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Research Article
Ameliorative Potential of Methanolic Extract of *Persea americana* Leaves on Carbon Tetrachloride Induced Hepatic and Renal Injury in Albino Rat

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Abstract

Background and Objective: *Persea americana* has been exploited in the management of several ailments in folkloric medicine. The present study, therefore, sought to investigate the medicinal potential of its leaves in the treatment of liver and kidney diseases. This was aimed at providing a cheap but potent alternative in the management of these diseases. **Materials and Methods:** Rats were randomly placed into five groups and exposed to 3 mL kg^{-1} b.wt., of CCl_4 except for group I which served as the control. Exposed animals were treated with graded doses of *P. americana* and silymarin except for group II animals that were left untreated for fourteen days. Biomarkers of liver and kidney functions such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) as well as bilirubin, creatinine and urea were determined in the serum and organs homogenates. Moreover, serum levels of selected inflammatory markers, malondialdehyde (MDA), lactate dehydrogenase and antioxidant enzymes were also measured. **Results:** Treatment with methanolic extract of *P. americana* leaves attenuated the toxicity imposed by CCl_4 in a dose-dependent manner as reflected in the restoration of all deranged biochemical parameters including the distorted histoarchitecture of hepatic, renal and cardiac tissues of experimental animals. **Conclusion:** Considering the avalanche of antioxidant phytochemicals present in the methanolic extract of *P. americana* leaves, coupled with biochemical and histopathological observations, the plant is a highly promising therapeutic alternative that can be exploited for the treatment of renal, hepatic diseases.

Key words: *Persea americana*, liver, kidney, carbon tetrachloride, renal, hepatic diseases

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The plant kingdom largely contributes to human health before the advent of synthetic medicines[1]. Plants have been used as important ingredients in folkloric medicine and drug formulations in pharmacy. The use of medicinal plants in the treatment of diseases has come a long way and appears to be prominent, especially in developing economies[2-4]. It has been suggested that only about 30% of human diseases have reliable treatment, plants with proven efficacy have been used to augment both physical and mental health[5], Perhaps, this might explain the rationale behind the astronomical rise in search of pharmacologically active principles from plants that can serve as a therapeutic alternative in the treatment of diseases[6].

Avocado (*Persea americana*) belongs to the family of Lauraceae family. Reports indicate that *P. americana* is rich in antioxidant vitamins and essential nutrients[7,8]. Specifically, the leaf extract of avocado contains a high level of potassium and glutathione which may explain its role in the regulation of blood pressure and free radicals scavengers respectively[9,10]. Several medicinal benefits of avocado have been reported. Moreover, avocado is rich in antioxidant phytochemicals including phytosterols and the β-sitosterol which are responsible for its antihyperlipidemic properties[11-13]. Its leaves extract has been reported to possess analgesic, anti-hypertensive, antidiabetic, anti-convulsant, anti-ischemic and anti-inflammatory potentials[14-17]. Most importantly the lethal dosage of avocado extract is very high attesting to its safety when used in medication[18]. Besides, aqueous extracts of *P. americana* leaves have shown potent vasorelaxant, antiulcerogenic and anti-diarrheal properties in experimental animals[19]. Avocado oil has demonstrated effective antibiotic action against fungal and bacterial infections[20].

Despite the enormity of research works on the health benefits of the fruit and seed of avocado, there are relatively low reports on the medicinal potentials of its leaves. Hence, there is a dire need to investigate and possibly explore the therapeutic potentials of its leaves in the management of liver, kidney and heart-related diseases which are fast becoming a global issue of public health concern.

MATERIALS AND METHODS

Study area: The present study lasted for four months (February to May, 2021) and was carried out in Ado-Ekiti, Ekiti State, Nigeria.

Plant extraction: Leaves of *P. americana* were harvested from a farm in Ado Ekiti and authenticated at the Department of Plant Science, Ekiti State University, Ado Ekiti with voucher number UHAE2020071 deposited at the university herbarium. Fresh leaves of *P. americana* were rinsed with distilled water and air-dried. The pulverized sample was weighed and stored airtight in a container. Forty percent homogenate of the powdered leaves was obtained by extraction with 80% (v/v) methanol for 72 hrs to obtain a clear supernatant which was carefully decanted. The supernatant was freeze-dried, weighed, labelled as crude extract and kept airtight in the refrigerator. The crude extract was then reconstituted with distilled water for animal treatment.

Animals experiment: Experimental animals were used following established guidelines (Revised NIH Publications 1978, No. 8023) while ethical approval was obtained from the Committee on Care and Use of Experimental Animal Resources, Office of Research and Development, Ekiti State University, Ado Ekiti, Nigeria. Twenty Wistar albino rats with a mean weight of 170 g were purchased from the animal breeding colony of the College of Medicine, Ekiti State University, Ado-Ekiti. Five groups of four animals per group were created. Experimental animals were housed in separate cages made of iron- mesh at temperature (24±1°C), relative humidity and 12/12 hrs light and dark cycle. Animals were allowed unrestricted daily access to food and drinking water *ad libitum*. Throughout the experimental period, rat beddings were routinely turned over daily to maintain good hygiene.

