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Research Article

Effect of *Phyllantus amarus* Leaf Extract on the Kidney of Alloxan-Induced Diabetic Wistar Albino Rats

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ABSTRACT

Background: Some plants are toxic, but not all plants or compounds are equally toxic to all parts of a living system. This is because many chemicals' harmful effects only appear in specific organs, which are their intended targets for toxicity. The study aims to evaluate how an ethanolic leaf extract of *Phyllantus amarus* affects the kidneys of alloxan-induced diabetic albino rats.

Methods: A total of 25 albino rats of either sex weighing 86–150 g were assembled and divided into five groups (control, 1, 2, 3, and 4) of five rats each. The control group received no treatment and no induction with diabetes; group 1 received *P. amarus* treatment without induction; group 2 received *P. amarus* treatment and was induced with diabetes on the eighth day of treatment; and groups 3 and 4 were induced with diabetes and received *P. amarus* treatment. For 21 days, rats in groups 1, 2, and 3 received 300 mg/kg body weight of *P. amarus* leaf extract orally, while group 4 received 500 mg/kg body weight of the extract, and the control group received only water. For biochemical parameters, blood samples were collected in plain containers. Standard methods were used to measure urea, creatinine, albumin, and protein levels.

Results: When compared to the control, there was a significant increase in the mean urea level in the groups that received 300 mg/kg body weight of *P. amarus* (4.170 ± 23 *vs.* 5.770 ± 55, 6.000 ± 12, and 5.900 ± 60; P>0.05). However, there was no significant difference in creatinine, albumin, or protein levels in alloxan-induced rats compared to the control group for any of the groups. Again, there was no significant increase in the mean weight of the rats after the administration of *P. amarus*.

Conclusions: Therefore, caution should be exercised when consuming *P. amarus* because prolonged use may result in changes in urea concentration.

Keywords: *Phyllantus amarus;* Diabetes; Kidney; Urea; Creatinine; Albumin; Protein

Abbreviations: *P. amarus*: *Phyllantus amarus*; LD_{50}: Median lethal Dose; B.wt: Body weight

INTRODUCTION

Plant materials as sources of medical compounds have continued to play a vital role in the wellbeing of human health since antiquity. Many modern drugs and processed scientific medicines are of plant origin, and man has used herbs for the treatment of many diseases [1]. It has been generally recognized that traditional medicine and medicinal plants are routinely used as a foundation for the upkeep of good health in the majority of developing countries, with roughly 80% of the world's population depending on herbal medicine [2].

In Nigeria, herbal medicine has been extensively used for many years in the treatment of a broad series of diseases. Some of the used plants include *Phyllantus amarus* (stone breaker), *Bryophyllum pinnatum* (never die), *Ficus exasperata* (sand paper), *Phyllantus*

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niruri (gale of the wind), *Coriandrum sativum*, *Nauclea latifolia* (African peach), and *Azadirachta indica* (neem tree), among others [3-8]. Most of these plants' parts are prepared using the leaves, root, stem, callus, aerial parts, or whole plant.

Phyllantus amarus (*P. amarus*) is a small, erect tropical annual herbal plant that is widely distributed worldwide [9]. In Nigeria, it is used for various treatments in folklore medicine. It is called "Iyin Olobe" in Yoruba, "Ebebenizo" in Bini, and "Oyomokeisoamankedem" in Efik [10]. Additionally, research on the leaf extract of *Phyllanthus amarus* has revealed anti-hepatitis B activity, hepatoprotective, anti-cancerous, and antibacterial characteristics [11].

Renal disease has been found to be the ninth leading cause of death, and renal failure is one of the most common clinical symptoms [12]. Renal failure refers to the deterioration of excretory functions of the kidney, and it is usually characterized by a decrease in Glomerular Filtration Rate (GFR) resulting in the abnormal accumulation of blood urea nitrogen and serum creatinine, which must be excreted out of the body system. Hence the levels of urea, creatinine, and uric acid are used as important indices in the diagnosis of kidney function [13]. Nephrotoxicity is the result of the impairment or destruction of kidney function by exogenous or endogenous toxicants, which results in suboptimal kidney-specific detoxification and excretion [14]. However, the exact adverse effect of *Phyllantus amarus* on the kidney is yet to be elucidated.

