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Effect of ethanolic leaves extract of *phyllantus amarus* on cisplatin induced nephrotoxicity in albino rats

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ABSTRACT: Cisplatin is a potent front-line antitumor drug characterized by dose-limiting nephrotoxicity resulting to withdrawal from treatment by patient. This research was therefore designed to investigate the mitigating effect of ethanolic leave extract of *phyllantus amarus* on cisplatin induced nephrotoxicity in albino rats. Thirty six (36) rats were divided into 9 groups (n=4rats) labeled A to I with group A (water control) treated with 0.2ml of distil water, B(vehicle control) administered 0.2ml of olive oil, C and D were orally treated with 150 and 300mg/kgBW leave extract of *P. amarus* only dissolved in olive oil for 14day respectively, 0.7ml of cisplatin was administered to rats in group E while groups F and G , H and I orally received 150 and 300mg/kgBW leave extract of *P. amarus* dissolved in olive oil for 7 and 14days respectively after the administration of cisplatin to the rats. The rats were sacrificed on days 7 and 14, blood samples were collected into heparinized sample bottles for biochemical investigation of sodium ion (Na^+), potassium ion (K^+), Chloride ion (Cl^-), bicarbonate ion (HCO_3^-), creatinine and urea levels. Qualitative phytochemical screening of *P.amarus* was also carried out. The result revealed significant decrease ($p<0.05$) in plasma concentrations of K^+, Cl^-, creatinine and urea in groups F,G, H and I when compared to negative control(E) value and significant increase ($p<0.05$) in plasma concentrations of Na^+ and HCO_3^- when compared to negative control value. Group H for all the parameters under consideration showed a non significant difference ($p>0.05$) (almost close to oil control value) when compared to group A value, indicating restoration to normal value. The result of phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, cardenolides, steroids, tannins, carbohydrate and anthraquinones. Conclusively, ethanolic leave extract of *P. amarus* demonstrated mitigating effect of cisplatin induced nephrotoxicity in albino wistar rats © JASEM.

Keyword: *Phyllanthus amarus,* nephrotoxicity, sodium ion (Na^+), potassium ion (K^+), Chloride ion (Cl^-), bicarbonate ion (HCO_3^-), creatinine and urea © JASEM

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Introduction

Phyllanthus amarus (family: Euphorbiaceae) is widely distributed in all tropical and subtropical regions of the planet (Edeoga *et al*., 2006) and Paleobotanical studies have not found the exact geographic origin of this plant. It is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993). In tropical Africa it occurs in most countries. In Nigeria, it is called "Oyomokeisoamank edem" in Efik, "Iyin Olobe" in Yoruba and "Ebebenizo" in Bini (Etta, 2008) *P. amarus* has various groups of compounds such as alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols. Genetic diversity plays an important role in plant conservation and their survival in adverse conditions. Study on phytochemicals of wild populations of plants at different altitudes were performed, and it is not conclusive whether the observed variations are a response of individual plants to environmental factors related to altitude or a genetic adaptation of the populations growing at different altitudes to their specific environment (Ruhland and Day, 2000; Zidorn and Stuppner, 2001; Zidorn *et al*., 2005). Khan *et al.*, (2011) studied on 19 populations of this species collected from different geographical regions of India and discovered phytochemical diversity was found more in leaves followed by seeds, stem and roots from one geographical region to another region. This informed the need to embark on the phytochemical screening of this species located in South-South geographical region of Rivers State, Nigeria despite the fact that several works have been done on the phytochemical screening of the plant. *P. amarus* extract also has good antioxidant properties which help in removal of free redicals from the human body (Mhaskar *et al.*, 2000; Raphael and Kuttan, 2003; Londhe *et al.*, 2009; Harikumar *et al.*, 2009; Rai *et al*., 2007). The phenolic constituents of *P. amarus* mitigated the effect of anti-mycin A-induced mitochondrial

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apoptotic cascade (Guha *et al*., 2010). *Phyllanthus amarus* is used in the treatment for kidney/gallstones, other kidney related problems, appendix inflammation, and prostate problems (Heyde, 1990). This plant is traditionally used around the world in the treatment of liver ailments and kidney stones as derived from the Spanish name 'chanca piedra' means "stone breaker or shatter stone." The alkaloid extract demonstrated smooth muscle relaxation specific to the urinary and biliary tract which the researchers surmised facilitates the expulsion of kidney and bladder calculi (Miller, 1998; Calixto, 1984). However, much work has not been reported on the ameliorating effect of alcoholic leave extract of *Phyllanthus amarus* on cisplatin induced nephrotoxicity.

