Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Sida acuta leaf extract attenuates oxidants-induced animal model of nephrotoxicity and hepatotoxicity

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Abstract

Background: Curative potential of *Sida acuta* Burm. f. (Malvaceae) leaves on hepatotoxicity and nephrotoxicity induced by carbon tetrachloride (CCl_4) and rifampicin was studied. This was aimed at providing a potent therapeutic alternative to conventional drugs in the management of liver and kidney diseases.

Methodology: Carbon tetrachloride (3 ml/kg bw) and rifampicn (250 mg/kg bw) were administered to induce liver and kidney damage in selected groups of albino rats. Graded doses of *Sida acuta* leaves extract as well as silymarin (200 mg/kg bw) were then post-administered to experimental animals placed into eight groups of five animals each. Biomarkers of oxidative stress such as lipid profile, alanine aminotransferase (ALT), aspartate aminotransferase (ALT), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase (CAT), urea, uric acid, bilirubin and malonidialdehyde (MDA) were determined. Histopathological observations of both renal and hepatic tissues of experimental animals were also performed.

Results: Animals exposed to CCl_4 and rifampicin without treatment exhibited significant derangement in lipid profile, elevated levels of ALT, ALT, ALP, urea, uric acid, bilirubin and MDA in the serum and tissues homogenates. Superoxide dismutase and catalase activities were significantly inhibited while level of GSH was depleted. Following treatment with *S. acuta* extract, all deranged parameters including histological alterations were restored in a dose-dependent manner comparable to animals treated with silymarin.

Conclusion: Biochemical and histopathological data obtained from the present study confirmed that *Sida acuta* is a potential antioxidant plant that can be exploited in the management of liver and kidney dysfunctions.

Keywords: *Sida acuta*, Kidney, Liver, Antioxidant, Histopathology, Lipid profile, Biochemical parameters

Introduction

Oxygen-centered chemical species collectively known as reactive oxygen species (ROS), are endogenous metabolic by-products of specific biochemical processes in the biological system [45, 56]. ROS are required for vital processes such as apoptosis, immunity, cellular differentiation, protein phosphorylation and activation of transcriptional factors among others. In spite of their usefulness, ROS level must be kept low in the physiological system, to avoid its deleterious effects on critical biological macromolecules such as nucleic acids, proteins and lipids [51, 71]. Accumulating pieces of evidence have shown that these free radicals are the major molecular culprits in the onset, progression and complications of several diseases such as cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases ([63]; Kumari et al., 2018). The mitochondria are enriched with an intrinsic ROS scavenging capacity [20]. However, its endogenous ROS scavenging ability is inadequate to mop up free radicals generated during oxidative phosphorylation and electron transport chain [2,

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17]. Consequently, endogenous enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) as well as other non- enzymic antioxidants act synergistically to ensure protection of critical macromolecules from ROS-induced cellular damage ([10]; Collin, 2019). Despite the myriad of endogenous antioxidants in the physiological system, ROS production from multiple sources often overwhelm their antioxidative capacity ultimately resulting in oxidative stress. It has been suggested that the etiology of all known diseases is traceable to oxidative stress (Kumari et al., 2018). Considering the critical role of the liver and kidney in biotransformation and excretion respectively, it is evident that these organs are more susceptible to oxidative attack.

Globally, there are approximately 2 million deaths linked to liver disease per year [39]. Cirrhosis and liver cancer currently constitute 1.16 and 0.788 million deaths respectively, making them the 11th and 16th most common causes of death annually [39]. When combined, liver diseases account for 3.5% of all annual deaths worldwide. This data indicates a progressive increase in liver-related mortality when compared to 3% of all deaths in the year 2000. Similarly, kidney dysfunction has been identified as a global public health problem of emerging concern, affecting over 750 million persons worldwide. The burden of kidney disease varies substantially across the world [21, 29]. Chronic kidney disease (CKD) predisposes patients to complications such as kidney failure, cardiovascular disease, and untimely death [33]. In 1990, CKD was ranked as 27th global cause of death, however, by 2020, it has progressed to rank 18th global cause of death being next to HIV/AIDS [28]. A sustainable panacea to this ugly trend must therefore be sought urgently to avoid further escalation.