Animal treatment

Groups	Treatment
I	Negative control (PC), distilled water only
II	Positive control (NC), 3 mL CCl_4 single administration
III	3 mL CCl_4+200 mg kg^{-1} b.wt., *P. americana*
IV	3 mL CCl_4+400 mg kg^{-1} b.wt., *P. americana*
V	3 mL CCl_4+200 mg kg^{-1} b.wt., *Silymarin*

Dissection of rats: Decapitation of animals was done under very mild cold ether anaesthesia and rapidly dissected to excise the liver, kidney and heart which were separately trimmed of fat. The tissues were washed in distilled water, 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, supernatants were decanted and kept chilled by refrigeration. Whole blood was obtained by cardiac puncture into an EDTA bottle and left to stand for 1 hr 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.

Phytochemical screening: Methanolic extract of *P. americana* leaf was subjected to preliminary phytochemical screening to test for the presence of flavonoids, saponins, terpenoids, tannins and cardiac glycosides following established protocols.

Determination of biochemical parameters

Measurement of aspartate aminotransferase (AST) activity: The activity of aspartate aminotransferase (AST) in the serum and tissue homogenates was assayed[21]. Briefly, 0.1 mL of homogenates and serum was added in separate test tubes to phosphate buffer (100 mM, pH 7.4), α-oxoglutarate (2 mM) and L-aspartate (100 mM). The mixture was incubated for 30 min at 37°C after which 0.5 mL of 2,4-dinitrophenylhydrazine (0.02 M) was added and left for 20 min at 25°C. Thereafter, 5.0 mL of 0.4 M NaOH was added to the reaction mixture. The mixture was left to stand for 5 min and its absorbance read at 546 nm against the reagent blank.

Measurement of alanine aminotransferase (ALT) activity: Alanine aminotransferase was assayed as described by Ogunmoyole *et al.*[21]. Briefly, 0.5 mL of reagent 1 (R1) containing 100 mM phosphate buffer pH 7.4, 2.0 M α-oxoglutarate and 0.2 M L-alanine were added to 0.1 mL of serum and tissues homogenates and the mixture incubated for 30 min at 37°C. Thereafter, 0.5 mL of reagent 2 (R2) containing 0.02 M 2,4-dinitrophenylhydrazine was added and the resulting mixture and re-incubated for 20 min at 20°C. Finally, the reaction mixture was left to stand for 5 min at 25°C after the addition of 5.0 ml NaOH. The absorbance of the resulting solution was then read at 546 nm.

Assay for alkaline phosphatase activity: Alkaline phosphatase (ALP) was assayed[22], which employs the use of ρ-nitrophenyl phosphate as a substrate. In this method, the amount of phosphate ester that is split within a given period is a measure of the phosphatase enzyme activity. ρ-Nitrophenyl phosphate (ρ-NPP) is hydrolyzed to p-nitrophenol and phosphoric acid at a pH 10.1. The yellow colour of the reaction mixture is read at 400 nm as a measure of the enzyme activity.

Assay for catalase (CAT): Catalase activity in the liver, kidney, heart and serum of experimental animals was assayed[23]. Two hundred microliters each of five-fold dilution of serum, kidney and liver homogenates serum was measured into a reaction mixture of 2 mL of (800 mmol) hydrogen peroxide and 2.5 mL of potassium phosphate buffer. Thereafter, 0.5 mL of appropriate enzyme dilution was quickly introduced to the mixture and agitated by gentle swirling in a flat bottom flask at 25°C. One millilitre aliquot of the reaction mixture was then withdrawn and blown into a 1 mL dichromate/acetic acid reagent at 60 sec intervals. Catalase activity was estimated as the amount of hydrogen peroxide consumed per minute per mg protein:

$$H_2O_2 \text{ consumed} = 800\text{-Concentration of } H_2O_2 \text{ remaining}$$

The concentration of H_2O_2 remaining was extrapolated from the standard curve for catalase activity.

Assay for glutathione peroxidase (GPx) activity: GPx activities in the sample were determined according to the method described by Meghri *et al.*[24]. Five hundred microliter of phosphate buffer was pipetted into two test tubes for labelled reagent and blank. Ten microliters of sodium azide, 0.2 mL of GSH and 0.1 mL of hydrogen peroxide were added to both test tubes. Six hundred microliter of distilled water was added to another test tube containing the extract tube while 0.11mL of distilled water was added to the blank test tube. The reaction mixture was mixed thoroughly and incubated for 3 min, after which TCA was added and centrifuged at 3000 rpm for 5 min. Two milliliters of dipotassium phosphate and 1 mL of DNTB were added to 1 mL of supernatant decanted after centrifugation. The absorbance of the resulting solution was then read at 412 mm against the blank.

Urea determination: Urea level in the serum and tissue homogenates was determined according to the method of Tekin and Seven[25] following instructions obtained from Randox kit, UK.

Creatinine determination: Serum creatinine was measured as described by Tekin and Seven[25] following the instruction obtained from Randox kit, UK.