Cisplatin is a major antineoplastic drug used for the treatment of solid tumors. Its chief dose limiting side effect is nephrotoxicity; 20% of patients receiving high-dose cisplatin have severe renal dysfunction.(Marie, and Prasad, 2003) The mechanism for this renal cell injury has been the focus of intense investigation for many years, and recent studies proposed injury pathway as follows: 1) accumulation of cisplatin mediated by the transport pathway; 2) metabolic conversion of cisplatin into nephrotoxins and accumulation in kidney cells; 3) DNA injury; 4) cell transport system alterations; 5) mitochondrial dysfunction 6) oxidative and nitrosative stress; 7) inflammatory response; 8) activation of mitogen-activated protein kinase (MAPK); and 9) activation of apoptotic pathways(Luis and Ademar, 2013). It has been suggested that cisplatin conjugates with reduced glutathione (GSH) in the liver, reach the kidneys as a platinum-GSH conjugate, which is cleaved primarily to a toxic metabolite mostly by gamma-glutamyl transpeptidase (GGT), an enzyme located in the brush border of the renal proximal tubule. The formed metabolite is highly reactive with thiol/platinum compounds that interact with macromolecules and eventually lead to renal cell death.(Wainford *et al.,* 2008)11 Substantial evidence indicates that oxidative stress is involved in renal injury secondary to cisplatin administration. ROS production, depletion of antioxidant systems, and stimulation of renal accumulation of lipid peroxidation products have been listed as the main mechanisms associated with cisplatin-induced nephrotoxicity. The present study therefore, investigated the effect of alcoholic leaves extract of *Phyllanthus amarus* on cisplatin induced nephrotoxicity in albino rat model.

MATERIALS AND METHODS

Experimental Animals: Thirty six (36) albino rats of both sexes weighing 175-200g were purchased from the Animal House of Department of Biochemistry, University of Port Harcourt, Choba Park and feed with growers feed and water *ad libitum*

*Collection and Identification of Plant***:** *Phyllantus amarus* was harvested at Choba Park, University of Port Harcourt and identified by Dr. Edwin Nwosu of Plant Science and Biotechnology Department, University of Port Harcourt with herbarium no 0609.

*Phytochemical Screening***:** Qualitative determination of the secondary metabolites was done using standard procedure as described by Sofoware (1993).

*Preparation of Plant Extract***:** The leaves of the plant were thoroughly washed and air dried for one week and shredded for blending in a warring blender to a fine powder. The powder was mixed in 95% ethanol in 1:10 ratio and allowed to stand for 48hours.Thereafter, the suspension was filtered using a Whatman no 1 filter paper and the filtrate was evaporated to remove ethanol in a water bath at 45^{o}C to obtain an oily gel like extract which was weighed and stored in a refrigerator.

Determination of LD_{50}: Two doses of 150 and 300mg/kgBW of *p. amarus* extracts were the choice in this work in reference to LD_{50} determination of *phyllantus amarus* reported by Rajesh and Ramteej (2009).

Experimental Design: Thirty six (36) albino wistar rats weighing 175-200g of both sexes were divided into 8 groups (n=4rats) and kept in plastic cages. The groups were labeled A to I. Group A (water control) received 0.2ml of distil water only administered orally through an orogastric tube for 14 day. Group B (oil control) was treated with 0.2ml of olive oil orally once daily. Groups C and D (positive control) were administered orally with 150 and 300mg/kgBW oily gel of *p. amarus* extract dissolved in 0.2ml olive oil respectively using orogastric tube daily for 14 days, group E received 0.7ml of cisplatin administered intraperetoneally while groups F and G, H and I were treated orally with 150 and 300mg/kgBW of ethanolic leave *p. amarus* extracts dissolved in 0.2ml olive oil for 7 and 14days respectively after the administration of 0.7ml of cisplatin to all the rats in these groups.

*Collection of Blood and Preparation of Plasma***:** The rats were withdrawn from the cages in each of the group on days 7 and 14 and placed in a desiccator

containing cotton wool soaked in chloroform to partially anaesthetize the rats. Blood samples were obtained by cutting the jugular vein of the rat on the neck by means of surgical blade and put in anticoagulant sample bottle smeared with lithium-heparin . The blood samples were spun at 5000rpm using MSE centrifuge to obtain plasma.

*Chemicals and Reagents***:** All chemicals and reagents used were of analytical grade.

*Biochemical Investigation***:** Determination of Electrolyte: The electrolytes (sodium ion Na^+, potassium ion K^+ ,Chloride ion Cl^- and bicarbonate ion HCO_3^-) were carried out using Ion Selective Electrode(ISE) Humalyte machine (Human Germany). Creatinine and urea levels in plasma were determined by the method of Edmund and David (2009).