MATERIALS AND METHODS

Chemical reagent

All chemicals and reagents used in the study were of analytical grade.

Plant materials

Fresh leaves of *Phyllantus amarus* were collected from the premises of Yaba College of Technology, Yaba, and at Shakiru Adeboye Street, Ogudu, Ojota, Lagos State, South-west Nigeria, in July 2019. The plant was identified and authenticated at the department of botany herbarium, University of Lagos, Lagos, by Dr. Nodza George, and a voucher specimen (LUH 8423) was given for future references.

Plant extract preparation

The *P. amarus* leaves collected were sorted out by removing the dead and dried ones. The leaves were air dried for 7 days and then transported to the oven to dry at 45°C until they became crispy. The oven-dried leaves were coarsely powdered and packaged in airtight containers until they were used. The extract was prepared by the maceration of six hundred grams (600 g) of the coarsely powdered leaves in 95% ethanol (5 litres) at room temperature for 72 hours while being stirred vigorously at intervals. The suspension was then filtered using Whatman no. 1 filter paper, and a rotary evaporator was used to concentrate the extract, after which it was evaporated to dryness in the water bath at 45°C to yield a dry crude extract (39.4 g), and the percentage yield calculated was 6.6%.

The extract obtained was transferred to an airtight, sterile universal container and stored at 4°C until used.

Phytochemical screening

Phytochemical screening of the plant extract was carried out for the presence of some classes of natural products using the standard procedure as described [15]. The following qualitative tests were carried out on aliquots of the plant extract: Tests for saponins, tests for phenolic compounds, tests for alkaloids, tests for flavonoids, tests for carbohydrates, tests for glycosides, and tests for amino acids. This was done at the College Central Research Laboratory, Yaba College of Technology, Yaba, Nigeria.

Acute toxicity study

An acute toxicity study was carried out following OECD 425 guidelines. Twenty-five (25) Wistar albino rats weighing 19-32 g of both sexes were divided based on their weight into five (5) groups of five (5) rats per group. Graded doses of the extract dissolved in 1 ml of Tween-20 were administered to the rats in groups 1, 2, 3, 4, and 5 in single oral doses of 250 mg/kg, 500 mg/kg, 700 mg/kg, 1000 mg/kg, and 1500 mg/kg b.wt, respectively, for 24 hours using an oral cannula. The rats were observed for the first 2 hours, during which behavioral parameters and mortality were closely observed, and subsequently for toxic symptoms for 24 hours.

Experimental animal handling and treatment

Twenty-five (25) Wistar albino rats of either sex weighing 86-150 g were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Nigeria. The rats were maintained under standard environmental conditions (27°C ± 2°C, 12 h light/dark cycle) and were fed commercial rat chow and given water ad libitum. They were acclimatized to the laboratory environment for 7 days before the actual commencement of the study. They were marked for easy identification. Administration of the extracts and drugs was between 8:00 and 10:00 am daily.

Induction of diabetes in Wistar albino rats

A freshly prepared solution of alloxan monohydrate (140 mg/kg) was induced intraperitoneally in overnight fasted rats of groups 3 and 4, while group 2 was induced on the eighth day of treatment with the plant extract. The use of appropriate doses of alloxan monohydrate, according to the body weight of the animals, allowed acute or mild diabetes to be established in experimental animals. Diabetes was confirmed by ascertaining the glucose concentration in the blood of the rats 48 hours after alloxan monohydrate injection, which was found to have increased two- to three-fold from its normal value. Blood glucose was measured in mg/dL with a glucometer (On Call Plus II, USA) through an orbital puncture in the tail vein of the rats.

