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Research article

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 aliments in folkloric medicine. The present study therefore investigates the restorative potential of its leaves extract on hepatic and renal assault induced by CCl_4 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 CCl_4 only. Groups III, IV and V animals were administered 3 ml/kg b/w of CCl_4 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, malonidialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) as well as lipid profile were also measured. Results showed that exposure to rifampicin and CCl_4 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 CCl_4 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 wreck 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), antimalarial (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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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*

Akojenu, 2000) as well as in the treatment of several skin infections (Adeneye et al., 2006; Kassuya et al., 2005). It possesses hepatoprotective (Toyin et al., 2008; Raphael et al., 2002b; Kumar and Kuttan, 2005) anti-carcinogenic (Rajeshkumar et al., 2002), anti-inflammatory (Kiemer et al., 2003), antiasthmatic (Oluwafemi and Debiri, 2008; Idowu et al., 2009) and antidiabetic (Raphael et al., 2002) properties. Considering the vast medicinal relevance of the plant coupled with the challenge of an ever-increasing burden of liver and kidney diseases in developing nations, such as Nigeria, there is a dire need to evaluate the medicinal potentials of *P. amarus* leaves that can be exploited as a viable, cheap and efficient alternative to conventional drugs currently used in the management of renal and hepatic diseases.

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 discarded. The crude extract was obtained from the filtrate by freeze-drying, weighed and kept in an airtight container in the refrigerator. Subsequently, the crude extract was reconstituted with appropriate volume of distilled water and administered to experimental animals.

2.2. Reagents and chemicals

Adrenaline, malonidialdehyde (MDA), phosphotungistic 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 accomondated in separate cages made of iron- meshed at ambient temperature (24 ± 1 °C), 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 °C 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 °C. 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 diphosphate, 10 mM imidazole pH 6.6, 20 mM glucose, 30 mM creatine phosphate, 20 mM N-acetyl-cysteine, 2.0 mM ethylene diaminetetraacetate, 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 °C. 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-oxoglutarate (2 mM) and L-aspartate (100 mM). The resulting mixture was incubated at 37 °C 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 °C. Five milliliter 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-oxoglutarate 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 °C. Five hundred microliter of reagent 2 (R2) made up of 0.02 M 2, 4-dinitrophenylhydrazine was then added and the resulting solution re-incubated for 20 min at 20 °C. Finally, 5.0 ml of NaOH was added to the reaction mixture and left to stand for 5 min at 25 °C. Absorbance of the resulting solution was then read at 546 nm.

