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Biochemical Effect of Ethanolic Extract of *Phyllantus Amarus* (L.) On Gentamicin-Induced Liver and Kidney Damage in Rats

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ABSTRACT

Hepato protective and nepro protective activity of ethanolic extract of *Phyllanthus amarus* leaves was assessed. Twenty five wistar albino rats were used, comprising of five groups of five animals each and were fed with growers' match and water freely. Rats in groups B and C were administered 200 and 300 mg/kg, respectively, of ethanol extract of *P. amarus* orally for twenty one days. Groups A and E received normal saline water for the same period, while group C received 100 mg/kg of vitamin C. On the 21st day the of study groups (B, C, D and E) were administered single dose of 80 mg/kg of gentamicin intraperitoneally, fasted for 24 h, then sacrificed on the 22nd day and blood samples were collected through cardiac puncture. Samples were used to determine total protein, urea, total bilirubin and direct bilirubin concentrations using standard kits. The results showed a significant increase p<0.05 in the level of total protein in rat group A (normal control) compared to group E (positive control). Also, the level of total protein increased significantly in rats groups B, C and D pretreated with extracts and vitamin C, respectively, compared to group E. However, there was significant decrease p<0.05 in the levels of urea, total bilirubin and direct bilirubin in extract pretreated rats groups (B and C), compared to untreated rats group E. Conclusively, the findings indicated that the administration of ethanol extract of *P. amarus* at these concentrations confers hepato protective and nephron protective effects on gentamicin-induced rats.

Key words: Hepato protective, Nephro protective, Hepato protective and *P. amarus*.

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INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in their chemical constituent which produces a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Itoro et al., 2013). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food meant for pregnant and nursing mothers for medicinal purpose (Sonia et al., 2014). *Phyllanthus amarus* is a broad spectrum medicinal plant that has received world- wide recognition (Chanderewar and Dhongade, 2013). In Nigeria, it is called "Oyomokeisoamankedem" in Efik, "Iyin Olobe" in Yoruba and "Ebebenizo" in Bini (Etta, 2008; Obianime and Uchie, 2008). *P. amarus* is generally employed to reduce pain, expel intestinal gas, to stimulate and promote digestion, as anti-helminthes to expel intestinal worms and act as a mild laxative. *P. amarus* also has antiseptic, diuretic, antiviral, anti-diabetic, hypotensive and antipyretic properties, and is also used in the treatment of jaundice, diarrhoea, dysentery, wound, ulcers and urogenital diseases (Patel et al., 2011; Srividiya et al., 1995).

The plants of the genus *Phyllanthus* are widely distributed in most tropical and subtropical countries and have long been used in traditional medicine to treat chronic liver disease (Liu and Huang, 2003). *P. amarus* has been reported to contain, alkanoids, flavonoids, germanuin, tannin, phyllantine, phypohyllanthine, quercetin, isoquercitrin, astraglin, geranin, corilagin, rutin and 3-0-gluscopyyranoside (Adeneye and Benebo,

2008). *P. amarus* is useful in ophthalmia, gonorrhea sores, swelling and itchiness and hypoglycaemic in nature. It promotes liver functions (Ogunyemi, 2008; Adegoke et al., 2010). The need to investigate the hepato protective and nephron protective nature of *P. amarus* is due to the need to ascertain how well locally available plants in Nigeria can protect the liver and kidney from damage. The objective of this study was to investigate the effect of *P. amarus* in gentamicin- induced organ damage in rats

MATERIALS AND METHODS

Animals

Twenty five (25) male Wistar rats, with average mass of 150 ± 1.0 g were purchased from the Department of Pharmacology Animal House and kept in the Department of Biochemistry Animal House in College of Health Sciences, Niger Delta University. They were randomly assigned into five groups: A, B, C, D and E (n=5) in each group. They were fed with growers' mash obtained from Edo feed and flour mill limited, Ewu, Edo State and given water liberally. The rats gained maximum acclimatization (2 weeks) before actual commencement of the experiment.