Treatment

Twenty-five (25) Wistar albino rats of both sexes were divided into five (5) groups based on their weight and sex and were given the following treatment:

The control group, which are the normal, healthy rats, were given water and feed only.

Group 1: Non-diabetic rats were given 300 mg/kg body weight of *P. amarus* extract dissolved in 1% Tween-20 once daily.

Group 2: Received 300 mg/kg body weight of *P. amarus* extract dissolved in 1% Tween-20 once daily and 140 mg/kg body weight of Alloxan monohydrate intraperitoneally only on the eighth day of treatment.

Group 3: Diabetic rats were given 300 mg/kg body weight of *P. amarus* extract dissolved in 1% Tween-20 once daily.

Group 4: Diabetic rats were given 500 mg/kg body weight of *P. amarus* extract dissolved in 1% Tween-20 once daily.

The plant extract was given by oral administration using an oral cannula for 21 days. The weights and glucose levels of the rats during periods of treatment were recorded at the beginning of the experiment and at weekly intervals. Following the 21st dose of extract treatment, blood samples were collected from the rats at the veins of the eyes using a capillary tube for biochemical analysis. The rats were then sacrificed by cervical dislocation.

Statistical analysis

The results were expressed as mean ± SEM. Data obtained from the groups was analyzed using one-way Analysis of Variance (ANOVA), followed by Dunnet's test to detect intergroup differences. The level of significance was determined at $P<0.05$, which is a 95% confidence level.

RESULTS

Phytochemical screening

Table 1 shows the phytochemical composition of an ethanolic leaf extract of *P. amarus*. The table revealed the presence of saponins, phenolic compounds, alkaloids, flavonoids, carbohydrates, and glycosides, while amino acids were not detected.

Table 1: Qualitative phytochemical composition of ethanolic leaf extract of *Phyllantus amarus*.

Phytochemicals	Inferences
Saponins	+
Phenolic compounds	+
Alkaloids	+
Flavonoids	+
Amino acids	-
Glycosides	+
Carbohydrates	+

Key: + = Positive, - = Negative

Acute toxicity

Table 2 shows the result of an acute oral toxicity study (LD_{50} determination) of an ethanolic leaf extract of *P. amarus* in Wistar albino mice. The result revealed the non-toxic nature of P. *amarus*; no change in observed physical parameters such as changes in fur, mucous membrane of the eyes, behavioral patterns, palpitation, or mortality was recorded in all treated groups after 24 hours of administration with the plant extract. This indicates that the LD_{50} is greater than 1500 mg/kg.

Table 2: Acute oral toxicity of ethanolic leaf extract of *Phyllantus amarus* in Wistar albino mice.

Groups	Mean weight (g)	Dosage/Kg Body weight	Behavioral changes	Mortality
1 (n=5)	19.6 g	250 mg/kg	None	0
2 (n=5)	20.4 g	500 mg/kg	None	0
3 (n=5)	22.0 g	700 mg/kg	None	0

4 (n=5)	24.4 g	1000 mg/kg	None	0
5 (n=5)	25.4 g	1500 mg/kg	None	0

Body weight

The change in the average body weights of alloxan-induced diabetic albino rats is presented in Table 3. There was no significant decrease (P value>0.05) in the average body weights of albino rats before induction and treatment with the plant extract before the experiment. Also, there was no significant increase (P value>0.05) in the average weights of albino rats given the plant extract and induced with diabetics at sacrifice.

Table 3: Effect of *Phyllantus amarus* on weight of alloxan-induced diabetic albino rats.