2.8. Assay for akaline 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 MgCl₂, 1.0 M diethanolamine buffer pH 9.8,
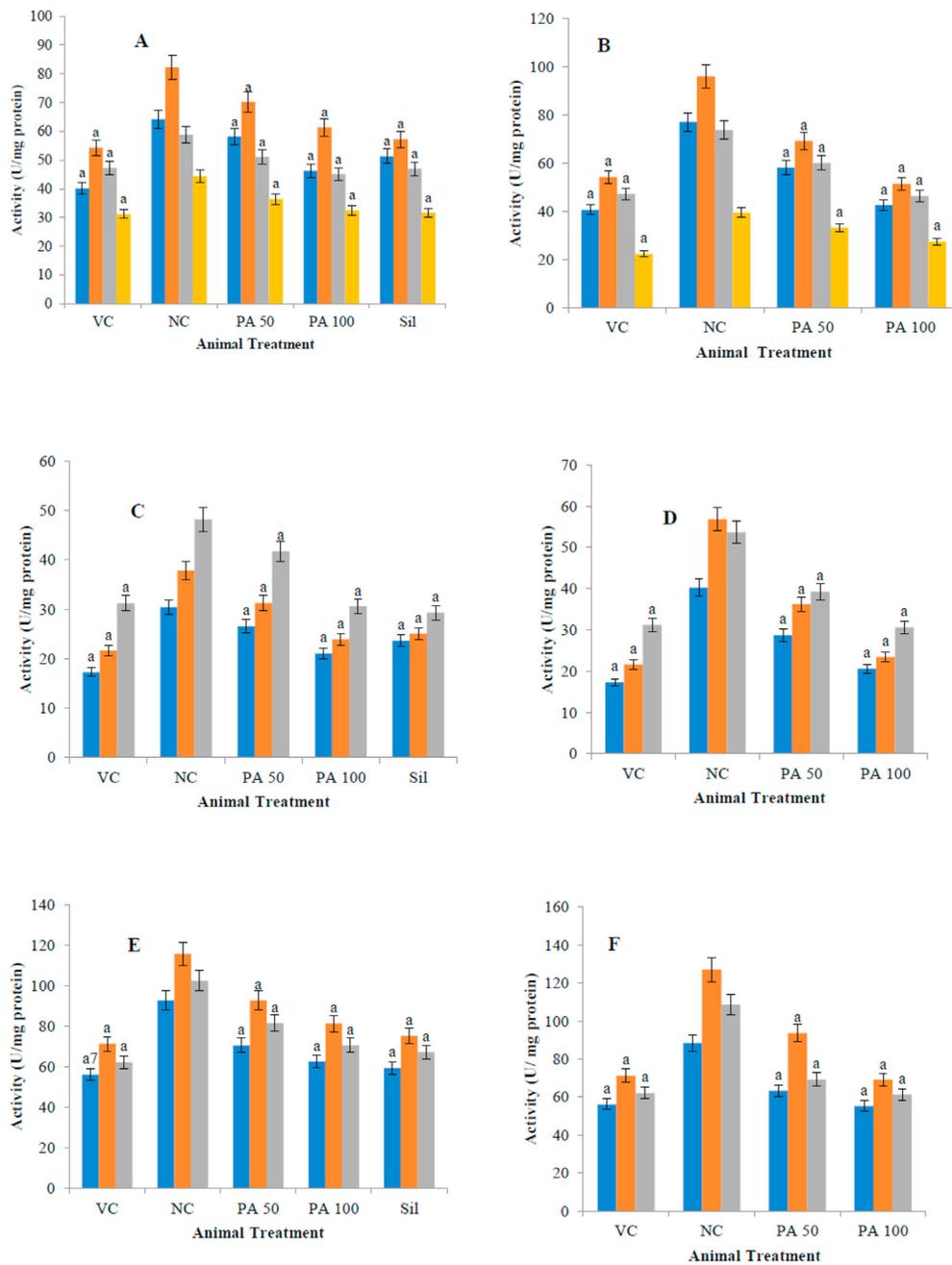
Figure 1. Effect of P. amarus leaf extract on ALT, AST, ALP and total bilirubin of albino rats exposed to CCl4 and rifampicin toxicity. Data represents mean ± SEM values of four independent experiments performed in triplicate. 'a' represents significant difference (P<0.05) from the positive control - (NC); VC - negative control; PA - P. amarus; Sil - Silymarin. Rif – Rifampicin. ALP- ALT- AST- T.BIL- . A and B are liver of CCl4 and rifampicin - challenged rats respectively. C and D are kidney of CCl4 and rifampicin - challenged rats respectively; E and F are serum of CCL4 and rifampicin - challenged rats respectively.

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 (1)

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X Concentration of standard}$$

(1)