Determination of cytokines

ELISA assay: Competitive ELISA kit was used to quantify IL-1β and IL-6, while TNF-α was measured using amplification ELISA following the manufacturer's instructions. One hundred microliters of serum was were added to the microplates followed by incubation for 2.5 hrs after which washing

four times using the buffer provided by the manufacturer. In other to measure IL-1β and IL-6, a target-specific antibody (anti-IgG) was used to coat the microplates used before the addition of serum to allow for interaction between the serum peptides and antibody in the microplate. A reagent provided by the manufacturer (TMB Reagent) was then added to each well and the mixture was incubated for 30 min, resulting in the formation of a chromogenic product whose absorbance was read at 450 nm. A similar procedure was followed in the measurement of TNF-α according to the manufacturer's instructions.

The absorbance was directly proportional to the amount of biotinylated peptide-streptavidin-HRP and inversely proportional to the amount of serum target peptide. A standard curve of the inflammatory markers was generated according to manufacturer's instructions (IL-6 = 1.37-1,000 pg mL^{-1}, IL-1β = 0.48-100 pg mL^{-1} and TNF-α = 0.31-20 pg mL^{-1}), while the concentration of each of the markers in the serum was measured according to manufacturer's instruction.

Histopathological examination of tissues: A standard laboratory protocol for paraffin embedding was used to treat the formalin preserved tissues. Tissue sections were cut into a size of 4 mm before fixing on the slides. The slides were deparaffinized in p-xylene and rehydrated in ethanol gradient (100, 80, 70 and 50%) and rinsed with water. Slides were stained in hematoxylin for 5 min and rinsed with water and counterstained in eosin, mounted in DPX, cover-slipped and viewed with Leica slide scanner (SCN 4000, Leica Biosystems, Wetzlar, Germany).

Statistical analysis: All experimental data were expressed as Mean±SEM. Data were analyzed using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 14 for windows. The level of significance was at $p<0.05$.

RESULTS

Table 1 shows the result of preliminary phytochemical screening. Although it was qualitative, it reveals that flavonoid is the most abundant phytochemical methanolic extract of *P. americana* leaves. This was followed by phenols and terpenoids, which were present in moderate amounts while steroids, tannins, phenolics and saponins were present in low amounts. Cardiac glycosides were absent in the extract.

Table 2 shows the effect of *P. americana* leaves on urea and creatinine levels in the serum and kidney homogenates of experimental animals. Exposure to 3 mL kg^{-1} CCl_4 caused an increase in serum urea from 0.19±0.01 in normal animals to 0.90±0.00. Treatment with *P. americana* at 400 mg kg^{-1} b.wt., restored the serum urea to 0.21±0.01, while treatment with silymarin at 100 mg kg^{-1} b.wt., restored the serum urea to 0.15±0.01. Serum creatinine increased from 44.01±1.25-83 after exposure to a toxicant. Treatment with *F. americana* leaves at 400 mg kg^{-1} b.wt., reduced the serum creatinine from 83.34±3.72-43.16±1.12 mg dL^{-1} while silymarin at 100 mg kg^{-1} b.wt., reduced it to 51.04±1.10 mg dL^{-1}. A similar trend was obtained in the kidney homogenate.

Table 3 reveals the effect of *P. americana* leaves extract on glutathione peroxidase (GPx) and catalase (CAT) in serum and selected organs of the albino rat. Results generally indicate that GPx levels which were 73.29±2.60, 64.74±0.70, 76.51±3.84 and 92.17±2.49 in the kidney, heart, liver and serum, respectively in normal animals were reduced to 45.39±1.00, 34.31±0.91, 44.21±1.41 and 39.58±1.31, respectively following exposure to CCl_4. Treatment with *P. americana* at 400 mg kg^{-1} b.wt., restored the activities of the enzyme to 64.53±2.74, 65.21±2.66, 66.45±2.61 and 63.04±1.28 in the kidney heart, liver and serum, respectively relative to exposed animals that were not treated. A similar trend was observed when catalase activity was measured.

Table 4 shows the effect of *P. americana* leaves extract on selected biomarkers of liver injury. It clearly shows that exposure to CCl_4 led to an increase in ALT from 64.13±2.81 and 35.01±0.52 (for normal animals) to 81.51±2.61 and 52.00±1.32 in the serum and liver respectively. Treatment with *P. americana* leaves at 400 mg kg^{-1} b.wt. and silymarin at 100 mg kg^{-1} b.wt., reversed ALT level to 61.61±10.72 and 61.30±2.71, 41.52±1.54 and 41.00±2.36 in the serum and liver, respectively. The same trend was observed for AST, ALP and total bilirubin in the serum and liver.

