Phyllanthus amarus extract restored deranged biochemical parameters in rat model of hepatotoxicity and nephrotoxicity

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ABSTRACT

Phyllanthus amarus has been exploited for the management of several ailments in folkloric medicine. The present study therefore investigates the restorative potential of its leaves extract on hepatic and renal assault induced by CCl₄ and rifampicin respectively. Eight groups (I-VIII) containing five animals each were created for the experiments. Group I were fed with normal commercial pellet only, while group II were exposed to single intraperitoneal injection of 3 ml/kg b.w. of CCl₄ only. Groups III, IV and V animals were administered 3 ml/kg b/w of CCl₄ and treated with 50, 100 mg/kg b. w. of P. amarus and 100 mg/kg b.w of silymarin respectively. Group VI animals were orally exposed to 250 mg/kg b/w of rifampicin only while groups VII and VIII were treated with 50 and 100 mg/kg b. w. P. amarus respectively for 14 days after the initial exposure to 250 mg/kg b/w rifampicin.

Liver and kidney function tests such as alanine amino transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, urea and uric acid were determined in the serum and organs homogenates. Moreover, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) as well as lipid profile were also measured. Results showed that exposure to rifampicin and CCl₄ respectively caused a marked derangement in lipid profile as well as decrease in SOD and CAT activity relative to the negative control. Administration of both toxicants also caused a marked increase in serum ALT, AST, ALP, urea, uric acid and creatine kinase compared to the negative control. Treatment with P. amarus attenuated the toxicity imposed by rifampicin and CCl₄ on the liver and kidney in a dose-dependent fashion. All biochemical indices measured were restored to values comparable with animals treated with silymarin. Histopathological results of the hepatic and renal tissues from the various groups of experimental animals gave credence to the curative effects of P. amarus leaf extract on damaged liver and kidney cells. Put together, P. amarus is a potential medicinal plant with similar potency to conventional drugs currently in use for the treatment liver and kidney diseases. Hence, it is a viable therapeutic alternative that can be exploited for the treatment of renal and hepatic diseases.

1. Introduction

Medicinal plants have been recognized as veritable therapeutic agents since they house a cocktail of secondary metabolites (phytochemicals) with vast medicinal benefits (Sandberg and Corrigan, 2001). Plants with medicinal potentials are useful both as raw materials for the maintenance of sound health as well as in the treatment of diseases (Schulz et al., 2001; Calixto, 2000). Consequently, the role of plant-based drugs for alleviating and/or eradicating diseases is increasing globally (Bhat, 2014).

The etiology of almost all diseases have been linked to deleterious imbalance between free radicals production and the body's antioxidant capacity to neutralize them. Whenever reactive oxygen species (ROS) are produced in excess of the endogenous antioxidant capacity to mop them, oxidative stress becomes inevitable. During oxidative stress, free radicals wreak havoc on critical macromolecules such as protein, nucleic acids, lipids and carbohydrates. This oxidative attack ultimately results in pathological conditions depending on the organs attacked (Alugoju et al., 2015).

Phyllanthus amarus (Euphorbiaceae) is an annual tropical plant with several medicinal benefits in folkloric medicine. Several reports have shown that P. amarus exhibits numerous medicinal properties including: antihepatitis (Thyagarajan et al., 1988), antimarial (Tran et al., 2003), anti-viral (Notka et al., 2004; Pramyothin et al., 2007; Burkill, 1994), antibacterial (Mazumder et al., 2006), antidiarrheal (Odetola and

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2. Materials and methods

2.1. Preparation of plant extract

P. amarus leaves were obtained from a private farm in Ado Ekiti, botanically identified and authenticated at the Department of Plant Science, Ekiti State University, Ado Ekiti. Its voucher specimen with number UHAE2020072 was deposited at the university herbarium. Its leaves were then air-dried, pulverized, weighed and stored in an airtight container.