*Statistical Analysis***:** Results were analyzed using (SPSS) version 15. Values are expressed in Means±Standard Deviation (M±SD).The data were expressed using descriptive statistics and Analysis Of Variance (ANOVA). Multiple comparisons for the groups were done using Post Hoc Turkey (HSD) to test for the level of significance between means. A $p<0.05$ was considered to be statistically significant

RESULT AND DISCUSION

Table1. Effect of ethanolic leave extract of *phyllantus amarus* on some biochemical parameters

	Experimental groups (M±SEM)								
	Water Control	Vehicle control	*P amarus* control for 14 days		Negative control (Cisplatin)	(Cisplatin + *P amarus* Extract Treated for 7days		(Cisplatin + *P amarus*Extract Treated for 14days	
Parameters	Group A 0.2ml	GROUP B (0.2ml)	GROUP C (150mg/kg)	GROUP D (300mg/kg)	GROUP E	GROUP F (150mg/kg)	GROUP G (300mg/kg)	GROUP (150mg/kg)	GROUP I (300mg/kg)
Na ion	141.75±0.63^{c}	139.75±0.85^{c}	140.50±0.50^{c}	142.50±1.04^{c}	133.25±1.03^{ab}	134.25±0.63^{ab}	136.50±0.65^{a}	139.00±0.91^{c}	139.75±0.85^{c}
K ion	4.80±0.09^{bc}	4.12±0.11a^{c}	4.60±0.11^{c}	4.68±0.14^{c}	6.13±0.09^{ab}	5.22±0.14^{bc}	5.25±0.25^{bc}	4.60±0.15^{c}	4.38±0.09^{c}
Cl ion	105.25±0.75^{b}	88.00±0.91^{ac}	95.50±15.55^{ac}	95.00±12.91^{ac}	112.00±12.25^{b}	105.00±35.11^{b}	98.00±10.80^{bc}	96.50±17.08^{abc}	91.50±6.46^{ac}
HCO_3 ion	21.00±0.41	23.00±0.41^{c}	19.50±0.65	20.25±0.25	17.63±0.47^{b}	19.25±1.89	20.25±0.85	21.50±1.04	22.00±±0.58^{c}
Urea	3.60±0.32^{c}	3.30±0.21^{c}	4.13±0.17^{c}	4.23±0.20^{c}	7.60±0.70^{ab}	5.33±0.35ab^{c}	2.85±0.12^{c}	4.30±0.09^{c}	3.40±0.34^{c}
Creatinine	65.25±1.31^{c}	65.25±2.50^{c}	66.25±2.50^{c}	68.25±2.21^{c}	106.6±6.23^{ab}	114.50±9.84^{ab}	100.25±3.35^{ab}	84.00±3.19^{c}	74.00±2.68^{c}

All data are represented in Mean±Standard Error of Mean (M±SEM)
Superscript a represents significant difference when compared to water control
Superscript b represents significant difference when compared to vehicle control
Superscript c represents significant difference when compared to negative control
Superscript d represents significant difference when vehicle control compared to *p.amarus* extract treated groups only

Table 2. Result of the phytochemical screening of ethanolic leave extract of *phyllantus amarus.*

Secondary Plant Metabolites	Test	Result
Alkaloids	Drangedorfs	+ve
	Mayers	+ve
	Hagers	+ve
Flavoniods	Shinodas	Not determined
	Lead acetate	+ve
	Alluminium chloride	+ve
	Alkaline	+ve
Tannis	Iron (iii) Chloride	+ve
Anthraquinone	Free Anthraquinone	+ve
	Combined Anthraquinone	+ve
Triterprenoids/steroids	Lman-buchner	+ve
	Salvoski	+ve
Carbohydrate	Molsch	+ve
	Kedde	+ve
Cardenolide	Keller kilani	+ve
	Kedde	ND
Sapinins	Fronthing	+ve
	Haemolysis	ND
	Emulsion	+ve