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 was 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
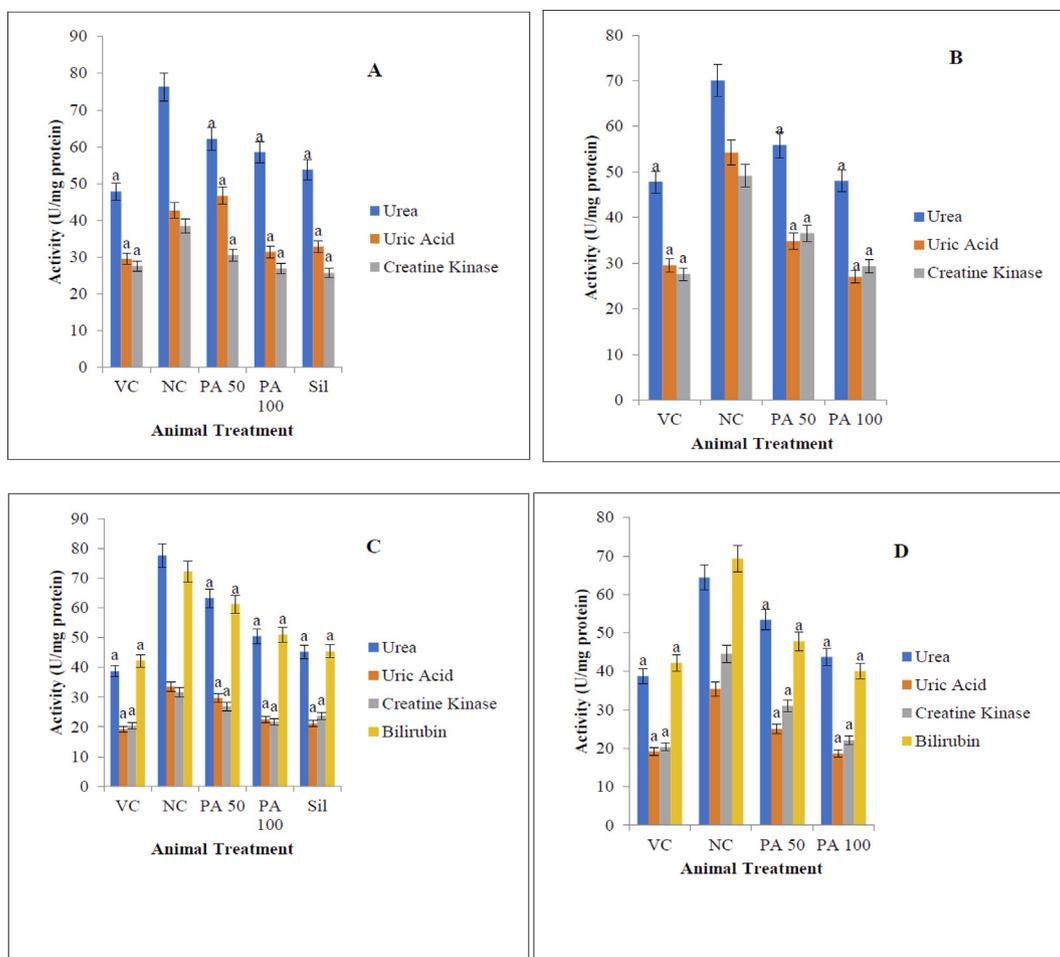
Figure 2. Effect of *P. amarus* leaf extract on selected biomarkers of albino rats exposed to CCl₄ and rifampicin toxicity. Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a' represents significant difference (p<0.05) from the positive control - (NC); VC - negative 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.

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)

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 $_{sample}$) and standard (A$_{standard}$) was then read within 1 h at 500 nm against the reagent blank.

$$\text{Triglyceride concentration (mg / dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{X Concentration of s tandard} \qquad (2)$$

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

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).

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

$$(3)$$

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
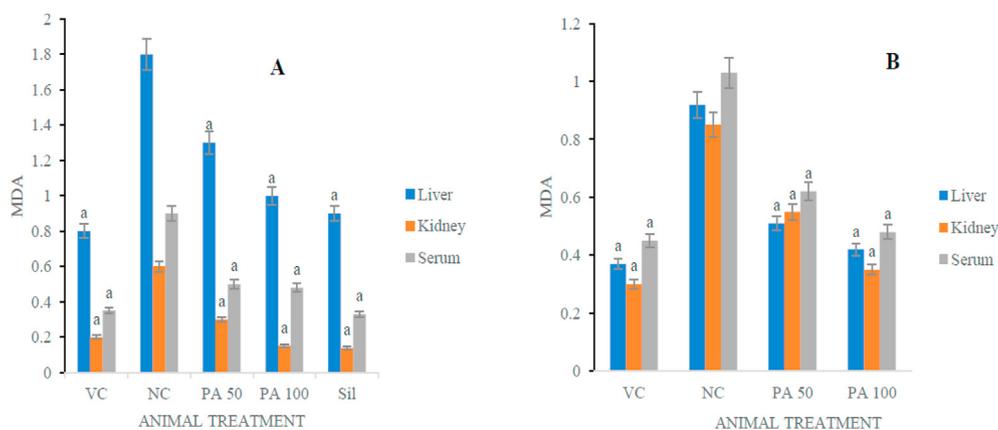
Figure 3. Effect of *P. amarus* leaf extract on lipid peroxidation in albino rats exposed to CCl₄ and rifampicin toxicity. Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a' represents significant difference (p < 0.05) from the positive control - (NC); PC- negative control; PA- *P. amarus*; Sil- Silymarin. A and B represent animals challenged with CCl₄ and rifampicin 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/acetic acid reagent at 60 s intervals. Catalase activity was estimated as the amount of hydrogen peroxide consumed per minute per mg protein (4).