325 g of the powdered leaves was extracted in 1000 ml of 80% (v/v) ethanol for 72 h. The mixture was then filtered on cheese cloth to get the supernatant which was kept in an airtight container and residue that was trimmed of fat. The tissues were washed in distilled water, and blotted with clean filter paper, weighed and homogenized in 0.1 M phosphate buffer (pH 7.4) to obtain a 10% homogenate. The resulting homogenates were centrifuged separately at 3000 rpm at 4 ºC for 30 min. After centrifugation, the supernatant was decanted and kept chilled by refrigeration. Whole blood was obtained by cardiac puncture into an EDTA bottle and left to stand for 1 h at room temperature. Serum was obtained from the whole blood by centrifugation at 3000 rpm for 15 min at 25 ºC. The serum was then obtained as supernatant and refrigerated for subsequent estimation of serum biochemical parameters.

2.2. Reagents and chemicals

Adrenaline, malondialdehyde (MDA), phosphotungstic acid, magnesium acetate, creatine phosphate, potassium phosphate, hydrogen peroxide, ethylene diamine tetraacetate (EDTA), Ellman’s reagent and reduced glutathione (GSH) Other chemicals and reagents used were of high grade analytically and were obtained from reputable commercial suppliers. All biochemical kits were obtained from Randox Chemical Ltd. England.

2.3. Animals protocol

All experimental animals were used in accordance with established guidelines (Revised NIH Publications 1978, No. 8023) and ethical approval of the Committee on Care and Use of Experimental Animal Resources, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria. Forty wistar albino rats with mean weight of 170 g were purchased from the animal breeding colony of the Department of Science Technology, Federal Polytechnic, Ado - Ekiti. Eight groups of five animals per group were created as described in Table 1. Experimental animals were accommodated in separate cages made of iron- meshed at ambient temperature (24 ± 1 ºC), relative humidity, and 12/12-h light and dark cycle. Animals were given unrestricted daily access to food and drinking water ad libitum. Throughout the experimental period, rat beddings were routinely turned-over on a daily basis to maintain good hygiene.

2.4. Dissection of rats

Decapitation of animals was done under cold ether anesthesia and rapidly dissected to excise the liver and kidney which were separately trimmed of fat. The tissues were washed in distilled water, and blotted with clean filter paper, weighed and homogenized in 0.1 M phosphate buffer (pH 7.4) to obtain a 10% homogenate. The resulting homogenates were centrifuged separately at 3000 rpm at 4 ºC for 30 min. After centrifugation, the supernatant was decanted and kept chilled by refrigeration. Whole blood was obtained by cardiac puncture into an EDTA bottle and left to stand for 1 h at room temperature. Serum was obtained from the whole blood by centrifugation at 3000 rpm for 15 min at 25 ºC. The serum was then obtained as supernatant and refrigerated for subsequent estimation of serum biochemical parameters.

2.5. Assay for creatine kinase (Ck-Mb) activity

Creatine kinase activity was assayed according to Reitman and Franke (1957). One milliliter of imidazole buffer containing: 2.0 mM nicotinamide adenine dinucleotide phosphate, 10 mM imidazole pH 6.6, 20 mM glucose, 30 mM creatine phosphate, 20 mM N-acetyl-cysteine, 2.0 mM ethylene diamineteraacetate, 2.0 mM adenosine diphosphate, 5.0 mM adenosine monophosphate, 10 mM magnesium acetate, 10 µM DAPP, 2.0 ku/L glucose-6-phosphate dehydrogenase and 2.15 ku/L hexokinase was pipetted into a thermostatic cuvette and heated to 37 ºC. This was followed separate addition of 50 µl each of serum and tissues homogenates and thorough agitation of the resulting mixture. Absorbance at 340 nm was read immediately for 5 min at 30 s interval, followed by the estimation of the change in absorbance per min.

2.6. Measurement of aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was assayed following the method of Reitman and Franke (1957). One hundred microliter each of serum, liver and kidney was added to phosphate buffer (100 mM, pH 7.4), α-oxyglutarate (2 mM) and L-aspartate (100 mM). The resulting mixture was incubated at 37 ºC for 30 min. Five hundred microliter of 2, 4-dinitrophenylhydrazine (0.02 M) was then added to the reaction mixture and left for 20 min at 25 ºC. Five microliter of 0.4 M NaOH was then introduced to the mixture and its absorbance at 546 nm was read after 5 min against the reagent blank.