Plants use for nutritional, ornamental and medicinal purposes is as old as mankind and perhaps marked the commencement of human civilization. A wide array of conventional drugs in use today have their origin traceable to natural compounds primarily synthesized for plants defense against predators (phytochemicals) but exploited by man for various medicinal purposes.

Sida acuta Burm.f (Malvaceae) is a small perennial plant whose bark, leaves, flowers and seeds have been exploited in folkloric medicine for the treatment of several disorders ([38, 61]; Mgbemena et al., 2015). Although, all parts of the plant are therapeutically important, its leaves are the most frequently used by traditional medical practitioners. *S. acuta* leaves have been known for its analgesic, antipyretic, antiemetic, diuretic, demulcent, anthelmintic, antiulcer, anti-rheumatic properties [1, 35, 42]. Specifically, extract of *S. acuta* leaves is a potent therapeutic agent in the treatment of male sexual dysfunction [41, 52]. Reports have indicated that its root extract is a potent anti-aging, anti-hypertensive, anti-tuberculosis and immunomodulating agent [22, 24, 27, 30, 31, 38, 44, 55, 58]. There is a dire need to identify medicinal plants with comparable therapeutic efficacy to conventional drugs routinely used for the management of liver and kidney diseases. Extracts of such plants would serve as viable, affordable and locally-made alternative for the management of liver and kidney diseases. Hence, this study.

Materials and methods

Plant materials

Sida acuta leaves were obtained within Ekiti State University Campus, authenticated at the Department of Plant Science, Ekiti State University, Ado Ekiti with herbarium number UHAE2020073 and a voucher specimen deposited. The leaves were air- dried in the laboratory, pulverized, weighed and then stored in an airtight container.

Reagents and chemicals

All reagents and chemicals were all of high analytical grade purchased from Randox Diagnostic, England. All parameters were determined using Randox kits.

Extraction of the extract

One hundred grams (100 g) of the powdered leaves was soaked in 500 ml of 80% ethanol for 72 h. It was then filtered using a cheese cloth, and freeze-dried to obtain the crude ethanolic extract. The extract was then kept airtight in a closed container and kept inside the refrigerator at 4 °C. The dose of administration was determined based on previous work by Benjumea et al. [5] and Sreedevi et al. [61].

Animals handling

Forty wistar albino rats of average weight 170 g were obtained from the animal house of the Department of Science Technology, The Federal Polytechnic, Ado Ekiti and housed in clean wire meshed cages under standard conditions temperature (24 ± 1 °C), relative humidity, and 12 / 12-h light and dark cycle. The animals were allowed free access to food (commercial palletized diet from Vital Feed Mill) and drinking water ad libitum daily. Rat beddings were cleaned and replaced everyday throughout the period. Ethical approval was obtained from the office of Research and Development, Ekiti State University, Ado Ekiti. Animals were handled in accordance with recommendation of Committee on Care and Use of Experimental Animals. Animals were randomly placed into eight groups of five animals per group and treated as shown in Table 1.

Table 1 Animal grouping and treatments

Group	Treatment
NC	distilled water only
PCI	3 ml/kg bw CCl_4 only
PCI + SA (50 mg/kg bw)	3 ml/kg b.w CCl_4 + 50 mg/kg bw. *Sida acuta*
PCI + SA (100 mg/kg bw)	3 ml/kg b.w CCl_4 + 100 mg/kg bw. *Sida acuta*
PCI+ Silymarin (100 mg/kg bw)	3 ml/kg b.w CCl_4 + 100 mg/kg bw. Silymarin
PCII	250 mg/kg b.w. Rifampicin only
PCII + SA (50 mg/kg bw)	250 mg/kg b.w. Rifampicin + 50 mg/kg bw *Sida acuta*
PCII + SA (100 mg/kg bw)	250 mg/kg b.w. Rifampicin + 100 mg/kg bw *Sida acuta*