$$H_2O_2 \text{ consumed} = 800 - \text{Concentration of } H_2O_2 \text{ remaining} \qquad (4)$$

Concentration of H_2O_2 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)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. Malonidialdehyde (MDA) level in the supernatant was expressed as µmole MDA per mg protein taking the molar absorptivity of MDA-thiobarbituric chromophore as 1.56×10^5/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 Winsten 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
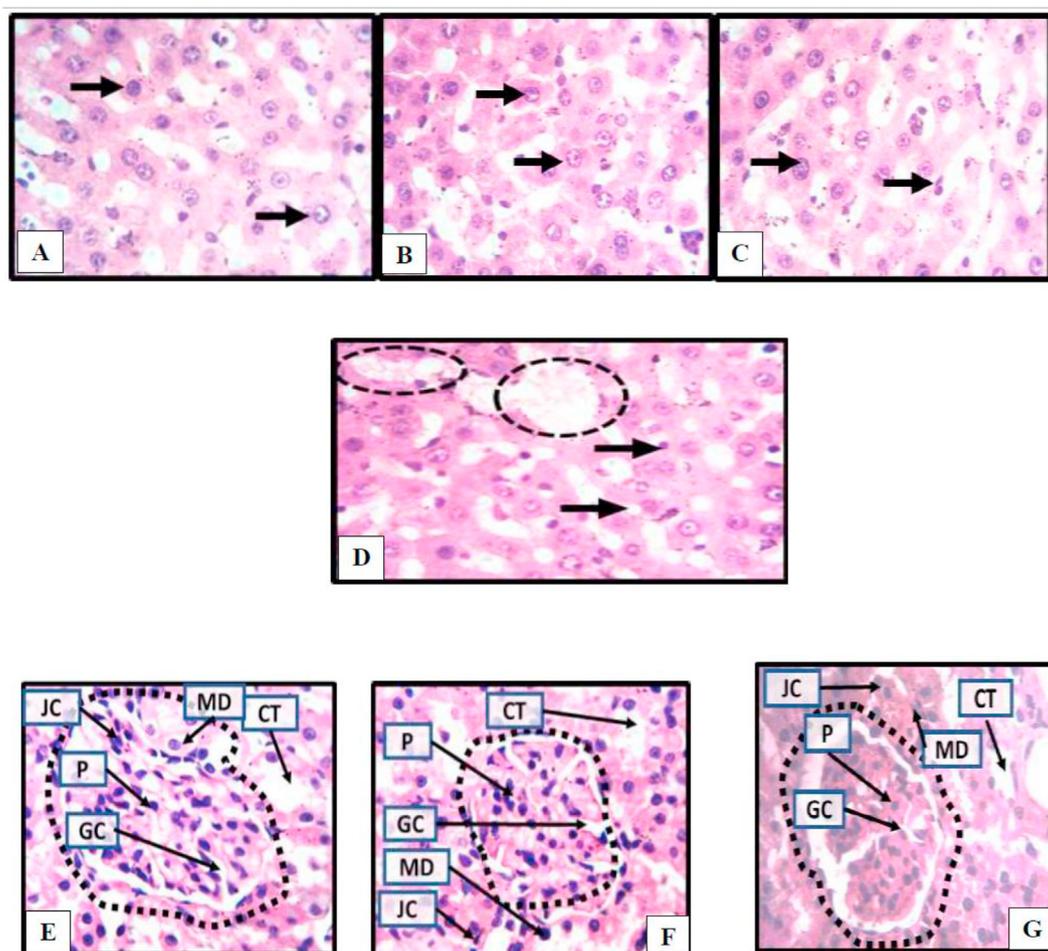
Figure 4. Photomicrograph of the liver Fig. (A–D) and kidney Fig. E–G) of albino rat under different experimental conditions. *A- liver photomicrograph of negative control animals (fed with normal feed and distilled water only):* Histomorphology of the hepatic tissue normal with distinct hepatocytic nuclei well positioned in their cytoplasmic space. There is no observable histopathological alteration in the liver tissues. *B- liver photomicrograph of animals exposed to with* 3 ml/kg CCl_4: Cholestatic fatty liver was observed, with distorted hepatic histoarchitecture. *C- liver photomicrograph of animals exposed to* 3 ml/kg CCl_4 *and treated with* 100 mg/kg *P. amarus:* Normal liver histoarchitecture was noticed. There are no histopathological alterations in the histological presentation of the hepatic tissues. *D - liver photomicrograph of animals exposed to* 3 ml/kg CCl_4 *and treated with* 100 mg/kg *Silymarin:* The nuclei of hepatocytes are well stained and properly positioned in the cytoplasm. There are no histopathological alterations in the histological presentation of the liver tissues. *E - kidney photomicrograph of animals exposed to distilled water only:* There renal corpuscle was normal, with appropriate cellular delineation, distribution, density and staining intensity. No apparent histopathological alteration was observed. *F - kidney photomicrograph of animals administered with* 250 mg/kg *rifampicin only:* Normal renal histoarchitecture was distorted with glomerular atrophy. Renal tubular degeneration coupled with intraluminal exfoliation and granular cast formation as well as pyknosis of the nuclei were observed. *G- kidney photomicrograph of animals exposed to* 250 mg/kg *rifampicin and treated with* 100 mg/kg bw of *P. amarus:* Normal renal corpuscle with typical cellular delineation, distribution, density and staining intensity. No apparent histopathological alteration. *CT - Convoluted tubule, GC- glomerular capillaries, P- podocytes, JC- juxtaglomerular cells MD- macula densa cells.*