2.7. Measurement of alanine amino transferase (ALT) activity

ALT activity was measured following the method of Reitman and Franke (1957). Five hundred microliter of reagent 1 (R1) made up of 100 mM phosphate buffer pH 7.4, 2.0 M α-oxyglutarate and 0.2 M L-alanine were added separately to 0.1 mL of serum and tissues homogenates in different test tubes and the mixture was incubated for 30 min at 37 ºC. Five hundred microliter of reagent 2 (R2) made up of 0.02 M 2, 4-dinitrophenylhydrazine was then added to the reaction mixture and the resulting solution re-incubated for 20 min at 20 ºC. Finally, 5.0 ml of NaOH was added to the reaction mixture and left to stand for 5 min at 25 ºC. Absorbance of the resulting solution was then read at 546 nm.

2.8. Assay for alkaline phosphatase (ALP) activity

ALP activity in the tissue homogenates as well as the serum was determined according to the method of Englehardt (1970) using biochemical assay kits (Randox laboratories, UK). One milliliter of reagent A made up of 0.5 mM MgCl_2, 1.0 M diethanolamine buffer pH 9.8,

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Table 1. Animal treatment.

| Groups | Treatment |
|--------|-----------|
| I: Negative Control (PC) | Distilled water only |
| II: Positive Control (NC) | 3 ml CCl₄ single administration |
| III | 3 ml CCl₄ + 50 mg/kg b.w. P. amarus |
| IV | 3 ml CCl₄ + 100 mg/kg b.w. P. amarus |
| V | 3 ml CCl₄ + 100 mg/kg b.w. Silymarin |
| VI | 250 mg/kg b.w. rifampicin alone for a single administration |
| VII | 250 mg/kg b.w. rifampicin + 50 mg/kg P. amarus |
| VIII | 250 mg/kg b.w. rifampicin + 100 mg/kg P. amarus |
substrate and 10 mM p-nitrophenol phosphate, was added separately to 0.02 mL of liver, kidney and heart homogenates and thoroughly mixed. Absorbance of the assay mixture was then monitored at 405 nm for 3 min at 1 min interval.

2.9. Serum lipid profile analysis

2.9.1. Total cholesterol

Total cholesterol level in the tissues homogenates and serum was estimated according to the method of Trinder (1969) using commercially available kits from Randox laboratories, United Kingdom. Ten microlitres each of cholesterol standard, serum and tissues homogenates were measured into separate test tubes with labels. One milliliter of working reagent made up of: 0.25 mM 4-Aminoantipyrine, 0.08 M Pipes buffer pH 6.8, 6.0 mM phenol; 0.5 U/mL peroxidase; 0.15 U/mL cholesterol esterase ion and 0.10 U/mL cholesterol oxidase was measured into all the tubes. After thorough mixing, the test tubes were incubated for 10 min at 25 °C. Absorbance of sample (Asample) was read at 500 nm against the reagent blank. Total cholesterol (mg/dl) was then estimated

\[
\text{Total cholesterol (mg/dl) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}} \times \text{Concentration of standard}
\]

2.9.2. Determination of triglyceride concentration

Levels of triglycerides in the tissue homogenates and serum was determined based on Tietz (1995). Ten microliter each of triglyceride standard, serum, kidney and liver homogenates were measured into test tubes with labels. Thereafter, 1.0 mL of working reagents; R1a (buffer) containing 17.5 mM magnesium-ion, 40 mM Pipes buffer pH 7.6; 0.0055 M 4-chlorophenol and enzyme reagent (R1b) which consists of 1.0 mM...
ATP, 0.4 U/mL glycerol-kinase, 0.5 mM 4-amino phenazone; 150 U/mL lipase, 0.5 U/mL peroxidase and 1.5 U/mL glycerol-3-phosphate oxidase and was measured into the labelled test tubes. The mixture was incubated at 25 °C for 10 min after thorough mixing. Absorbance of the reaction mixture was then measured at 546 nm against the blank. Concentration of triglyceride (mg/dL) was then estimated (2).