+ve=present
-ve=absent
ND= Not Detected

*Conclusion***:** Result from Table 2 showed significant elevation ($p<0.05$) in plasma concentrations of K^+, Cl^-, creatinine and urea in group E when compared to water control value and significant decrease ($p<0.05$) for Na^+ and HCO_3^- suggesting cisplatin induce kidney damage as reported by Meyer and Madias (1994) that "even with vigilant hydration, approximately one-third of patients treated with cisplatin have transient elevation of blood urea nitrogen levels or other evidence of kidney damage in the days following cisplatin treatment." Chu *et al*., (1993) reported accidental overdoses, all of which have led to renal failure, confirm the potency of cisplatin as a renal toxin in humans. dos Santo *et al*., (2012) and Pabla and Dong (2008) also observed decreases of 20% to 40% in glomerular filtration 10 days after drug intravenous administration, and are followed by increased levels of creatinine, reduced glomerular filtration rates (GFR), hypomagnesemia, and hypokalemia. Toxic effects of cisplatin occur primarily in the proximal tubule, particularly in S3 segment of the tubular epithelial cells; glomeruli and distal tubules are affected subsequently (Luis and Ademar, 2013). The pathophysiological mechanism of cisplatin-induced tubular damage is complex and involves a number of interconnected factors, such as accumulation of cisplatin mediated by membrane transportation, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction, oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers, and activation of apoptotic pathways (dos Santo *et al*., 2012 ; Pabla and Dong 2008; Sánchez-González *et al*.,2011).3-5 Significant decreases($p<0.05$) were observed in the plasma concentrations of K^+, Cl^- , creatinine and urea and significant increases($p<0.05$) in plasma concentrations of Na^+ and HCO_3^- for extract treated groups F,G,H and I when compared to group E indicating recovery of kidney function on administration of the two doses of the extract for 7 and 14 days. For all the parameters investigated, the plasma levels showed non significant difference ($p>0.05$) when group A was compared to I demonstrating restoration of kidney function in a time and dose dependent manner. Result from Table 1 revealed the presence of alkaloids, flavonoids, saponins, cardenolides, steroids, tannins and anthraquinones as secondary metabolites found in the plant. It has been reported that oxidative stress is involved in renal injury secondary to cisplatin administration. ROS production, depletion of antioxidant systems, and stimulation of renal accumulation of lipid peroxidation products have been listed as the main mechanisms associated with cisplatin-induced nephrotoxicity. These mechanisms cause the activation of oxidative metabolism by stimulating the production of ROS by impaired mitochondria, including superoxide anions (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH); they may also impair antioxidant defense mechanisms such as GSH, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Nitrosative stress is involved in cisplatin-induced renal damage. Studies revealed that the cellular effects of ROS are amplified by high production levels of nitric oxide (NO), possibly as a result of induced synthesis of nitric oxide synthase (iNOS), leading to the continuous formation of peroxynitrites (ONOO-), which contribute to cisplatin-induced renal damage by reacting with superoxide anions. The increased production of reactive oxygen and nitrogen species after cisplatin administration results in significant damage to cell structure and function, including lipid peroxidation, protein nitration, enzyme inactivation, and DNA breaks. Consequently, this phenomenon leads to cell dysfunction and signaling for the activation of both apoptotic and cell survival pathways, causing kidney damage and cell death.(Ramesh and Reeves, 2005; Cetin *et al.*,2006; Santos *et al.,* 2007: Chirino *et al.*, 2008) *P. amarus* extract also has good antioxidant properties which help in removal of free redicals from the human body (Mhaskar *et al.,* 2000; Raphael and Kuttan, 2003; Londhe *et al.*, 2009; Harikumar *et al.*, 2009; Rai *et al*., 2007). The phenolic constituents of *P. amarus* mitigated the effect of anti-mycin A-induced mitochondrial apoptotic cascade (Guha *et al*., 2010). Several active compounds have been identified in *P. amarus* extract. Lignins like phyllanthin and hypophyllanthin, flavonoids like quercetin and astragalin, ellagitannins like amarinic acid and hydrolyzable tannins like phyllanthisiin D isolated from this plant were reported to possess antioxidative properties (Foo and Wong, 1992). Phyllanthus amarus can detoxify the free radicals and has an antioxidant activity and increase the rate of regen-eration of necrosed cells (Tagrajan *et al*.,1990; Oudhia and Tripthi,2002). Several experimental models demonstrated the renal protection offered by antioxidants such as dimethylthiourea (DMTU), melatonin, selenium, vitamin E, and N-acetylcysteine, to name a few (Luis and Ademar, 2013). However, use of these drugs is limited by side effects and cost. In addition, concerns about possible

interference with the antitumor activity of cisplatin should limit its use to clinical trials in tumors other than those listed above (Luis and Ademar, 2013). Interestingly, the doses of choice in this work did not show any significant effect in all the parameters investigated when the vehicle control was compared to the extract treated groups suggesting safety of the extract on the kidney. Conclusively, ethanolic leaves extract of *p. amarus* demonstrated significant mitigation of the changes induced by cisplatin on the kidney tissues of albino rats.

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