2010.21.1.93.

Nwanjo HU, Oze G, Okafor MC, Nwosu D, Nwankpa P. 2007. Protective role of *Phyllantus niruri* extract on serum lipid profiles and oxidative stress in hepatocytes of diabetic rats. Afr J Biotechnol 6 (15): 1744-1749. DOI: 10.5897/AJB2007.000-2256.

Oudhia P, Tripthi RS. 2002. Prospect of cultivation of medicinal plants in Chhattisgarh, India. In: Crop Improvement, Production Technology, Trade and Commerce 211-235. Sci Tech Publishing LLC, Houston, USA

Palmer BF. 2014. Regulation of potassium homeostasis. Clin J Am Soc Nephrol 10 (6): 1050-1060. DOI: 10.2215/CJN.08580813.

Showkat AG. Ehtishamul H, Abid H, Yasrib Q, Zahi M, Bilal, AZ, Akbar M, Mahammad AZ. 2011. Carbon tetrachloride induced kidney and lung tissues damage and oxidative activities of aqueous rhizome extract of *Podophyllum hexandrum*. BMC Complement Altern Med 11 (17): 1472-6882. DOI: 10.1186/1472-6882-11-17.

Vasudevan DM, Sreekumar S, Kanan V. 2011. Biochemistry for medical students. Jaypee Brothers Medical Publishesr Limited. 6th Edition. New Delhi, India.

Yakubu OE, Abu MS, Akighir J, Onuche JI, Arabi A. 2021. Comparative determination of total antioxidant effects of ethanol extract of *Phyllanthus amarus* leaves. Asian J Nat Prod Biochem 19: 81-85. DOI: 10.13057/biofar/f190206.

Yakubu OE, Nwodo OFC, Shaibu C, Tatah SV, Abah MA, Sunday G. 2019. In vitro determination of antioxidant activities of the fractions obtained from *Adansonia digitata* L. (baobab) stem bark ethanolic extract using different parameters. Curr Trends Biomed Eng Biosci 17 (5): 555973. DOI: 10.19080/CTBEB.2019.17.555973.

Yakubu OE, Obot AC, Dawoye Y. 2016. Effect of aqueous extract of *Hymenocardia acida* leaves on Aluminium chloride-induced toxicity in male albino rats. J Anal Pharm Res 3 (2): 00049 DOI: 10.15406/japlr.2016.03.00049.