EFFECT OF *PHYLLANTHIUS URINARIA* AQUEOUS LEAF EXTRACT AGAINST ACYCLOVIR-INDUCED NEPHROTOXICITY IN WISTAR RATS.

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ABSTRACT
Background: Renal damage presents a significant health concern, commonly occurring due to exposure to toxic substances. *Phyllanthius urinaria*, known for its medicinal benefits, demonstrates various pharmacological actions, including anti-oxidative and anti-inflammatory effects.
Aim: This research, investigates the protective properties of aqueous extract of *Phyllanthius urinaria* leaves against Acyclovir-induced kidney toxicity in Wistar rats.
Methodology: Twenty-four (24) adult Wistar rats grouped into four groups were administered different treatment regimens involving oral administration of Acyclovir, *Phyllanthius urinaria*, or a combination of both for thirty-one days.
Results: It revealed a rise in urea and creatinine levels in rats exposed to Acyclovir, indicating kidney injury. However, treatment with *Phyllanthius urinaria* substantially reduced these levels, indicating a protective effect against Acyclovir-induced nephrotoxicity. Acyclovir exposure resulted in diminished activity of antioxidant enzymes, whereas *Phyllanthius urinaria* treatment significantly enhanced antioxidant levels. Histological analysis revealed signs of nephrotoxicity in Acyclovir-exposed rats, whereas those treated with *Phyllanthius urinaria* exhibited preserved kidney structure. When the extract was given along with acyclovir, the extract was able to protect the renal tissue from the toxic effect of the acyclovir.
Conclusion: The study concludes that *Phyllanthius urinaria* effectively alleviates Acyclovir-induced nephrotoxicity. These results highlight the potential therapeutic application of *Phyllanthius urinaria* in mitigating kidney damage associated with Acyclovir exposure.
Keywords: *Phyllanthius urinaria,* Acyclovir, Kidney, Nephrotoxicity, Wistar Rats

INTRODUCTION
Renal injury, have been a major concern for health worker globally because of its renal impairment effect, thereby causing malfunctioning of the body system. Among the myriad agents known to induce renal damage, is acyclovir. Acyclovir (ACV), an antiviral drug, is the drug of choice in the management of herpes simplex virus infections. Acyclovir-induced nephrotoxicity can manifest in various ways, affecting approximately 12–48% of cases. (Keeney *et al*., 1982; Bean *et al*., 1985).
Phyllanthus amarus Schumach. & Thonn. (Family: Euphorbiaceae) is widely found in all tropical and subtropical regions of the planet (Edeoga *et al*. 2006). It originates from the Caribbean area of the United States as a vicarious species of *P. abnormis* and has further spread around the tropics by trading vessels (Webster, 1957). The plant is indigenous to the rainforests of the Amazon and other tropical countries such as China, India, the Bahamas, (Morton 1981), Philippines (Chevallier, 2000). It is a communal pan-tropical weed that grows in moist, shady and sunny places (Cabieses, 1993).

Furthermore, different region have different names that they called the plant. 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 South America, it has been used to remove gall bladder stones, and in the treatment of respiratory infections (Foo 1992), cardiovascular diseases (Chevallier, 2000), antihepatitis (Thyagarajan et al., 1988), antimalarial (Tran et al., 2003), anti-viral (Notka et al., 2004; Pramyothin et al., 2007; Burkill, 1994)), antibacterial (Mazumder et al., 2006) anti-carcinogenic (Rajeshkumar et al., 2002), anti-inflammatory (Kiemer et al., 2003) as well as a remedy for influenza around the globe (Foo and Amarulone 1993). These activities have been attributed to the flavonoids, terpenes, benzenoids, lignans, lipids, vitamin C, steroids etc, that have been purified from the plant (Taylor 2003). Some of its other constituents include Mg, PO, K, Ca, ascorbic acid, Fe, Zn, thiamine, niacin and riboflavin (Okiki *et al* 2015).

Considering the medicinal importance of the extract and the increasing prevalence of renal diseases, it is of importance to investigate if the extract can be of therapeutic importance in the management of kidney disease.

The aim of the study was to investigate the effect of *Phyllanthius urinaria* aqueous leaf extract against acyclovir-induced nephrotoxicity in Wistar rats.