These treatments were done every day for fourteen days. Thereafter, all animals were fasted for 24 h before decapitation under cold anesthesia

Preparation of homogenates

Animals were fasted twenty-four hours before sacrifice. All experimental rats were decapitated following ARRIVE guidelines to harvest the liver and kidney which were trimmed of fat, blotted with filter paper and weighed. Ten percent homogenate of each of the tissues was made with 10 mM potassium phosphate buffer pH 7.4 tissue. The homogenates were then centrifuged at 3000 rpm at for 30 mins at 4 °C, decanted and the clear supernatant obtained was kept at 4 °C for subsequent biochemical analyses. Whole blood was collected by cardiac puncture in EDTA bottles and allowed to stand for 1 h. Thereafter, it was centrifuged at 3000 rpm for 15 min at 25 °C to obtain the serum.

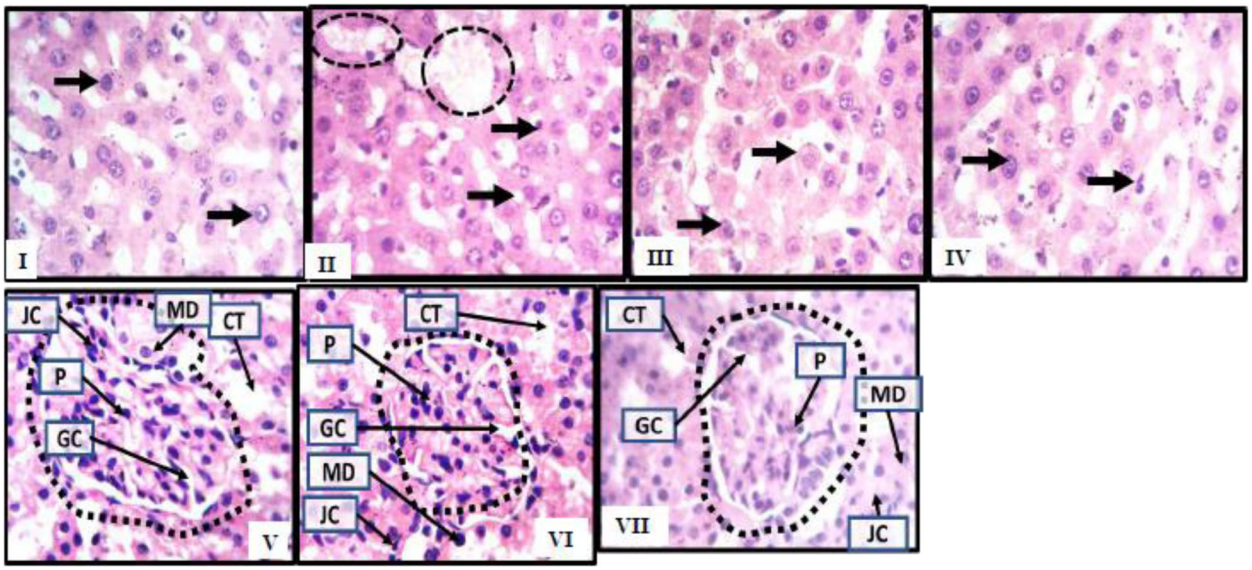

Fig. 1 Photomicrograph of the liver Fig. (I-IV) and kidney Fig. (V-VII) of wistar albino rat under various experimental treatments. I- *liver photomicrograph of rats fed with normal feed and distilled water only:* Normal histomorphology of the hepatic tissue, distinct hepatocyte nuclei correctly located in the cytosol (black arrows). No noticeable histopathological distortion in the liver tissues. *II- liver photomicrograph of rats administered with 3 ml/kg bw CCl_4 only*: Distorted liver histoarchitecture (black arrows) with noticeable fatty liver (broken circles). *III- liver photomicrograph of rats exposed to 3 ml/kg bw CCl_4 and treated with 100 mg/kg S. acuta:* Liver histoarchitecture was normal. No noticeable histopathological distortion was found in the hepatic tissues. IV - liver photomicrograph of rats exposed to 3 ml/kg bw CCl_4 and treated with 100 mg/kg bw Silymarin: Distinctly stained and well positioned hepatocytic nuclei was observed. No histopathological distortion of the liver histoarchitecture. V - kidney photomicrograph of rats administered with distilled water only: Cellular delineation, distribution density and staining were appropriate. No apparent histopathological distortion was observed. VI - kidney photomicrograph of rats administered with 250 mg/kg bw rifampicin only: Marked distortion in renal histoarchitecture coupled with glomerular atrophy. Tubular degeneration, formation of pathological cast, intraluminal exfoliation and observable pyknosis of the nuclei were noticeable. *VII- kidney photomicrograph of rats administered with 250 mg/kg bw rifampicin and treated with 100 mg/kg bw of S. acuta:* 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