Groups	Dosage	Weight before experiment (g)	Weight at sacrifice (g)	Weight difference (g)
Control	-	130.00 ± 5.54	156.00 ± 9.86	26.00 ± 4.32
1	300 mg/kg	86.40 ± 4.12	105.50 ± 4.50	19.10 ± 0.38
2	300 mg/kg	93.20 ± 2.22	117.50 ± 0.50	24.80 ± 1.72
3	300 mg/kg	107.60 ± 4.79	148.33 ± 13.54	40.73 ± 8.75
4	500 mg/kg	111.80 ± 2.33	172.00 ± 0.50	60.20 ± 1.83

The results are expressed as mean SEM (n=5) and are significant when compared to the normal control (P value<0.05). *Control group received only water without induction or leaf extract. Group 1-received 300 mg/kg body weight of *Phyllantus amarus* leaf extract only; group 2-received 300 mg/kg body weight of leaf extract and was induced with diabetics on the eighth day of treatment; group 3-induced with diabetics and treated with 300 mg/kg body weight of leaf extract; and group 4-induced with diabetics and treated with 500 mg/kg body weight of leaf extract. All are allowed free water ad libitum.

Kidney (renal) function parameters

The results obtained from the parameters are shown in Table 4. The concentration of urea in the rats administered the extract was significantly increased (P value<0.05) compared with the control, except for group 4, which showed no significant change compared with the control. Creatinine, protein, and albumin show no significant change (P>0.05) as compared to the control.

Table 4: Effect of *Phyllantus amarus* leaf extract on kidney (renal) function parameters of alloxan-induced diabetic albino rats.

Groups	Dosage	Urea (mmol/l)	Creatinine (umol/l)	Albumin (g/l)	Protein (g/l)
Control	-	4.17 ± 0.23^{a}	48.00 ± 0.58^{a}	39.33 ± 0.68^{a}	70.00 ± 4.58^{a}
1	300 mg/kg	5.77 ± 0.55^{b}	46.00 ± 1.73^{a}	38.67 ± 2.03^{a}	77.67 ± 3.18^{a}
2	300 mg/kg	6.00 ± 0.12^{c}	38.33 ± 1.76^{a}	40.00 ± 1.73^{a}	71.00 ± 1.16^{a}
3	300 mg/kg	5.90 ± 0.60^{d}	71.00 ± 11.68^{a}	40.00 ± 1.53^{a}	74.67 ± 2.03^{a}
4	500 mg/kg	3.53 ± 0.15^{a}	52.33 ± 5.70^{a}	39.00 ± 1.16^{a}	63.33 ± 1.76^{a}

Results expressed as Mean ± SEM (n=5), significant at (P<0.05) as compared to normal control. Values with the same superscript with the control are not significantly difference.

DISCUSSION

The results of the analysis of the ethanolic leaf extract of *P. amarus* as presented in Table 1 indicate the presence of saponins, phenolic compounds, alkaloids, flavonoids, glycosides, and carbohydrates, which revealed the presence of medically important bioactive compounds.

Most medicinal plants used in traditional medicine lack toxicological records because most herbs are believed to be safe compared to synthetic drugs. Scientifically, this evidence is not accepted, hence the need to carry out toxicological studies on *P. amarus* leaf extract using Wistar albino mice, so as to determine the potential health risk in humans caused by the plant extract.

However, on administration of the plant extract at doses of 250, 500, 700, 1000, and 1500 mg/kg b.wt to the albino mice, there was no change in the mice and no mortality was recorded (Table 2). This indicates that *P. amarus* leaf extract is safe for consumption because the lethal dose is above 1500 mg/kg b.wt. Hence, 300 and 500 mg/kg b.wt for the main study.

The present study was designed to evaluate the effect of an ethanolic leaf extract of *P. amarus* on the kidneys of alloxan-induced diabetic albino rats. The kidney is one of the important organs that performs vital functions for the healthy survival of the body. The kidney helps to maintain homeostasis in the body by reabsorbing important materials and excretory waste products.