kidney (creatine kinase, urea and uric acid) (Figure 2) were significantly raised in the animals administered with CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 and rifampicin. This indicates that both CCl_4 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

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	Tissues	NC (distilled water only)	PC I (CCl$_4$ Only)	CCl$_4$ + (50mg/kg PA)	CCl$_4$+ (100mg/kg PA)	CCl$_4$+ (100mg/kg Silymarin)	PC II (Rif. Only)	Rif + (50mg/kg PA)	Rif + (100mg/kg PA)
T. chol (mg/dL)	Liver	56.08±2.09[a]	89.12±1.73[b]	67.33±2.07[c]	60.27±1.12[d]	52.33±1.34[e]	97.23±1.42[b*]	65.14±1.06[c*]	56.15±1.06[d*]
	Kidney	30.07±2.18[a]	41.86±2.09[b]	32.64±1.10[c]	28.39±1.06[d]	38.63±2.23[e]	56.71±1.18[b*]	42.15±1.17[c*]	26.29±1.20[d*]
	Serum	52.16±2.19[a]	72.44±1.86[b]	63.04±1.23[c]	57.69±1.63[d]	58.27±1.56[e]	87.46±1.38[b*]	68.44±1.20[c*]	55.36±1.11[d*]
Trig. (mg/dL)	Liver	31.33±1.07[a]	70.25±1.24[b]	52.37±1.06[c]	43.53±1.23[d]	39.43±1.13[e]	86.50±0.42[b*]	60.33±1.22[c*]	42.67±1.03[d*]
	Kidney	11.82±0.34[a]	19.47±0.42[b]	16.49±0.75[c]	12.68±0.34[d]	13.43±0.29[e]	38.68±1.18[b*]	31.55±1.25[c*]	24.58±1.21[d*]
	Serum	37.51±1.39[a]	61.41±1.28[b]	52.56±1.11[d]	44.38±1.48[d]	40.14±1.72[e]	70.39±1.45[b*]	56.84±1.00[c*]	34.39±1.06[d*]
HDL (mg/dL)	Liver	24.72±0.44[a]	46.07±0.53[b]	35.36±0.96[c]	25.31±0.74[d]	26.31±0.59[e]	18.26±0.24[b*]	22.26±0.46[c*]	25.46±0.61[d*]
	Kidney	8.26±0.17[a]	5.31±0.87[b]	6.32±0.59[c]	7.86±0.54[d]	8.42±0.68[e]	3.07±0.02[b*]	4.06±0.08[c*]	4.59±0.01[d*]
	Serum	9.42±0.31[a]	6.37±0.43[b]	7.04±0.22[c]	7.93±0.17[d]	8.79±0.29[e]	5.10±0.07[b*]	6.38±0.07[c*]	7.20±0.95[d*]
LDL (mg/dL)	Liver	33.76±1.27[a]	41.27±1.53[b]	35.18±1.15[c]	29.80±0.88[d]	33.04±0.08[e]	57.81±0.11[b*]	48.17±0.45[c*]	42.55±0.38[d*]