Table 5 revealed the effect of *P. americana* on lactate dehydrogenase (LDH) and anti-inflammatory markers. It shows that serum LDH was increased from 142.18±2.00 to

Table 1: Preliminary phytochemical screening of methanolic extract of *Persea americana* leaf

Phytochemicals	Presence
Saponin	+
Flavonoids	+++
Phenolics	+
Terpenoids	++
Tannins	+
Cardiac-glycosides	-
Steroids	+

++: Present in moderate, +++: Present in more quantity and -: Absent

Table 2: Effect *Persea americana* leaf extract on selected kidney parameters in the rat model exposed to CCl_4 toxicity

Parameters	Tissues	Control	CCl_4 only	CCl_4+200 mg of *Persea americana*	CCl_4+400 mg of *Persea americana*	CCl_4+silymarin
Urea mg dL^{-1}	Kidney	0.38±0.01[a]	1.23±0.01[b]	0.49±0.00[a]	0.36±0.01[a]	0.37±0.02[a]
	Serum	0.19±0.01[a]	0.90±0.00[b]	0.52±0.01[a]	0.21±0.01[a]	0.15±0.01[a]
Creatinine mmole L^{-1}	Kidney	71.34±1.74[a]	91.42±2.43[b]	84.17±1.32[a]	75.3±1.54[a]	75.03±2.56[a]
	Serum	44.01±1.25[a]	83.34±3.72[b]	53.23±2.33[a]	43.16±1.12[a]	51.04±1.10[a]

Data represent Mean±SD of two independent experiments performed in triplicate

Table 3: Effect *Persea americana* leaf extract on selected anti-oxidants enzymes in the rat model exposed to CCl_4 toxicity

Parameters	Tissues	Control	CCl_4 only	CCl_4+200 mg of *Persea americana*	CCl_4+400 mg of *Persea americana*	CCl_4+silymarin
GPX U mg^{-1} protein	Kidney	73.29±2.60[a]	45.39±1.00[b]	55.27±3.20[a]	64.53±2.74[a]	74.35±1.82[a]
	Heart	64.74±0.70[a]	34.31±0.91[b]	49.07±2.46[a]	65.21±2.66[a]	55.23±1.34[a]
	Liver	76.51±3.84[a]	44.21±1.41[b]	51.25±1.88[a]	66.45±2.61[a]	54.28±1.05[a]
	Serum	92.17±2.49[a]	39.58±1.31[b]	60.94±1.51[a]	63.04±1.28[a]	55.99±2.18[a]
Catalase U mg^{-1} protein	Kidney	0.46±0.02[a]	0.14±0.03[b]	0.31±0.02[a]	0.47±0.01[a]	0.45±0.00[a]
	Heart	0.24±0.05[a]	0.03±0.01[b]	0.21±0.02[a]	0.24±0.03[a]	0.22±0.02[a]
	Liver	0.21±0.01[a]	0.04±0.00[b]	0.17±0.01[a]	0.22±0.04[a]	0.19±0.02[a]
	Serum	0.20±0.07[a]	0.03±0.00[b]	0.11±0.04[a]	0.21±0.03[a]	0.17±0.01[a]

Data represents Mean±SD of two independent experiments performed in triplicate

Table 4: Effect *Persea americana* leaf extract on selected liver parameters in the rat model exposed to CCl_4 toxicity

Parameters	Tissues	Control	CCl_4 only	CCl_4+200 mg of *Persea americana*	CCl_4+400 mg of *Persea americana*	CCl_4+silymarin
ALT U L^{-1}	Serum	64.13±2.81[a]	81.51±2.61[b]	74.25±2.89[a]	61.61±10.72[a]	61.30±2.71[a]
	Liver	35.01±0.52[a]	52.00±1.32[b]	44.15±1.62[a]	41.52±1.54[a]	41.00±2.36[a]
ALP U L^{-1}	Serum	53.54±2.15[a]	68.14±2.11[b]	55.04±1.80[a]	51.22±0.32[a]	58.40±1.85[a]
	Liver	62.24±2.52[a]	85.15±1.21[b]	73.11±2.61[a]	62.29±1.24[a]	62.63±1.94[a]
AST U L^{-1}	Serum	64.13±2.49[a]	97.01±1.72[b]	68.70±2.26[a]	61.13±2.11[a]	63.13±2.22[a]
	Liver	55.30±1.63[a]	81.72±5.91[b]	69.74±1.49[a]	52.52±0.44[a]	58.13±1.33[a]
T.BIL mg dL^{-1}	Serum	88.38±4.235[a]	112.51±0.84[b]	97.04±3.99[a]	85.54±2.96[a]	87.33±2.64[a]
	Liver	62.23±0.33[a]	92.34±1.29[b]	77.62±2.02[a]	62.05±2.24[a]	66.35±0.23[a]

Data represents Mean±SD of two independent experiments performed in triplicate

Table 5: Effect *Persea americana* leaf extract on selected pro- and anti-inflammatory markers in the rat model exposed to CCl_4 toxicity