MATERIALS AND METHODS

Plant collection

Leaf *of Phyllanthius urinaria* was collected from a farm in Ikhin town in Owan east local government area of Edo state, Nigeria. It was authenticated at the herbarium in the Department of Plant Biology and Biotechnology, University of Benin (UNIBEN), Benin City, Edo state, Nigeria. The leaf was shade dry for 5 days. It was then dried with an oven at a temperature of 40^0C for about 20 minutes and then pulverized into powder form using the British Milling Machine. The powdered sample was weight to be 100g.

The powdered material was macerated by soaking the100g powdered sample of the extract in 2.0L of water for 24 hours with constant shaking and stirring every eight hours (8). Filtration was carried out to separate the residue from the filtrate and the filtrate was concentrated over water bath using crucibles to obtain a gel like extraction which was then stored in a bottle that was kept inside a freezer.

The animals were kept in the cages for two weeks (14 days) before the experiment for effective acclimatization. They had access to standard livestock feed and clean drinking water freely. Animals were housed in a clean plastic cage under natural light, good ventilation.

Animal grouping and treatment

Twenty-five (24) adult Wistar rats weighing between 150-240 g were randomly assigned into six (6) groups (n=4). Group A – control; Group B - 432 mg/kg body weight of Acyclovir only; Group C - 500 mg/kg body weight of *Phyllanthius urinaria*; Group D - 1000 mg/kg body weight of *Phyllanthius urinaria*; Group E - 500 mg/kg body weight of *Phyllanthius urinaria* and 432 mg/kg body weight of acyclovir; Group F - 1000 mg/kg body weight of *Phyllanthius urinaria* and 432 mg/kg body weight of acyclovir. The administration lasted for thirty-one (31) days and was done orally using an orogastric tube.

Kidney Function Assessment*:* Kidney function assessment were carried out as previously described; Urea (Chaney and Marbach, 1962) and creatinine (Bartels and Bohmer, 1972).

Evaluation of Oxidative Stress Markers*:* They were carried out as previously described; Catalase (CAT) [Cohen *et al.* 1970]; Superoxide dismutase (SOD) [Misra and Fridovich 1972].

Histological Assessment: The tissues were processed according to the method of Drury and Wallington (1980) for Hematoxylin and Eosin staining.

Photomicrography*:* The stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) and photomicrographs were taken at x100 magnification using an attached Eakins 14MP digital microscopic camera, model 2307su, manufactured by Eakins Microscope Store, UK.

STATISTICAL ANALYSIS

All data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) Version 25 (SPSS, Inc., Chicago, Illinois, USA) and relevant statistical values were obtained. The values of the treated groups were compared with those of non-treated group using the one-way analysis of variance (ANOVA) and the T-test method. Values of P < 0.05 were considered significant. LSD was used as the post-hoc test.

RESULTS

Table 1: Biochemical Parameters of the experimental animals

	Group A	Group B	Group C	Group D	Group E	Group F	P value
Urea (mg/dl)	12.0±0.801	85.0±0.643*	17.0±0.313	15.0±0.101	18.0±0.842#	22.0±0.457#	0-0227
Creatinine ((mg/dl)	0.1±0.312	4.0±0.138*	0.7±0.022	1.0±0.024	1.0±0.041#	0.9±0.045#	0.0132

*Represent p<0.05 when compared to control

represent p,< 0.05 when compared to rats exposed to Acyclovir

There was a significant increase (p<0.05) in urea and creatinine concentration in Acyclovir-exposed rats. However, treatment with *Phyllanthius urinaria* significantly decreased (p<0.05) urea and creatinine concentrations

Table 2: showing antioxidant levels in the Kidney of the experimental animals.