Urea plays an important role in the metabolism of nitrogen-containing compounds and is the main nitrogen-containing substance in the urine of mammals. When urea is high in the blood, it can result in tissue breakdown, such as hemorrhage. From the present study, the results obtained (Figure 1) showed *P. amarus* leaf extract at a dosage of 300 mg/kg body weight in group 1 caused a significant increase ($P<0.05$) in urea level concentration as compared to the control group. Also, alloxan-induced diabetic rats in groups 2 and 3 showed a significant increase ($P<0.05$) in urea level concentration treated with 300 mg/kg body weight of *P. amarus* leaf extract, while there was no significant decrease ($P>0.05$) in urea level concentration in group 4 treated with 500 mg/kg body weight of the extract compared to the control group. These results indicate treatment with plant extracts is not dose-dependent on concentration.

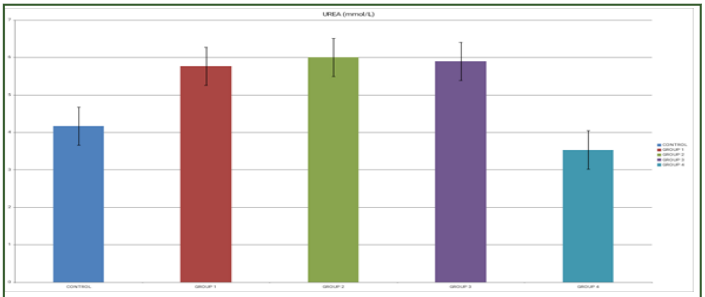

Figure 1: Effect of *Phyllantus amarus* leaf extract on the urea level of alloxan-induced diabetic albino rats.

Creatinine is an excretion product of muscle activity that circulates in the blood. Its elimination is exclusively renal; hence, there is a correlation between creatinine levels and renal function [16]. Most creatinine that is eliminated by the kidneys is freely filtered in renal glomeruli, and a small fraction is filtered by the tubular component, indicating good renal glomerular function. The results showed no significant decrease ($P>0.05$) in creatinine level in group 1 treated with 300 mg/kg body weight of *P. amarus* extract as compared with the control group. Alloxan induced diabetic rats in group 2 showed no significant decrease ($P>0.05$) in creatinine level treated with 300 mg/kg body weight of the plant extract, and diabetic rats in group 3 showed no significant increase ($P>0.05$) in creatinine level treated with 300 mg/kg body weight. Also, group 4 showed no significant increase ($P>0.05$) in creatinine levels of the induced diabetic rats compared to the control group (Figure 2).

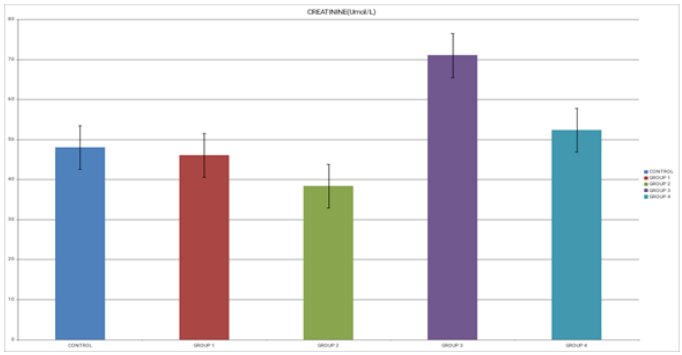

Figure 2: Effect of *Phyllantus amarus* leaf extract on the creatinine level of alloxan-induced diabetic albino rats.

Albumin is the most prevalent circulating protein in plasma. In healthy human patients, it makes up half of the plasma's total protein content (3.5 g/dL to 5 g/dL) [17]. It is present in high concentrations in the blood, and when the kidneys are functioning properly, virtually no albumin is lost in the urine. The kidneys, however, start to lose their capacity to preserve albumin and other proteins if they become ill or injured. This usually occurs with chronic conditions like diabetes. Nephrotic syndrome causes the kidneys to lose extremely large levels of albumin. The results indicate the level of albumin concentration did not decrease significantly ($P>0.05$) in group 1, and group 2 showed no significant increase ($P>0.05$); also, group 3 showed no significant increase in albumin level, while group 4 showed no significant decrease in albumin level compared to the control group (Figure 3).