Parameters	Tissues	Control	CCl_4 only	CCl_4+200 mg kg^{-1} b.wt. of *Persea americana*	CCl_4+400 mg kg^{-1} b.wt. of *Persea americana*	CCl_4+silymarin
LDH U L^{-1}	Serum	142.18±2.00[a]	221.50±0.11[b]	187.12±2.51[a]	147.16±3.01[a]	152.26±2.72[a]
IL-4 ng L^{-1}	Serum	163.23±2.28[a]	134.21±1.59[b]	158.51±3.71[a]	164.05±1.17[a]	167.31±5.23[a]
IL-6 pg mL^{-1}	Serum	31.07±1.22[a]	11.34±0.42[b]	35.21±1.82[a]	41.62±1.11[a]	29.55±0.98[a]
IL-1B pg mL^{-1}	Serum	6.27±1.97[a]	1.11±0.12[b]	2.92±0.93[a]	4.97±0.51[a]	5.72±0.52[a]
TNF-a	Serum	28.60±1.67[a]	40.91±0.71[b]	31.35±1.5[a]	21.41±0.10[a]	29.63±1.78[a]

Data represents Mean±SD of two independent experiments performed in triplicate

221.50±0.11 following exposure to the toxicant. Treatment with *P. americana* leaves extract and silymarin reversed the serum LDH level to 147.16±3.01 and 152.26±2.72, respectively. Meanwhile, the serum level of TNF-α was increased from 28.60±1.67 to 40.91±0.71 after exposure to CCl_4. Treatment with *P. americana* (400 mg kg^{-1} b.wt.) and silymarin (100 mg kg^{-1} b.wt.) ameliorated the toxicity by restoring serum TNF-α to 21.41±0.10 and 29.63±1.78, respectively. A similar trend was observed for other inflammatory markers.

Figure 1 and 2 show the effect of *P. americana* leaves on lipid peroxidation in liver, serum, kidney and heart homogenates of animals initially exposed to CCl_4 toxicity. Exposure to CCl_4 resulted in an MDA rise from 0.41 and 0.57 in normal animals to 0.48 and 0.79 in the serum and liver, respectively. Treatment with *P. americana* leaves extract and silymarin reversed the toxicity imposed by reducing MDA to (0.32, 0.46 in the serum) and (0.42 and 0.53 in the liver) respectively. A similar trend was observed when heart and kidney MDA was measured.

Histopathological analysis results: Figure 3a-c describe the photomicrograph of the histoarchitecture of the liver (3a), kidney (3b) and heart (3c) of animals exposed to CCl_4 toxicity and treated with *P. americana.* From the Figures, CCl_4 exposure resulted in distorted histoarchitecture, vacuolar and

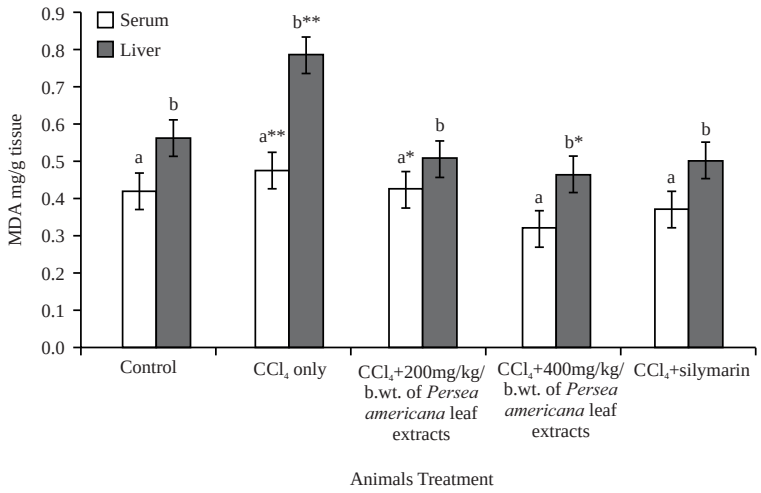

Fig. 1: Effect of *Persea americana* on the liver and serum malondialdehyde (MDA) level in animals exposed to CCl_4

Data represents Mean±S.D of two independent experiments performed in triplicate, a** and b** represents a significant difference from the controls a and b, respectively and P.a: *P. americana*

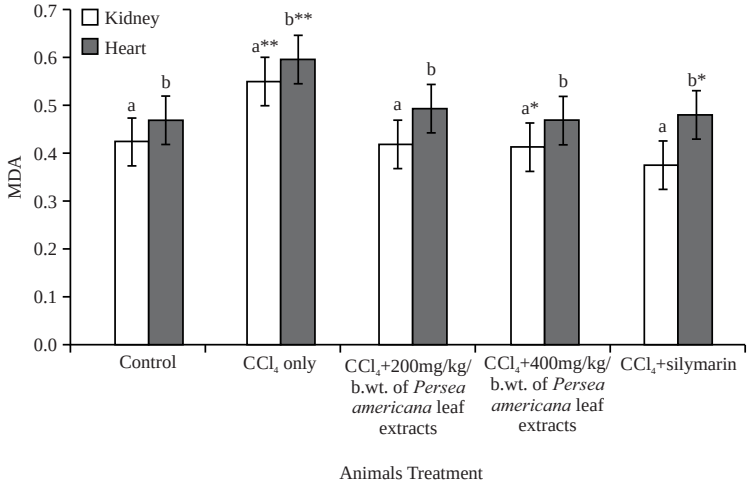

Fig. 2: Effect of *Persea americana* on the heart and kidney malondialdehyde (MDA) level in animals exposed to CCl_4

Data represents Mean±S.D of two independent experiments performed in triplicate, a** and b** represents a significant difference from the control a and b, respectively and P.a: *P. americana*

myocardial degeneration as well as the obstructed glomerulus. Treatment with *P. americana* and silymarin extract restored normal histoarchitecture of the liver, kidney and heart of experimental animals in a manner comparable with animals treated with silymarin.