Table 2 Effect of *S. acuta* extract on hepatic, renal and serum lipid profile of rats exposed to CCl_4 and rifampicin

Parameter	Tissues	NC	PC I	PCI + SA (50 mg/kg bw)	PCI +SA (100mg/kg bw)	PCI + silymarin (100mg/kg bw)	PC II	PCII + SA (50 mg/kg bw)	PCII + SA (100 mg/kg bw)
T. Chol (mg/dl)	Liver	56.08± 2.09^{a*}	89.12±1.73^{a}	64.17±1.34^{a*}	57.22±1.65^{a*}	52.33±1.34^{a*}	97.23±1.42^{b}	62.14±1.16^{b*}	55.41±1.27^{b*}
	Kidney	30.07± 2.18^{a*}	41.86 ± 2.09^{a}	33.22±2.03^{a*}	27.63±1.97^{a*}	38.63±2.23^{a*}	53.24±1.03^{b}	44.25±0.40^{b*}	35.64±0.69^{b*}
	Serum	52.16± 2.19^{a*}	72.44 ±1.86^{a}	63.30±2.09^{a*}	54.16±1.63^{a*}	58.27±1.56^{a*}	87.46±1.38^{b}	58.43±1.59^{b*}	55.39±1.24^{b*}
Trig. (mg/dl)	Liver	41.33±1.07^{a*}	70.25± 1.24^{a}	50.28±1.20^{a*}	43.79±1.31^{a*}	39.43±1.13^{a*}	86.50±0.42^{b}	60.08±0.85^{b*}	40.81±0.92^{b*}
	Kidney	11.82±0.34^{a*}	19.47± 0.42^{a}	16.40±0.56^{a*}	13.05±0.62^{a*}	13.43±0.29^{a*}	26.48±0.33^{b}	20.86±0.29^{b*}	13.08±0.43^{b*}
	Serum	37.51±1.39^{a*}	61.41± 1.28^{a}	47.58±1.31^{a*}	38.29±1.09^{a*}	40.14±1.72^{a*}	70.39±1.45^{b}	62.03±0.87^{b*}	34.13±1.16^{b*}
HDL (mg/dl)	Liver	24.72± 0.44^{a*}	16.07 ± 0.53^{a}	18.63±0.58^{a*}	23.09±0.69^{a*}	26.31±0.59^{a*}	18.26±0.24^{b}	20.44±0.24^{b*}	23.64±0.31^{b*}
	Kidney	8.26 ± 0.17^{a*}	5.31 ± 0.87^{a}	7.68±0.92^{a*}	8.38±0.71^{a*}	8.42±0.68^{a*}	6.08±0.63^{b}	6.92±0.53^{b*}	7.17±0.72^{b*}
	Serum	9.42 ± 0.31^{a*}	6.37 ± 0.43^{a}	7.40±0.36^{a*}	8.48±0.39^{a*}	8.79±0.29^{a*}	5.10±0.07^{b}	6.18±0.06^{b*}	7.79±0.04^{b*}
LDL (mg/dl)	Liver	33.76±1.27^{a*}	41.27 ± 1.53^{a}	36.47±1.37^{a*}	35.20±1.84^{a*}	33.04±0.62^{a*}	57.81±0.11^{b}	40.59±0.12^{b*}	31.67±0.15^{b*}
	Kidney	13.26± 0.73^{a*}	21.60 ± 0.82^{a}	17.52±0.45^{a*}	13.14±0.60^{a*}	12.13±0.48^{a*}	31.27±0.57^{b}	27.50±0.27^{b*}	19.48±0.38^{b*}
	Serum	22.36± 0.32^{a*}	34.26 ± 0.53^{a}	28.63±0.43^{a*}	23.52±0.76^{a*}	24.67± 0.72^{a*}	39.53±0.68^{b}	31.04±0.50^{b*}	20.75±0.70^{b*}