	Group A	Group B	Group C	Group D	Group E	Group F	P value
SOD (unit/mg protein)	57±0.045	21±0.133*	76±0.162	65±0.043	62±0.011#	60±0.027#	0.0037
Catalase (unit/mg protein)	77±1.371	18±0.277*	72±0.231	70±1.154	53±0.311#	50±0.224#	0.0004

*Represent p<0.05 when compared to control

represent p,< 0.05 when compared to rats exposed to Acyclovir

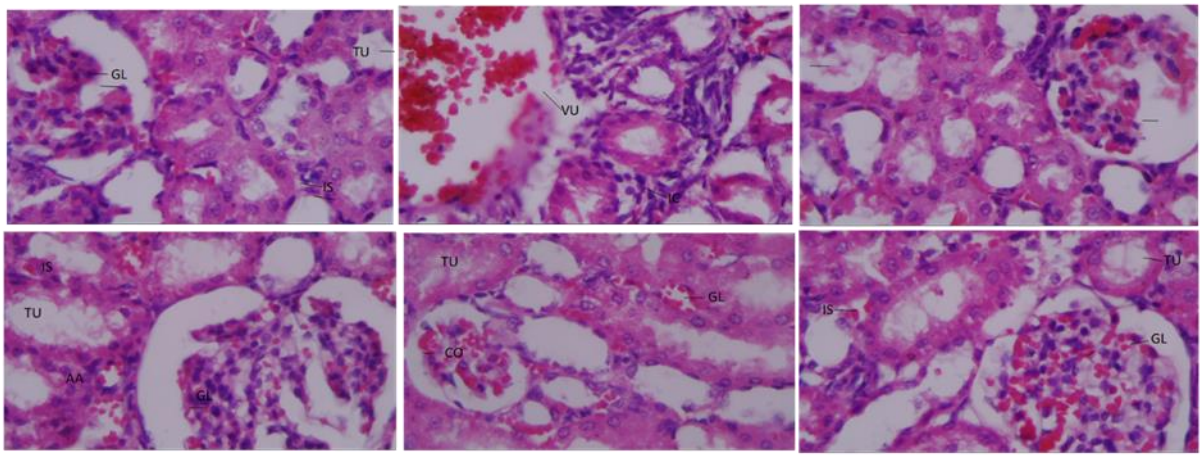

Figure 1: showing the kidney histology of **(A)** Control rats Composed of normal tissue architecture: tubules (TU), glomeruli (GL), interstitial space (IS); **(B)** Rat given 432 mg/kg body weight of Acyclovir only showing vascular congestion and ulceration (VU), heavy interstitial infiltrates of inflammatory cells; **(C)** Rat given 500 mg/Kg *Phyllanthius urinaria* only showing normal architecture: tubules (TU), interstitial space (IS), glomeruli (GL); **(D)** Rat kidney given 1000 mg/Kg *Phyllanthius urinaria* only showing normal architecture: interstitial space (IS), tubules (TU), arcuate artery (AA), glomeruli (GL); **(E)** Rat given 500 mg/Kg *Phyllanthius urinaria* + 432 mg/kg body weight of Acyclovir showing normal architecture: tubules (TU), glomeruli (GL, active interstitial congestion (CO); **(F)** Rat kidney given 1000 mg/Kg *Phyllanthius urinaria* + 432 mg/kg body weight of Acyclovir showing normal architecture: tubules (TU), interstitial space (IS), glomeruli (GL).

DISCUSSION

Renal diseases that diminish urea's glomerular filtration rate will lead to its retention in the blood (David *et al*., 2012). Urea and creatinine excreted by the kidneys, serve as one of the main diagnostic indicators of renal effectiveness. Findings from our study showed that exposure to Acyclovir resulted in a significant increase in urea and creatinine levels, suggesting potential renal injury. Such an elevation could implicate compromised kidney function, aligning with the well-documented nephrotoxic effects associated with acyclovir administration, in which there was a characteristic elevation of plasma urea, creatinine levels and abnormal urine sediments (Ahmad *et al* 1994, Vomiero *et al* 2002).

However, the introduction of *Phyllanthius urinaria* into the experimental regimen yielded noteworthy outcomes. Notably, the administration of *Phyllanthius urinaria* significantly mitigated the rise in urea and creatinine levels observed in the Acyclovir-exposed rats. This reduction hints at the potential renal-protective properties of *Phyllanthius urinaria*, suggesting its capacity to counteract the nephrotoxic effects induced by Acyclovir. Such findings underscore the therapeutic potential of *Phyllanthius urinaria* in safeguarding renal health and warrant further investigation into its mechanism of action in mitigating drug-induced nephrotoxicity.