Histopathological analysis of selected tissues of experimental rats (liver sections): The fixed liver tissues were sectioned (5-micron thickness) and liver sections were processed using the routine light microscopic techniques and stained with hematoxylin and eosin. Histological examinations were supplemented by biochemical assay analysis in Fig. 3a (i-v).

Histopathological analysis of selected tissues of experimental rats (kidney sections): The fixed kidney tissues were sectioned (5-micron thickness) and kidney sections were processed using the routine light microscopic techniques and stained with hematoxylin and eosin. Histological examinations were supplemented by biochemical assay analysis in Fig. 3b (i-v).

Histopathological analysis of selected tissues of experimental rats (heart sections): The fixed heart tissues were sectioned (5-micron thickness) and heart sections were processed using the routine light microscopic techniques and

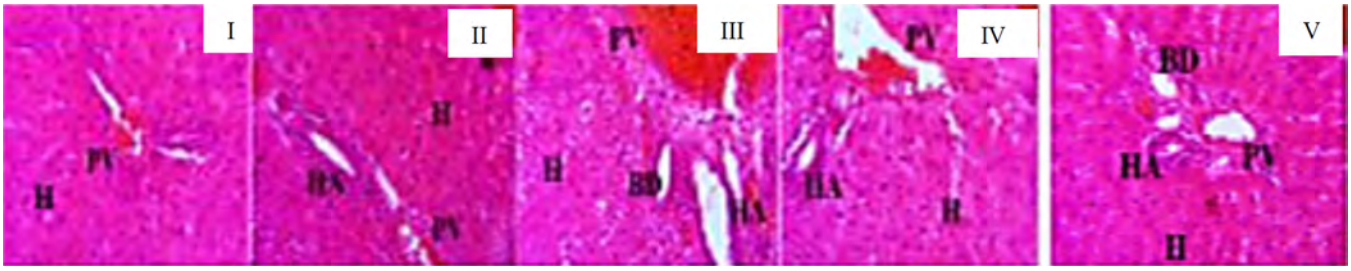

Fig. 3(a): Histoarchitecture of the liver of rats treated with *Persea americana* leaves extract after initial exposure to CCl_4 toxicity

i: Liver photomicrograph of negative control animals (fed with normal feed and distilled water only): Revealed normal arrangement of the hepatocyte, but slightly congested portal vein, ii: Liver photomicrograph of animals exposed to with 3 mL kg^{-1} CCl_4: Shows vacuolar degeneration, shrunken portal area and hyperplastic wall, iii: Liver photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 200 mg kg^{-1} *P. americana* revealed the normal arrangement of the hepatocyte, but slightly congested portal vein, iv: Liver photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 400 mg kg^{-1} *P. americana*: Revealed the normal arrangement of the hepatocyte, but slightly congested portal vein, v: Liver photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 200 mg kg^{-1} Silymarin: Revealed the normal arrangement of the hepatocytes, H: Hepatocyte, PV: Portal vein, BD: Bile duct and HA: Hepatic artery

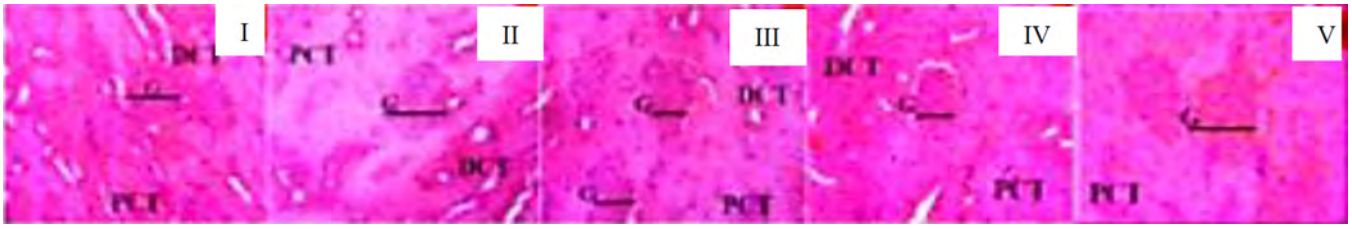

Fig. 3(b): Histoarchitecture of the kidney of rats treated with *Persea americana* leaves extract after initial exposure to CCl_4 toxicity

i: Kidney photomicrograph of negative control animals (fed with normal feed and distilled water only): Reveals glomerulus with no urinary space, ii: Kidney photomicrograph of animals exposed to with 3 mL kg^{-1} CCl_4: Reveals glomerulus with no urinary space, iii: Kidney photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 200 mg kg^{-1} *P. americana* reveals glomerulus with no urinary space, iv: Kidney photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 400 mg kg^{-1} *P. americana*: Revealed a normal bowman's capsule with distinct urinary space, v: Kidney photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 200 mg kg^{-1} Silymarin: Reveals vascular congestion and absence of urinary space of the kidney, G: glomerulus, PCT: Proximal convoluted tubules, DCT: Distal convoluted tubules, US: Urinary space and VP: Vacuolar pole