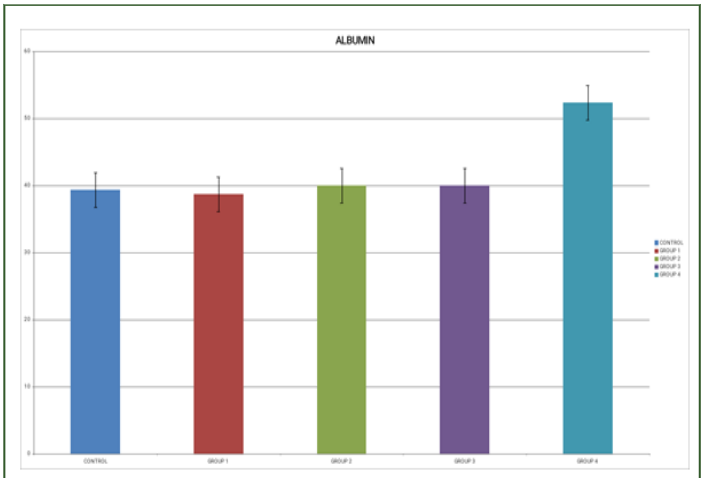

Figure 3: Effect of *Phyllantus amarus* leaf extract on the albumin level of alloxan-induced diabetic albino rats.

Proteins are large molecules that form the structural part of most organs and make up enzymes and hormones that regulate body functions. The results also showed (Figure 4) that there was no significant increase ($P>0.05$) in protein level in the groups treated with 300 mg/kg body weight of *P. amarus* leaf extract, while group 4 showed no significant decrease ($P>0.05$) in protein level compared to the control group.

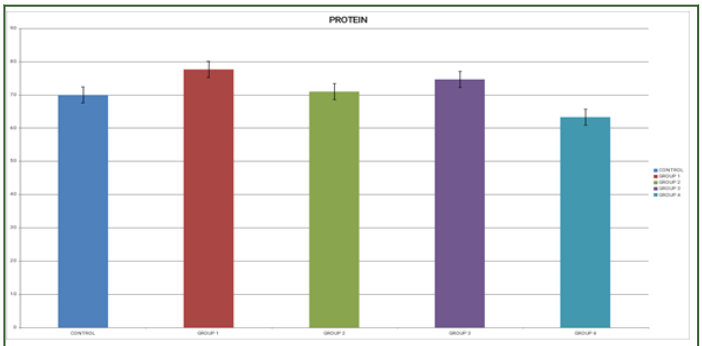

Figure 4: Effect of *Phyllantus amarus* leaf extract on the protein level of alloxan-induced diabetic albino rats.

Also, the results Table 3 showed no significant increase (P>0.05) in the mean weight in the induced diabetic albino rats compared to the control group, and this indicates that the plant extract does not have any effect on the weight, which shows the rats to be healthy and growing very well.

CONCLUSION

The acute toxicity of *P. amarus* carried out in albino mice showed no mortality or abnormalities. The biochemical results obtained from the present study revealed that the administration of an ethanolic leaf extract of *P. amarus* has no effect on the creatinine, albumin, or protein serum concentrations but has an effect on the urea concentrations of induced diabetic albino rats and hence can influence renal function. Therefore, it is recommended that caution be used in the intake of *P. amarus* because prolonged usage could lead to changes in the urea concentration excreted. Further studies should be carried out to examine the causes of these findings.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All appropriate ethical guidelines for the handling and use of animals in research have been followed in accordance to Lagos State University Research Ethics Policy.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

CONFLICT OF INTERESTS

The authors certify that no actual or potential conflict of interest in relation to this article exists.

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AUTHORS' CONTRIBUTIONS

QAA and OO collated and analyzed literature. TOO and AOO constructed all tables. QAA coordinated and wrote the paper. QAA and TOO revised the paper. All authors read and approved the manuscript.

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