Concerning the oxidative part of our experiment, there was a significant decrease ($p<0.05$) in Superoxide dismutase and Catalase activity in rats exposed to Acyclovir only. However, treatment with *Phyllanthius urinaria* significantly improved antioxidant activity as there was a significant increase ($p<0.05$) in Superoxide dismutase and Catalase activity.

The significant reduction in Superoxide dismutase (SOD) and Catalase (CAT) activities observed in rats exposed to Acyclovir highlights the potential onset of oxidative stress triggered by this antiviral medication.

The observed changes in the kidney morphology of ACV-treated rats may be a consequence of oxidative stress-induced kidney biomolecular damage (Dennis *et al*., 2017).

Oxidative stress causes lipid peroxidation (LPO). LPO is a process by which free radicals attack lipids especially polyunsaturated fatty acids causing alterations in the physical properties of cellular membranes leading to covalent modifications of proteins and nucleic acids stimulating cytotoxicity, cell necrosis and apoptosis (Gaschler *et al.,* 2017). The significant decline in SOD and Catalase activity observed in Acyclovir-exposed rats underscores a compromised antioxidant defense system, rendering cells susceptible to oxidative damage. Lu *et al*., reported elevated levels of the aforementioned renal biochemical markers in ACV-treated mice. The kidney accounts for about 60%–90% of ACV elimination. ACV is relatively insoluble in urine; it is filtered by glomeruli and secreted by renal tubules (Perazella, 1999). Therefore, ACV crystals can be deposited in renal tubules leading to the obstruction of nephron causing increased resistance to renal blood flow and the elevation of serum creatinine, urea, and uric acid levels (Lu *et al*., 2014, Perazella, 1999). Conversely, the significant increase in SOD and catalase activity after treatment with *Phyllanthius urinaria* suggests a potential mitigative impact on oxidative stress. *Phyllanthius urinaria*, renowned for its antioxidant properties, appears to bolster cellular defense mechanisms against ROS induced by Acyclovir. The significant enhancement in antioxidant enzyme activity implies that *Phyllanthius urinaria* may attenuate the deleterious effects of oxidative stress triggered by Acyclovir, thereby contributing to its observed protective effects against nephrotoxicity. This revelation underscores the significance of considering the antioxidant prowess of natural compounds, such as *Phyllanthius urinaria*, in mitigating drug-induced oxidative stress and its associated complications. The phytochemical profiling of the aqueous extracts of *P. amarus* indicated that it may contain the flavonoids quercetin and rutin, the lignans phyllanthin and hypophyllanthin, saponins and the phenolic compound gallic acid, all of which are potent drivers of protection (Putakala *et al*., 2017). This may account for the reason behind the protective effect of aqueous extract from *Phyllanthius urinaria* leaves against Acyclovir-induced kidney toxicity in Wistar rats.

Histological findings from this study showed that administration of Acyclovir caused kidney damage which was evident as prominent vascular congestion and ulceration, along with heavy interstitial infiltrates of inflammatory cells. These pathological changes signify the detrimental impact of Acyclovir on renal tissue integrity, indicative of nephrotoxicity). It can also be characterized by degenerative alterations in tubular epithelial cells such as tubular necrosis (Ahmad *et al* 1994, Vomiero *et al.,* 2002). The mechanisms by which acyclovir causes renal damage have been speculated to involve direct assault on renal tubular cells and oxidative stress (Vomiero *et al.,* 2002, Lu *et al.,* 2014).

However, the administration of *Phyllanthius urinaria* appeared to mitigate the detrimental effects of Acyclovir on renal tissue. Despite Acyclovir exposure, the kidneys exhibit relatively normal architecture, with minimal signs of vascular congestion or inflammation. The observed protective effects of *Phyllanthius urinaria* against Acyclovir-induced nephrotoxicity suggest its potential as a therapeutic adjunct in mitigating renal damage associated with antiviral therapy.

CONCLUSION

Results from this study showed that *Phyllanthius urinaria* ameliorates Acyclovir-induced nephrotoxicity in Wistar rats.

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