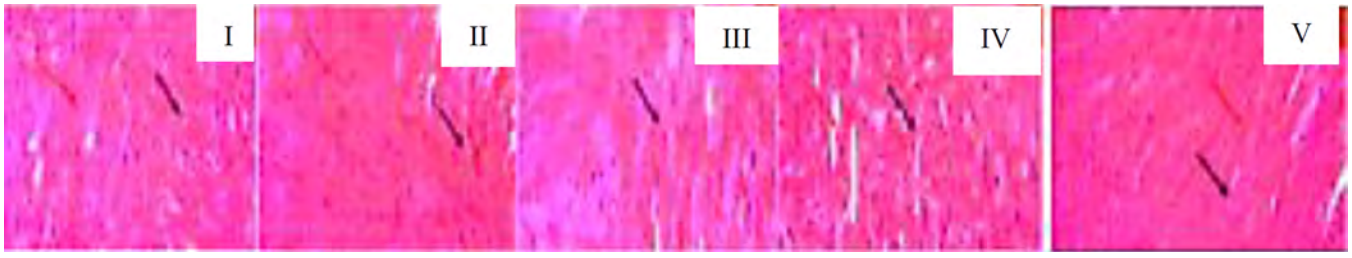

Fig. 3(c): Histoarchitecture of the heart of rats treated with *Persea americana* leaves extract after initial exposure to CCl_4 toxicity

i: Heart photomicrograph of negative control animals (fed with normal feed and distilled water only): Reveals distinct nuclei with scanty myocardium degeneration, ii: Heart photomicrograph of animals exposed to with 3 mL kg^{-1} CCl_4: Reveals focal areas of myocardium degeneration with increased extracellular space, iii: Heart photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 200 mg kg^{-1} *P. americana*: Reveals distinct nuclei with scanty myocardium degeneration, iv: Heart photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 400 mg kg^{-1} *P. americana*: Reveals distinct nuclei with scanty myocardium degeneration, v: Heart photomicrograph of animals exposed to 3 mL kg^{-1} CCl_4 and treated with 200 mg kg^{-1} Silymarin: Reveals normal slender muscle fibres that were densely packed and stained pick with centrally placed nuclei, Black arrow: Nuclei and Red arrow: muscle fibres

stained with hematoxylin and eosin. Histological examinations were supplemented by biochemical assay analysis in Fig. 3c (i-v).

DISCUSSION

Globally, the use of plants for prophylaxis and curative treatment of diseases is an age-long practice. To date, a significant proportion of the world population depends on herbal preparations as drugs[26]. The medicinal ability of plants is intricately linked to the presence of phytochemicals primarily synthesized for their protection from predators and pathogenic organisms. In the present study, both preliminary qualitative phytochemical screening and GC-MS analysis of the phytoconstituents of methanolic extract of *P. americana* revealed the presence of a wide variety of phytochemicals with proven medicinal efficacy (Table 1). Assuredly, these phytochemicals are responsible for the curative effects of the plant extract on animals exposed to hepato- and nephron-toxicant.

Urea and creatinine have been traditionally used as biomarkers of kidney function[27,28]. Recently, Lee *et al.*[29] reported the use of urea as a biomarker for measuring uremic retention and efficiency of solute removal via dialysis in the blood of patients with chronic kidney disease (CKD). In the present study, serum urea level was significantly increased in animals administered with CCl_4 without treatment (Table 2), suggesting that free radicals were more than the body's antioxidant capacity to mop them leading to the oxidative attack on the kidney and increased urea retention. However, treatment with avocado extract restored the serum urea and creatinine level to an amount comparable to normal animals and animals treated with silymarin, respectively. This is in line with the study reported by Ogunmoyole *et al.*[30].

Glutathione peroxidase (GPx) is an intracellular mitochondrial and cytosolic antioxidant enzyme involved in the catalytic lysis of hydrogen peroxides (H_2O_2) and lipid peroxides to water and alcohols, respectively[31]. On the other hand, catalase is a peroxisomal antioxidant enzyme that catalyzes the cleavage of H_2O_2 into the water and molecular oxygen, thereby mitigating the deleterious effect of free radicals. In the present study, activities of antioxidant enzymes that was initially depleted in exposed rats were restored in a manner comparable to animals treated with silymarin following oral administration of *P. americana* leaves extract (Table 3). This indicates that CCl_4 triggered an increased production of ROS over the endogenous antioxidant capacity to mop-up, thereby eliciting organ damage. However, treatment with *P. americana* leaves extracts caused a dose-dependent restoration of GPx and catalase in a fashion similar to the negative control animals and those treated with silymarin. Reports have indicated that increased activity of glutathione peroxidase and catalase correlates to decreased oxidative stress in experimental animals. In agreement with earlier reports, *P. americana* extract restored the enzymic antioxidants (GPx and CAT) in animals initially exposed to toxicant[32,33].