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls - ((PC I(CCl_4) 'a' and PC II (rifampicin)) 'b' respectively ; NC- negative control; SA- *S. acuta*.

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls – ((PC I(CCl4) 'a' and PC II (rifampicin)) 'b' respectively; NC- negative control; SA- *S. acuta*

Serum biomarkers

Determination of serum Creatine kinase (Ck-Mb) activity

Creatine kinase activity was determined according to the method of Reitman and Frankel [53]. One milliliter of imidazole buffer (10 mM, pH 6.6), creatine phosphate (30 mM), glucose (20 mM), N-acetylcysteine (20 mM), magnesium acetate (10 mM), ethylene diaminetetraacetic acid (2 mM), ADP (2 mM), NADP (2 mM), AMP (5 mM), DAPP (10 μM), G6PDH (≥2.0 ku/L) and HK(≥2.15 ku/L)) was measured into a thermostatic cuvette at 37 °C after which 50 μl of serum was added. Absorbance of the resulting mixture was read immediately at 340 nm for 5 min at 30 s interval. Change in absorbance per min was calculated then (ΔAbs/min) as:

CK-Mb Activity (IU/L) = (ΔAbs / min) × 6667.

Assay of aspartate aminotransferase (AST) activity

Aspartate Aminotransferase (AST) activity was determined following the method described by Reitman and Frankel [53]. Briefly, 0.1 ml of diluted sample of the serum, liver and kidney was mixed with phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L) and the mixture incubated for exactly 30 min at 37 °C. 0.5 ml of 2,4-dinitrphenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 25 °C. Thereafter, 5.0 ml of NaOH (0.4 mol/L) was added and absorbance read at 546 nm against the reagent blank after 5 min.

Assay of alanine amino transferase (ALT) activity

The principle described by Reitman and Frankel [53] was followed in assaying for ALT activity using

Table 3 Effect of *S. acuta* extract on heaptic and renal AST, ALT and ALP in rats exposed to CCl_4 and rifampicin