Plant Materials

Fresh leaves of *P. amarus* were collected from Pharmarcognosy herbarium, Faculty of Pharmacy, Niger Delta University, Bayelsa State. The plant was identified and authenticated by a Botanist, Prof. K. Ajibeshin of Department of Pharmarcognosy, Niger Delta University. The fresh *P. amarus* leaves were thoroughly washed weighted (200 g) and crushed with mortar and pestle. 500 ml of Ethanol was added into the crushed leaves of *P*. *amarus* and mixed thoroughly for 24 h in a 1000 ml jar. The extract was sieved and the juice was filtered using Whatman No 1 filter paper. The filtrate was concentrated by evaporating it using a water bath at a temperature of 40ºC. The resultant extract (20 g) was placed into small glass dishes and stored in a refrigerator for further studies.

Experimental Procedures and Extract Administration

Twenty five male wistar rats were randomly shared into 5 groups of 5 rats per group and ethanol extract of *P. amarus* administered orally through an orogastric tube on daily basis for 21 days as shown below; Group A: Saline water (normal control), Group B: 200 mmg/kg of extracts, Group C: 300 mg/kg extracts, Group D: 100 mg/kg vitamin C and Group E: Saline water (Positive control) Groups B, C, D and E were further administered single dose of gentamicin (80 mg/kg) intraperitoneal on the 21st day of study. Animals fasted for 24 h and on the 22nd day the animals were scarified and blood collected through cardiac puncture into well labeled universal bottles. The blood was centrifuged at 2500 rpm for 10 min. and the serum collected is used for biochemical analysis.

Biochemical Assay

Total protein was determined by colorimetric method (Biuret method), as modified by Gornallet et al. (1994) method. Bilirubin was estimated by colorimetric method of Jendrassik and Grof (1938). Serum Urea was estimated by Natelson et al. (1951) method.

Statistical Analysis

The results were expressed as mean± SD. Data were analyzed by one-way analysis of variance. Sequential differences among means were calculated at the level of $P< 0.05$, using the Turkey contrast analysis as needed.

RESULTS

The effects of *P. amarus* on gentamicin-induced rats are presented in Table 1. In this study, values obtained from group A (normal control) are compared to group E (positive control) and values of the extract treated groups B and C are compared to group E. There was significant increase in the weight of rats in group A (31.0±3.35) compared to rats in group E (24.5±8.95), $p<0.05$. A significant decrease was obtained in the weight of rats in extract treated groups (B and C), (14.4±10.86 and 12.3 ± 14.8), respectively, compared to group E. Total protein levels also increased significantly in rats in group A (91.84±3.86), compared to group E (75.94±3.45). Also, the total protein levels increased significantly in extract treated groups (B and C), (78.94 ± 4.58 and 82.41 ± 0.64), respectively, compared to group E. Urea level decreased significantly in rats group A (42.49 ± 3.60), compared to rats in group E (90.97 ± 10.07), also, significant decrease was obtained in extract treated groups (B and C), (42.89 ± 5.41 and 53.95 ± 5.07), respectively, compared to group E. Total bilirubin level decreased significantly in rat group A (2.424 ± 0.90) compared to group E (18.763 ± 5.43). Also, total bilirubin level decrease significantly in extract treated groups (B and C), (1.691 ± 0.16 and 1.826 ± 0.46), respectively, compared to untreated group E.

DISCUSSION

The quest to investigate the biochemical effects of ethanol extract of *P. amarus* on liver and kidney is due to its widely belief to have therapeutic application in folk medicine and scientific advancement through technology

Table 1. Biochemical effects of *P. amarus* on gentamicin- induced rats.