\[
\text{Triglyceride concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}
\]  

\hspace{1cm} (2)

2.9.3. High density lipoprotein (HDL-c)-cholesterol

Amount of HDL-c in the tissues and serum was determined following the method of Grove (1979). Reaction mixture containing 200 μL each of tissue homogenates, serum, and the cholesterol standard was mixed with 500 μL of the diluted precipitant R1 (0.55 mM phosphotungstic acid, 25 mM magnesium chloride) and left for 10 min at 25 °C. The reaction mixture was then centrifuged for 10 min at 4000 rpm. The clear supernatant obtained was decanted within 2 h and its cholesterol content was estimated by the CHOD-PAP reaction method as described below:

One thousand microliter of cholesterol reagent was measured and thoroughly mixed with 100 μL each of tissue homogenates and serum in separate test tubes. Contents of the test tube (1 mL of cholesterol reagent and 100 μL each of the cholesterol standard and supernatant) labelled as standard was thoroughly mixed and incubated at 25 °C for 10 min. Absorbance of the sample (A\text{sample}) and standard (A\text{standard}) was then read within 1 h at 500 nm against the reagent blank.

\[
\text{LDL cholesterol} = \frac{\text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL} - \text{cholesterol}}{\text{HDL}}
\]  

\hspace{1cm} (3)

2.9.4. Low density lipoprotein (LDL) - Cholesterol

Low-density lipoprotein in the tissue homogenates as well as serum was estimated using Eq. (3) of Friedwald et al. (1972).

2.10. Assay for catalase (CAT)

Catalase activity in the liver, kidney and serum of experimental animals was done by the method of Sinha (1972). Two hundred microliters

Figure 2. Effect of P. amarus leaf extract on selected biomarkers of albino rats exposed to CCl4 and rifampicin toxicity. Data indicates mean ± SEM values of four independent experiments performed in triplicate. ‘a’ represents significant difference (p<0.05) from the positive control - (NC); VC - negative control; PA - P. amarus; Sil-Silymarin. C.K.- Urea- Uric Acid- Bilirubin- A and B are kidney of CCl4 and rifampicin-challenged rats respectively. C and D are serum of CCl4 and rifampicin-challenged rats respectively.
each of five fold dilution of serum, kidney and liver homogenates serum was measure into a reaction mixture of 2 mL of (800 μmol) hydrogen peroxide and 2.5 mL of potassium phosphate buffer. Five hundred microliter of appropriate enzyme dilution was rapidly introduced to with the mixture and agitated by gentle swirling in a flat bottom flask at 25 °C. One milliliter aliquot of the reaction mixture was withdrawn and blown into 1 mL dichromate/acetate acid reagent at 60 s intervals. Catalase activity was estimated as the amount of hydrogen peroxide consumed per minute per mg protein (4).

\[
\text{H}_2\text{O}_2 \text{consumed} = 800 - \text{Concentration of } \text{H}_2\text{O}_2 \text{remaining}
\]  

Concentration of H₂O₂ remaining was extrapolated from the standard curve for catalase activity.

2.11. Assay for superoxide dismutase (SOD)

SOD activity was measured according to Misra and Fridovich (1972). An aliquot of a 10 fold dilution each of serum, liver and kidney homogenates was measured into 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and left to for equilibration in the spectrophotometer. 0.3 mM of freshly prepared adrenaline was added to the mixture agitated by rapid inversion to initiate enzymatic reaction. The blank contained all other components of the assay mixture except the enzyme (serum and tissue homogenates) which was replaced with distilled water. Absorbance of the mixture was monitored at 480 nm was monitored for 150 s at 30 s interval.