Parameter	Tissues	NC	PC I	PCI + SA (50 mg/kg bw)	PCI +SA (100mg/kg bw)	PCI + silymarin (100mg/kg bw)	PCII	PCII + SA (50 mg/kg bw)	PCII + SA (100 mg/kg bw)
AST (IU/l)	Liver	54.33±2.31^{a*}	82.31± 2.47^{a}	62.30±2.21^{a*}	50.23±2.08^{a*}	57.23±1.72^{a*}	96.14±2.41^{b}	68.28±1.21^{b*}	53.18±2.05^{b*}
	Kidney	21.60±2.07^{a*}	37.81± 1.02^{a}	32.47±1.30^{a*}	24.13±1.24^{a*}	25.06±1.25^{a*}	56.82±1.73^{b}	43.40±1.27^{b*}	24.66±1.12^{b*}
	Serum	71.26±1.71^{a*}	115.63± 1.67a	84.17±1.50^{a*}	73.29±1.68^{a*}	75.22±1.56^{a*}	127.13±2.60^{b}	90.27±1.17^{b*}	71.09±1.36^{b*}
ALT (IU/l)	Liver	54.72±1.43^{a*}	46.08±1.17^{a}	51.33±1.27^{a*}	54.72±1.43^{a*}	46.08±1.17^{a*}	77.23±1.42^{b}	56.40±1.29^{b*}	39.10±1.30^{b*}
	Kidney	17.27± 0.25^{a*}	30.44 ± 0.97^{a}	25.22±0.33^{a*}	18.51±0.57^{a*}	23.63±1.03^{a*}	40.29±1.77	36.29±1.23^{b*}	23.60±2.01^{b*}
	Serum	56.17± 1.98^{a*}	92.76 ± 1.23a	72.10±2.13^{a*}	64.40±2.22^{a*}	59.33±1.34^{a*}	88.45±2.43^{b}	60.45±3.21^{b*}	55.81±2.10^{b*}
ALP (IU/l)	Liver	47.25 ± 1.92^{a*}	58.78 ± 1.63^{a}	52.56±2.47^{a*}	45.32±2.17^{a*}	46.88±1.39^{a*}	73.84±0.81^{b}	60.22±0.68^{b*}	40.21±0.69^{b*}
	Kidney	31.21 ± 1.25^{a*}	48.17 ± 0.84^{a}	35.18±1.14^{a*}	32.56±0.52^{a*}	29.32±0.90^{a*}	53.66±1.98^{b}	44.72±0.74^{b*}	35.13±0.76^{b*}
	Serum	62.13 ± 3.12^{a*}	102.43 ± 4.20^{a}	85.50±2.42^{a*}	69.40±2.31^{a*}	67.11±2.09^{a*}	108.52±1.32^{b}	66.58±1.22^{b*}	64.18±1.27^{b*}

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls - ((PC I(CCl_4) 'a' and PC II (rifampicin)) 'b' respectively ; NC- negative control; SA- *S. acuta*.

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls – ((PC I(CCl_4) 'a' and PC II (rifampicin)) 'b' respectively; NC- negative control; SA- *S. acuta*

commercially available assay kit (Randox laboratories, UK). Reagent 1 (0.5 ml) containing phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l) and α-oxoglutarate (2.0 mol/l) was added to 0.1 ml of serum in a test tube and the mixture was incubated at 37 °C for 30 min. Exactly 0.5 ml of R2 containing 2, 4-dinitrophenylhydrazine (2.0 mmol/l) was added and the solution incubated again at 20 °C for 20 min. Finally, 5 ml of NaOH was added and the solution was allowed to stand for 5 min at room temperature and the absorbance was read at 546 nm. ALT activity serum was obtained from the standard curve provided in the kit.

Assay of alkaline phosphatase (ALP) activity

Assay of serum ALP was performed based on the method of Englehardt et al. [13] using commercial assay kits (Randox laboratories, UK) according to the instructions of the manufacturer. Exactly 1.0 ml of the reagent (1 mol/l diethanolamine buffer pH 9.8, 0.5 mmol/l $MgCl_2$; substrate: 10 mmol/l p-nitrophenol phosphate) was added to 0.02 ml of the serum sample and mixed. The absorbance was taken at 405 nm for 3 min at intervals of 1 min. ALP activity was determined using the formula U/l = 2760 x A405 nm/min.

Estimation of Total cholesterol level

Total cholesterol level was determined following the method of Trinder [66] using commercially available kits (Randox laboratories, UK). Ten microliter of standard and 10 μl serum samples were measured into labeled test tubes. 1000 μl of working reagent containing; Pipes buffer (80 mmol/l at pH 6.8), 4-aminoantipyrine (0.25 mmol/l), phenol (6 mmol/l), peroxidase (≥ 0.5 U/ml), cholesterol esterase ion (≥0.15 U/ml) and cholesterol oxidase (0.10 U/ml) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 10 min at room temperature. The absorbance of the sample (Asample) was taken at 500 nm against the reagent blank.