Groups	Treatments	Weight change rat (g)	Total protein g/dl	Urea mg/dl	Direct bilubirin mg/dl	Total bilubirin mg/dl
A	Normal control without gentamicin	31.0 ± 3.35[b]	91.84 ± 3.86[a]	42.49 ± 3.60[a]	0.227 ± 0.04[a]	2.424 ± 0.90[a]
B	200 mg/kg of extracts + 80 mg/kg gentamicin	14.4±10.86[a]	78.94 ± 4.58[b]	42.89 ± 5.41[a]	0.380 ± 0.15[a]	1.691 ± 0.16[a]
C	300 mg/kg of extracts + 80 mg/kg gentamicin	12.3 ± 14.8[a]	82.41 ± 0.64[c]	53.95 ± 5.07[b]	0.234 ± 0.02[a]	1.826 ± 0.46[a]
D	100 mg/kg of Vit. C + 80 mg/kg gentamicin	13.0 ± 11.1[a]	97.69 ± 4.60[d]	51.56 ± 9.28[b]	6.324 ± 1.56[b]	16.809 ± 3.63[b]
E	Saline + 80 mg/kg Gentamicin {positive control)	24.5 ± 8.95[c]	75.94±3.45[e]	90.97 ± 10.07[c]	7.493 ± 1.63[b]	18.763 ± 5.43[b]

Values are means of five determinations± SD. Values with different superscript in the column differ significantly (P<0.05).

has provided substantial evidence to support most of its medicinal claims (Sonia et al., 2014). The present study has further demonstrated the hepato protective and nephron protective potentials of this plant. The decrease in the level of serum total protein in rats group E (without extract) obtained in this study may be an indication of hepatotoxicity due to gentamicin administration. This finding was supported by earlier study of Abatan et al. (1996), while investigating the pathological effect of Lantana camara and *Dichapetalum madagascaiense* in goats that decreased level of serum total protein is an indication of hepatotoxicity. Nephro protective and cardio protective effect of *P. amarus* was shown to be evident from the study in which methanol extract of *P. amarus* leaves caused a significant decrease in the levels of total cholesterol, urea, total protein, uric acid, and prostatic, alkaline and acid phosphatases, AST and ALT in a dose dependent manner. Since increase in these enzymes is related to hepatic and heart disorders therefore their reduction shows that the leaves of *P. amarus* have hepato protective, nephron protective and cardio protective properties (Obianime and Uche, 2008). Also, hepato protective effects of aqueous extract from *P. amarus* on ethanol-induced rat hepatic injury was reported in *in vitro* study where *P. amarus* decreased the release of AST and ALT in rat primary cultured hepatocytes treated with ethanol (Pramyothin et al., 2007). Total protein level increased significantly in *P. amarus* extract treated groups (B and C) as shown in Table 1. Since protein degradation seems to occur by distinct mechanism, it may be suggested that *P. amarus* has protein inhibitory potency (Davies and Goldberg, 1986). Therefore the plant species may be a good source of medicine against diseases in which protein oxidation are involved such as toxic hepatitis (Njayou et al., 2008).

The decreased levels of total bilirubin and direct bilirubin reported in this study in rats groups B and C compared to group E, is in agreement with Al-Qarawi et al. (2004), who reported significant decrease in total bilirubin and direct bilirubin while investigating the hepato protective effect of extracts from dates (*Phoenix dactylifera* L) on carbon tetrachloride-induced hepatotoxicity in rats. There was a significant decrease ($p<0.05$) in the serum level of urea in extract treated rats (groups B and C) compared to rats in untreated group E. Urea is the main end product of protein degradation. Deamination of amino acids takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Some of the urea is bound to haemoglobin so its concentration in red blood cells is greater than in the plasma. Renal diseases which diminish the glomerular filtration leads to urea and increase in urea is seen in severe liver and kidney disease with destruction of cells leadings to impairment of the urea cycle (Ranjna, 1999). Therefore, the decreaselevels of urea in *P. amarus* extract treated groups are indication of possible nephron protective function of the extract. However, the effects of this extract are not concentration dependent as there was no significant difference in these parameters at 200 and 300mg/kg of extracts.