2.12. Estimation of reduced glutathione

Concentration of reduced glutathione (GSH) in the liver, kidney and serum was measured by the method of Beutler and Kelly (1963). Two hundred microliter of homogenates, 1.8 mL distilled water and 3 mL of precipitant were thoroughly mixed, incubated for 5 min at 25 °C and filtered. One milliliter of five fold dilution of the filtrate then mixed with 0.5 mL of Ellman’s reagent. The blank consists of all other assay components except the homogenates. Absorbance of the resulting solution was read at 412 nm against the blank.

2.13. Lipid peroxidation

Lipid peroxidation in the homogenates and serum of experimental rats was determined as described by Ohkawa et al. (1979) using Randox kits. One hundred microliter each of kidney and liver homogenates as well as serum were mixed separately with 2.5 mL reaction buffer and incubated for 1 h at 100 °C. After cooling, the mixture was centrifuged at 3,000 rpm for 10 min and decanted to obtain a supernatant. Absorbance of the supernatants was then read at 532 nm. Malondialdehyde (MDA) level in the supernatant was expressed as μmole MDA per mg protein taking the molar absorptivity of MDA-thiobarbituric chromophore as 1.56×10⁵/M/cm.

2.14. Estimation of urea, uric acid and total bilirubin

Amount of urea was determined by the method Veniamin and Vakirtz (1970). Total bilirubin was measured according to the diazonium salt method of Winstein and Cehelyk (1969). Uric acid level was determined using the uricase-peroxidase method by Fossati et al. (1980).

2.15. Estimation of total protein

Total protein in the tissue homogenates and serum was measured by Weichselbaum (1946) using commercially available kits (Randox laboratories, UK). One milliliter of Reagent R1 made up of sodium hydroxide (100 mM), Na–K-tartrate (18 mM), potassium iodide (15 mM) and cupric sulphate (6 mM) was mixed with 0.02 mL of the homogenates and incubated at 25 °C. Absorbance of the mixture was measured at 546 nm.

2.16. Statistical analysis

All experimental data were expressed as mean ± SEM. Dat were analyzed using One Way Analysis of Variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) by using SPSS 11.09 for windows. Level of significance was at p < 0.05.

3. Results

Generally, results indicate that both CCl₄ and rifampicin are toxic to both liver and kidney (Figures 1, 2, 3, and 4). P. amarus extract restored enzymatic activity of catalase, superoxide dismutase and creatine kinase which were initially lost after exposure to the toxicants (Table 2). Restoration was dose dependent and comparable to animals treated with standard drug (Table 2). Similarly, administration of CCl₄ and rifampicin respectively caused a marked derangement of lipid profile (triglycerides, low density lipoproteins (LDL), cholesterol and high density lipoprotein (HDL)) in experimental animals (Table 2). However, treatment with P. amarus extract reversed the derangement to a level comparable with the negative control and animals treated with silymarin respectively. Biomarkers of liver (AST, ALT, ALP, Total bilirubin) (Figure 1) and
kidney (creatine kinase, urea and uric acid) (Figure 2) were significantly raised in the animals administered with CCl4 and rifampicin without treatment. Treatment with *P. amarus* leaf extract reversed the trend by restoring values of these biomarkers to that comparable with animals treated with silymarin (Figure 2). Reduced glutathione (GSH) which was depleted by exposure to CCl4 and rifampicin respectively were raised back to values similar to negative control animals (Table 2) after treatment with *P. amarus* leaf extract. Finally, lipid peroxidation was markedly inhibited in a dose-dependent fashion by treatment with *P. amarus* extract (Figure 3).

**4. Discussion**

Globally, use of plants for prophylaxis and curative treatment of diseases is an age-long practice. Till date, a significant proportion of the world population depends on herbal preparations as drugs (Ogbera et al., 2010). *P. amarus* is an ubiquitous plant that has been exploited in folkloric medicine due to its vast medicinal potentials (Ankur et al., 2011).