Cholesterol concentration (mg/dl) was calculated as follows:

$$\text{Chol} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Evaluation of concentration of triglyceride

Triglycerides level in the serum and tissue homogenates was determined based on the method of Tietz [64] using commercially available kits (Randox

Table 4 Effect of *S. acuta* extract on selected antioxidant enzymes in the liver and kidney of rats exposed to CCl_4 and rifampicin

Parameter	Tissues	NC	PC I	PCI + SA (50 mg/kg bw)	PCI +SA (100mg/kg bw)	PCI + silymarin (100mg/kg bw)	PCII	PCII + SA (50 mg/kg bw)	PCII + SA (100 mg/kg bw)
SOD (μmol/min/mg protein)	Liver	6.14±0.31^{a*}	4.53±0.22^{a}	5.56±0.38^{a*}	6.25±0.21^{a*}	6.20±1.23^{a*}	2.36±0.19^{b}	4.43±0.16^{b*}	6.18±0.23^{b*}
	Kidney	3.48±1.03^{a*}	2.46±0.75^{a}	3.13±1.00^{a*}	3.27±0.89^{a*}	3.15±0.53^{a*}	1.93±0.19^{b}	2.07±0.20^{b*}	2.36±0.18^{b*}
	Serum	4.29±0.19^{a*}	3.17±0.10^{a}	3.28±0.18^{a*}	4.00±0.33^{a*}	4.34±0.21^{a*}	2.42±0.07^{b}	3.84±0.05^{b*}	4.14±0.02^{b*}
Catalase (μmol/min/mg protein)	Liver	4.70±0.56^{a*}	2.89±0.21^{a}	3.93±0.12^{a*}	4.81±0.23^{a*}	4.45±1.20^{a*}	1.63±0.16^{b}	3.27±0.15^{b*}	4.56±0.20^{b*}
	Kidney	2.77±0.15^{a*}	2.02±0.18^{a}	2.53±1.26^{a*}	2.74±0.16^{a*}	2.58±0.20^{a*}	1.88±0.26^{b}	2.04±0.29^{b*}	2.16±0.10^{b*}
	Serum	3.06±0.11^{a*}	2.38±0.10^{a}	2.95±0.66^{a*}	3.03±0.48^{a*}	2.93±0.20^{a*}	1.76±0.14^{b}	2.52±0.16^{b*}	3.09±0.20^{b*}
GSH ((μmol/mg protein)	Liver	5.77±0.06^{a*}	3.87±0.01^{a}	5.62±0.04^{a*}	5.89±0.06^{a*}	5.43±0.08^{a*}	2.93±0.08^{b}	4.11±0.10^{b*}	4.93±0.05^{b*}
	Kidney	3.23±0.07^{a*}	2.53±0.05^{a}	3.11±0.05^{a*}	3.25±0.09^{a*}	3.19±0.12^{a*}	3.04±0.02^{b}	3.09±0.04^{b*}	3.10±0.03^{b*}
	Serum	2.66±0.10^{a*}	1.57±0.22^{a}	2.04±0.10^{a*}	2.38±0.15^{a*}	2.49±0.98^{a*}	2.08±0.02^{b}	2.18±0.04^{b*}	2.37±0.03^{b*}

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls - ((PC I(CCl_4) 'a' and PC II (rifampicin)) 'b' respectively ; NC- negative control; SA- *S. acuta*.

laboratories, UK). Triglyceride standard (10 μl) and serum (10 μl) were measured into labeled test tubes. One (1 ml) of the working reagents; R1a (buffer) containing Pipes buffer (40 mmol/l, pH 7.6), 4-chlorophenol (5.5 mmol/l), magnesium-ion (17.5 mmol/l); R1b (enzyme reagent containing 4-amino phenazone (0.5 mmol/l), ATP (1.0 mmol/l), lipase ((≥150 U/ml), glycerol-kinase ((≥0.4 U/ml), glycerol-3-phosphate oxidase ((≥1.5 U/ml) and peroxidase (≥0.5 U/ml) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 10 min at room temperature. Absorbance was taken at 546 nm against the blank (Tietz, 1990). Triglyceride concentration (mg/dl) was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