CONCLUSION

This study has provided scientific evidence, whereby administration of ethanol extract of *P. amarus* to gentamicin- induced rats could reduce injury in the liver and kidney by decreasing the activity of the biochemical parameters. Our findings therefore support the claims that this herb may confer hepato protective and nephron protective effects on rats.

REFERENCES

Abatan MO, Arowolo ROA, Olorunsogo O (1996). Pathological effects of *Lantana camara* and *Dichapetalum*

madagascasiense in goats. Trop. Vet. Med. 14: 127-132.
Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro P (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. Int. J. Appl. Res. Natural Prod., 3:6.
Adeneye AA, Benebo AS (2008). Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J. Ethnopharmacol., 118(2): 318-323.
Al-Qarawi A, Mousa HM, Ali BH, Abdel-Rahaman H, El-Mougy SA (2004). Protective effect of extract from dates (*Phoenix dactylifera*. L) on carbon tetrachloride-induced hepatotoxicity in rats. Intern. J. Appl. Res. Vet. Med., 2: 176-180.
Chandewar A, Dhongade H (2013): Pharmacognostical and Phytochemical studies of *Phyllanthus amarus* leaves. Int. J.Biomed. Adv. Res. 4:383
Davies KJA, Goldberg AL (1987). Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanism in erythrocytes. J. Biol. Chem., 262(17): 8220-8226.
Etta H (2008). Effects of *Phyllanthus amarus* on litter traits in albino rats. Sci. Res. Essay 3(8):370-372.
Gornallet AG, Bardawill CJ, David MM (1994). Protein determination with Biuret reagent. J. Biochem., 117:751-756.
Itoro E, Ukana D, Ekaete D (2013). Phytochemical screening and nutrient analysis of *Phyllanthus amarus.* Asian J. Plant Sci. Res., 3: 116-122.
Jendrassik L, Grof P (1938). Simplified photometric method for Beshmung of bilirubin. Biochem. Z. 297: 81-89.
Lui R-LH, Huang YL (2003). Genus *Phyllanthus* for chronic hepatitis B virus infection: A systemic review. Viral Hepatitis (8): 358-36.
Natelson S, Scott ML, Beffa CA (1951). Rapid method for the estimation of urea in biologic fluids. Amer. J. Clin. Pathol., 21:275.
Njayou N, Fredric P, Moundipa A., Tchana N, Bonaventure T, Ngadjui F, Tchouanguep M (2008). Inhibition of microsomal lipid peroxidation and protein oxidation by extracts from plants used in BAMUN folk medicine (Cameroon) against hepatitis. Afr. J. Trad. Complement. Altern. Med. 5(3): 278-289.
Obianime, AW and Uchie, FI.(2008). The phytochemical screening and the effects of methanolic extract of *Phyllanthus amarus* leaf on the biochemical parameters of male guinea pigs. J. Appl. Sci. Environ. Manag. 12(4): 73-77.
Ogunyemi CM (2008). The use of phytomedicine as effective therapheutic agents in disease like siekle cell anemia. Pharmacol. Res., 63: 254-274.
Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK (2011). *Phyllanthus amarus* Ethnomedicinal uses phytochemistry and pharmacology: A review. J. Ethnopharmacol., 138(2):286-313.
Pramyothin P, Ngamtin C, Poungshompoo S, Chaichantipyuth C (2007). Hepatoprotective activity of *Phyllanthus amarus* Schum Thonn extract in ethanol treated rats: In vitro and in vivo studies. J. Ethnopharmacol., 14(2):169-173.
Ranjna C (1999). Practical Clinical Biochemistry Methods and Interpretation. 2nd Edn., p. 117.
Sonia V, Hitender S, Munish G (2014). *Phyllantus amarus* ; A review. J. Pharma. Phytochem., 3 (2): 18-22.
Srividiya N, Single RT, Hsu FL (1995). Diuretic, hypotensive and hypoglycemic effect of *Phyllanthus amarus*. Indian J. Expal. Bio., 33: 861-864.