Exposure of experimental animals to CCl4 and rifampicin respectively resulted in multiple oxidative damages to kidney and liver cells. Biochemical and histopathological observations confirmed the oxidative damage. Biomarkers of liver injury including AST, ALT and ALP were markedly increased in the serum of albino rats following exposure to CCl4 and rifampicin. This indicates that both CCl4 and rifampicin triggered an increased production of ROS in excess of the endogenous antioxidant capacity to mop-up, thereby eliciting organ damage. However, treatment with *P. amarus* leaves extract caused a dose-dependent restoration of ALT, ALP and AST in fashion similar to the negative control animals and those treated with silymarin. This agrees with the report of Surya et al. (2011). Phytochemical screening of *P. amarus* has shown that it is rich in flavonoids, alkaloids, major lignans, hydrolysable tannins and polyphenols (Kassuya et al., 2005; Morton, 1981; Srivastava et al., 2008; Chevallier, 2000; Maciel et al., 2007; Singh et al., 2009; Huang et al., 2003). Earlier reports have shown that phyllanthin, a major lignan present in abundance in *P. amarus* leaves is responsible for attenuation of liver toxicity (Chirdchupunseree and Pramyothin, 2010; Krithika and...
Trig. (mg/dL) Liver 31.33

GSH (nmol/ml) Liver 27.98

T. chol (mg/dL) Liver 56.08

- positive controls (PCI and II); C* and d* represent animals treated with 50 mg/kg bw and 100 mg/kg bw of NC- negative control; PA-

antioxidant capacity of P. amarus

is responsible for organ damage (Arun and Balasubramanian, 2011; Lodhi et al., 2018). Malondialdehyde (MDA) produced during lipid peroxidation is responsible for the production of superoxide anion, in excess of the antioxidant capacity of the liver, kidney and serum of albino rats separately exposed to CCl4 and rifampicin toxicity.

- Reduced glutathione is an intracellular non-protein thiol that coordinates the biochemistry of antioxidants defense. It is the major source of cellular reducing power, acting as free radicals scavenger, thereby arresting oxidative stress. It is also the model substrate for major enzymes of phase II detoxification of xenobiotics. Routinely, level of GSH has been used to predict the health status of an organism. The present study indicates that, administration of CCl4 and rifampicin caused a significant depletion of GSH in the serum and tissue homogenates. This is an indication that the two compounds (CCl4 and rifampicin) at the stated doses generated enough ROS that overwhelmed the endogenous antioxidant capacity of the animals. However, treatment with P. amarus extract markedly raised intracellular GSH concentration, thereby boosting the biochemical antioxidant pool of the cell. Generally, flavonoids had been reported to increase the concentration of reduced glutathione in the intracellular milieu via an up-regulation of glutamylcysteine synthetase, the rate limiting enzyme in the synthesis of GSH (Moskaug et al., 2005; Myhrstad et al., 2002). P. amarus is rich in flavonoids (Foo, 1993a; Foo and Wong, 1992; Londhe et al., 2008; Wongnawa et al., 2005; Morton, 1981), hence, its ability to boost the antioxidant capacity of experimental animals viz-a-viz GSH synthesis is due to the presence of its flavonoids (Chirdbunpares and Praminyoth, 2010).

- Superoxide dismutase has been suggested as the most vulnerable hepatic antioxidant enzyme in the event of an oxidative attack on the liver (Kharpate et al., 2007). It is the enzyme responsible for the conversion of ROS to H2O2 and eventually to water. Exposure to CCl4 and rifampicin caused a marked depletion in GSH relative to the negative control animals, suggesting toxicity. This marked depletion is traceable to the production of superoxide anion, in excess of the antioxidant capacity of the experimental animals (Mukherjee, 2002). However, treatment with P. amarus extract restored SOD activity to a level comparable to the production of superoxide anion, in excess of the antioxidant capacity of the experimental animals (Mukherjee, 2002). However, treatment with P. amarus extract restored SOD activity to a level comparable

Table 2. Effect of P. amarus leaf extract on lipid profile and other antioxidant parameter in the liver, kidney and serum of albino rats separately exposed to CCl4 and rifampicin toxicity.