ALT is one of the most commonly used markers of hepatic injury. Its elevation in the serum suggests an oxidative attack on liver cells leading to leakage from the cells to the blood. In the present study, the activity of ALT was significantly increased in the serum and other tissues homogenates following CCl_4 exposure (Table 4). However, treatment with graded doses of *P. americana* extract caused a restoration of ALT to levels comparable with negative control (unexposed animals) and those treated with silymarin. Studies have shown that flavonoids, terpenoids and steroids have a protective effect on the liver and kidney due to their antioxidants properties. Consequently, antioxidants' effects in the *P. americana* leaf extract could be responsible for the observed hepato- and nephroprotective. Preliminary phytochemical screening indicates that flavonoids are the most abundant phytochemical in the extract, suggesting that flavonoids contribute to a very large extent the antioxidant effects of *P. americana* leaf extract observed in the present study.

Aspartate aminotransferase is present in large amounts in the heart muscle tissues but is normally found in the liver, red blood cells, pancreas and kidney. AST is among the most sensitive markers employed in the diagnosis of organ damage. Oral administration of *P. americana* leaves extracts restored AST activities in rats exposed to CCl_4 toxicity (Table 4). This observation can be linked to the presence of flavonoids and saponin in the extract. Studies have shown that saponins have antioxidant effects in Wistar rats[34]. Flavonoids found in avocado leaf extracts can be used in the treatment of oxidative stress because flavonoids are potent free radical scavengers[35]. It implies that the extract can protect restore the heart, liver and kidney, hence it could be exploited in the management of diseases related to these organs. The significant reduction in serum AST activity by treatment with *P. americana* leaves extract may be correlated to its hepatoprotective effects. This effect was comparable to silymarin, suggesting that it can favourably compete with conventional drugs used for the treatment of diseases related to the liver. Bilirubin has been a routine marker of liver disease.

A linear relationship between bilirubin level and other diseases has been shown in recent years, bilirubin has been described as a potential biomarker of multiple pathological conditions. Cholestatic liver diseases are characterized by impairment of bile flow and accumulation of biliary constituents such as bile acids and bilirubin[36]. Bilirubin levels of rats induced with CCl_4 were significantly increased when compared to the control, suggesting that the toxicant disrupts heme metabolism. However, treatment of intoxicated rats with graded doses of *P. americana* leaves extract restored the bilirubin to a level comparable with animals treated with standard drugs (Table 4). This observation can be linked to the flavonoid content in the extract. showed a significant reduction in the levels of bilirubin in serum and liver when compared to the control.

Reports have established the central role of inflammatory cytokines, particularly continuous activation of NF-κB as the major pathogenic basis of liver diseases[37]. In the present study, exposure to CCl_4 resulted in the elevation and diminution of pro-and anti-inflammatory markers respectively (Table 5), suggesting that its mechanism of intoxication involves inflammation. This is in agreement with Schuster *et al.*[37]. Treatment with *P. americana* leaf extract raised the serum level of anti-inflammatory markers suggesting that the mechanism of restoration by the extract involves anti-inflammation.

Lipid peroxidation has been implicated in the onset of oxidative stress. In the present study, exposure to CCl_4 caused a significant increase in lipid peroxidation in the serum and organ homogenates (Fig. 1 and 2). This suggests that the free radical generated by the toxicant attacked the membrane lipids leading to oxidative stress. However, treatment with *P. americana* caused inhibited lipid peroxidation in the serum and organ homogenates (Fig. 1 and 2) suggesting that the plant is a potential antioxidant candidate in the management of oxidative stress-induced pathological conditions.

Histopathological observations found in the present study (Fig. 3a-c) are comparable with the findings of Dreher and Davenport[38]. Who reported scattered non-superlative inflammation in the liver following exposure to a toxicant. The histological changes were also in conformity with the earlier report of Dreher and Davenport[38] who observed the avocado leaves extract was consistent with the biochemical and gross pathological changes. The present study indicates that avocado leaf extract curtailed the toxic effects of CCl_4 on the liver, heart and kidney in a dose-dependent manner. This is an indication that the plant can be used in the management of diseases related to these organs.

CONCLUSION

The present study established that *P. americana* leaf extract mitigated the toxicity of CCl_4 on the liver, heart and kidney. This was premised on the restoration of all deranged biochemical and histopathological parameters determined, following the administration of graded doses of *P. americana* leaf extract. This effect was attributed largely to flavonoids and other phytochemicals present in the extract and showed the potential of *P. americana* leaf extract in the management of liver, kidney and heart diseases.

SIGNIFICANCE STATEMENT

This study discovered that leaves of *P. americana* can be beneficial in the management of liver, kidney and heart diseases. This will enable the researchers to uncover the critical areas of phytotherapy involving *P. americana* which other researchers have not captured. Eventually, a new theory on management of liver, kidney and heart diseases using *P. americana* leaves can be arrived at. This will provide a cheap, locally available therapeutic alternative in the management of multiorgan diseases especially in developing nations.

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