	Kidney	13.26±0.73[a]	21.60±0.82[b]	17.54±0.42[c]	14.83±0.36[d]	12.13±0.48[e]	22.74±0.27[b*]	17.28±0.15[c*]	14.32±0.12[d*]
	Serum	22.36±0.32[a]	34.26±0.53[b]	28.47±0.21[c]	23.08±0.41[d]	24.67±0.72[e]	39.53±0.68[b*]	33.29±0.76[c*]	27.42±0.61[d*]
SOD (µmol /min)	Liver	6.14±0.09[a]	4.53±0.73[b]	5.12±0.07[c]	6.07±0.12[d]	6.20±0.34[e]	2.36±0.04[b*]	4.19±0.06[c*]	5.26±0.16[d*]
	Kidney	3.84±0.18[a]	2.46±0.09[b]	2.89±0.10[c]	3.17±0.06[d]	3.15±2.23[e]	1.93±0.18[b*]	2.41±0.17[c*]	2.84±0.20[d*]
	Serum	4.29±0.19[a]	3.17±0.16[b]	3.55±0.23[c]	4.24±0.63[d]	4.34±0.15[e]	2.42±0.13[b*]	3.93±0.20[c*]	4.62±0.14[d*]
CAT (µmol /min)	Liver	4.70±0.07[a]	2.89±0.24[b]	3.82±0.06[c]	4.14±0.23[d]	4.45±0.13[e]	1.63±0.42[b*]	1.93±0.22[c*]	4.07±0.03[d*]
	Kidney	2.77±0.14[a]	2.02±0.12[b]	2.39±0.15[c]	2.61±0.14[d]	2.58±0.19[e]	1.88±0.18[b*]	2.21±0.25[c*]	2.68±0.21[d*]
	Serum	3.06±0.13[a]	2.38±0.28[b]	2.89±0.11[d]	2.86±0.18[d]	2.93±0.12[e]	1.76±0.14[b*]	2.97±0.10[c*]	3.10±0.06[d*]
GSH (µmol)	Liver	5.77±0.27[a]	3.87±0.13[b]	5.41±0.15[c]	5.81±0.18[d]	5.43±0.08[e]	2.93±0.11[b*]	3.86±0.15[c*]	5.14±0.18[d*]
	Kidney	3.23±0.13[a]	2.53±0.12[b]	3.08±0.12[c]	3.15±0.16[d]	3.19±0.15[e]	3.04±0.27[b*]	3.10±0.15[c*]	3.17±0.12[d*]
	Serum	2.66±0.32[a]	1.57±0.13[b]	1.90±0.21[c]	2.23±0.11[d]	2.49±0.17[e]	2.08±0.18[b*]	2.28±0.16[c*]	2.37±0.11[d*]
T. Protein (mg/mL)	Liver	2.64±0.08[a]	1.47±0.09[b]	2.07±0.10[c]	2.25±0.06[d]	2.33±0.03[e]	1.04±0.08[b*]	1.66±10.07[c*]	2.38±0.20[d*]
	Kidney	1.73±0.10[a]	0.76±0.07[b]	0.97±0.03[c]	1.23±0.02[d]	1.44±0.05[e]	1.06±0.13[b*]	1.20±0.12[c*]	1.47±0.11[d*]
	Serum	2.08±0.13[a]	1.26±0.05[b]	1.74±0.13[c]	1.95±0.12[d]	2.17±0.15[e]	1.02±0.31[b*]	1.42±0.20[c*]	1.91±0.11[d*]

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 PA respectively after initial exposure to rifampicin toxicity; NC- negative control; PA- *P. amarus;* Rif- rifampicin.