| Parameters   | T. chol (mg/dL) Liver | Kidney | Serum | Trig. (mg/dL) Liver |
|--------------|----------------------|--------|-------|---------------------|
| Liver        | 56.08±2.09a          | 30.07±2.18a | 52.16±2.19a |
| Kidney       | 41.86±2.09a          | 32.64±1.10b | 63.04±1.23b |
| Serum        | 60.27±1.12c          | 28.39±1.06b | 39.33±1.23b |

Data shows mean ± SEM values of four independent experiments performed in triplicate. ‘a’, ‘c’, ‘d’ and ‘e’ represent significant difference (p<0.05) from (b*) and (b”) - positive controls (PCI and II); C* and d* represent animals treated with 50 mg/kg bw and 100 mg/kg bw of NC- negative control; PA-

antioxidant capacity of P. amarus

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with the negative control and silymarin treated animals. This can be attributed to *Phyllanthus amarus*-triggered up-regulation in the synthesis of mitochondrial SOD via enhanced transcription of its DNA.

Catalase acts by decomposing H$_2$O$_2$ to water and oxygen thereby preventing its accumulation in the cell. In the present study, exposure to rifampicin and CCl$_4$ resulted in a marked decrease in catalase activity both in the serum and tissue homogenates of albino rats. Depletion in catalase activity as a result of exposure to both toxicants is traceable to a decrease in NADPH or increased lipid peroxidation or both (Arun and Balasubramanian, 2011). However, treatment with *Phyllanthus amarus* significantly restored activity of catalase both in the serum and tissue homogenates of albino rats. Depletion in catalase can be linked to *Phyllanthus amarus* triggered increase in HDL-C which is responsible for the transport of cholesterol to the liver (Der Gaag et al., 2001). On the other hand, administration of CCl$_4$ and rifampicin respectively triggered a marked increase in LDL-C level of experimental rats. Treatment with *Phyllanthus amarus* extract attenuated the LDL-C level and reversed it to level comparable with animals treated with the standard drug. The ameliorative effect of *Phyllanthus amarus* on the deranged lipid profile can be attributed to the polyphenols and flavonoids in the extract. Flavonoids have been reported to modulate the metabolism of lipid by inhibiting acyl coenzyme A: cholesterol O-acetyltransferase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in albino rats (El-Newary et al., 2016; Kurowska et al., 2000). Specifically, quercetin which is abundant in *Phyllanthus amarus* extract, has been reported to ameliorate hyperlipidemia, by enhancing lipolysis and up-regulation of beta oxidation pathway at the level of gene transcription (Abbass, 2011).

Selected markers of organ toxicity such as urea, uric acid and total bilirubin were significantly raised when animals were exposed to rifampicin and CCl$_4$ relative to the negative control animals. Treatment with *Phyllanthus amarus* restored the urea and uric acid levels to levels comparable with the negative control and those treated with silymarin. Histopathological examination of kidney and liver slices of experimental animals established the medicinal relevance of *Phyllanthus amarus* in the management of toxicant-induced hepatotoxicity and nephrotoxicity. Pramyothin et al. (2007) reported the beneficial role of *Phyllanthus amarus* in ethanol-induced model of hepatotoxicity. In line with this finding, treatment with *Phyllanthus amarus* histopathological observation of the liver and kidney slices showed a significant restoration of histoarchitecture of the liver and kidney cells.

In conclusion, *Phyllanthus amarus* leaf extract curtailed the toxic effects of CCl$_4$ and rifampicin on the liver and kidney respectively. Histopathological examination established the ameliorative potentials of *Phyllanthus amarus* leaf extract as reflected in the restoration of both liver and kidney histoarchitecture that were initially distorted by the toxicants. Liver and kidney function biomarkers which were grossly altered following exposure to the toxicants, were also restored dose-dependently in a manner comparable to animals treated with silymarin. Hence, *Phyllanthus amarus* has potentials that can be exploited in the management of liver and kidney diseases.

**Declarations**

**Author Contribution statement**

T. Ogunmoyole: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

M. Awodooju, S. Idowu and O. Daramola: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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**Data availability statement**

Data will be made available on request.

**Declaration of interests statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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