Verma, 2009a,b; Yadav et al., 2008). In line with these reports, the restorative potential of *P. amarus* leaf extract used in the present study is attributable to phyllanthin. Noteworthy is the fact, that there was no significant difference in therapeutic efficiency between *P. amarus* extract at 100 mg/kg bw and silymarin at the same dose. This finding is in line with Igwe et al. (2007) who reported the restorative potential of methanolic extract of *P. amarus* (whole plant) in chemically induced hepatotoxicity.

Exposure to CCl$_4$ and rifampicin resulted in an elevated lipid peroxidation, indicating that the major mechanism of toxicity of these compounds is via lipid peroxidation. Obviously, when free radicals are produced at a level beyond the antioxidant capacity of the system, they constitute a major threat to health. By virtue of their chemistry, free radicals are highly reactive because of an unpaired electron in the shell. In an attempt to gain stability, free radicals abstract protons from chemically stable macromolecules such as proteins, carbohydrates, nucleic acids and lipids, wrecking havocs. Polyunsaturated lipids, a major component of the biomembrane are more susceptible to ROS. Hence, an oxidative attack on membrane lipids could result in compromise of critical membrane functions. It has been suggested that malondialdehyde (MDA) produced during lipid peroxidation is responsible for organ damage (Arun and Balasubramanian, 2011; Lodhi et al., 2014; Das and Vasudevan, 2006). The present study established that, administration of *P. amarus* markedly restored the antioxidant capacity by inhibiting lipid peroxidation. Nikam et al. (2011) reported the antioxidant capacity of *P. amarus* to inhibit lipid peroxidation in ethanol-induced liver damage and attributed same to its high polyphenolic content. In line with this finding, inhibition of lipid peroxidation by *P. amarus* leaves extract is due to the presence of a wide array of polyphenols.

Reduced glutathione is an intracellular non-protein thiol that co-ordinates 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 CCl$_4$ and rifampicin caused a significant depletion of GSH in the serum and tissue homogenates. This is an indication that the two compounds (CCl$_4$ 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 milleu 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 (Chirdchupunseree and Pramyothin, 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 H$_2$O$_2$ and eventually to water. Exposure to CCl$_4$ 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

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

Catalase acts by decomposing H_2O_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 *P. amarus* significantly restored activity of catalase both in the serum and tissue homogenates by increasing the level of NADPH and inhibiting lipid peroxidation as earlier reported (Nazeema and Brindha, 2009).

Administration of CCl_4 and rifampicin respectively caused a marked derangement in lipid profile both in the tissue homogenates and serum and of albino rats. Specifically, total cholesterol level was markedly increased relative to negative control. This increment can be attributed to the activation of HMG-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis (Pari et al., 2015). However, when treated with *P. amarus,* total cholesterol in the liver and kidney as well as serum markedly reduced to a level comparable with animals treated with standard drug. This decrease in cholesterol can be linked to *P. 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 *P. amarus* extract attenuated the LDL-C level and reversed it to level comparable with animals treated with the standard drug. The ameliorative effect of *P. 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 the inhibiting acyl coenzyme A: cholesterol O-acyltransferase 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 *P. amarus* extract, has been reported to ameliorate hyperlipidemia, by enhancing lipolysis and upregulation 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 *P. 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 *P. amarus* in the management of toxicant-induced hepatotoxicity and nephrotoxicity. Pramyothin et al. (2007) reported the beneficial role of *P. amarus* in ethanol-induced model of hepatotoxicity. In line with this finding, treatment with *P. amarus* histopathological observation of the liver and kidney slices showed a significant restoration of histoarchitecture of the liver and kidney cells.

In conclusion, *P. 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 *P. 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, *P. 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.

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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