High density lipoprotein (HDL-c)-cholesterol assay

The method described by Grove [19] was adopted in estimation of the concentration of the HDL-cholesterol in the serum. Reaction mixture containing 200 μl of the serum, 200 μl of the cholesterol standard, 500 μl of the diluted precipitant R1 (0.55 mM phosphotungstic acid, 25 mM magnesium chloride) was mixed together and allowed to stand for 10 min at room temperature. It was then centrifuged for 10 min at 4000 rpm to obtain a clear supernatant. The clear supernatant was separated off within 2 h and the cholesterol content was determined by the CHOD-PAP reaction method. To 100 μl of the sample supernatant was 1 ml of cholesterol reagent added and mixed together in a test tube. The standard test tube contained 100 μl of the cholesterol standard supernatant and 1 ml of cholesterol reagent. The reagent mixture was mixed thoroughly and incubated for 10 min at 25 °C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was then measured at 500 nm against the reagent blank within 1 h.

$$\text{Conc.of HDL-Cholesterol} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentraion of standard}$$

Table 5 Effect of *S. acuta* extract on selected biomarkers of liver and kidney toxicity in rats exposed to CCl_4 and rifampicin

Parameter	Tissues	NC	PC I	PCI + SA (50 mg/kg bw)	PCI +SA (100mg/kg bw)	PCI + silymarin (100mg/kg bw)	PCII	PCII + SA (50 mg/kg bw)	PCII + SA (100 mg/kg bw)
Urea (mg/dl)	Kidney	47.82± 0.93^{a*}	76.32 ± 0.88^{a}	57.29±1.25^{a*}	47.60±1.31^{a*}	53.76±1.61^{a}	70.06±0.83^{b}	60.14±0.58^{b*}	49.61±0.34^{b*}
	Serum	38.77 ± 0.83^{a*}	77.64 ± 1.36^{a}	50.43±1.04^{a*}	37.09±1.22^{a*}	45.26 ± 0.76^{a}	64.39±0.62^{b}	54.41±0.53^{b*}	43.82±0.53^{b*}
Uric acid (mg/dl)	Kidney	29.54± 1.52^{a*}	42.65± 0.82^{a}	35.07±1.73^{a*}	30.22±1.58^{a*}	32.89± 1.62^{a}	54.23±0.78^{b}	40.63±0.67^{b*}	31.02±0.29^{b*}
	Serum	19.17 ± 0.24^{a*}	33.51 ± 0.52^{a}	23.62±0.67^{a*}	20.38±0.47^{a*}	21.17 ± 0.68^{a}	35.43±0.52^{b}	23.06±0.30^{b*}	21.06±0.37^{b*}
Creatine kinase (IU/L)	Kidney	27.61± 1.07^{a*}	38.44± 0.87^{a}	33.60±1.39^{a*}	30.50±0.98^{a*}	25.73± 0.85^{a}	49.17±0.94^{b}	39.23±0.83^{b*}	30.52±0.56^{b*}
	Serum	20.33 ± 0.30^{a*}	31.70 ± 0.29^{a}	26.55±0.32^{a*}	20.45±0.44^{a*}	23.66 ± 0.22^{a}	44.58±0.44^{b}	30.93±1.18^{b*}	19.88±0.41^{b*}
T. bilirubin (mg/dl)	Liver	31.18± 4.04^{a*}	44.37 ± 3.54^{a}	39.44±3.01^{a*}	30.50±2.53^{a*}	31.72±3.82^{a}	56.18±0.54^{b}	40.67±0.77^{b*}	33.76±0.72^{b*}
	Serum	42.25± 1.43^{a*}	72.18 ± 1.54^{a}	61.07±1.28^{a*}	44.23±1.54^{a*}	45.37±1.84^{a}	69.30±1.02^{b}	53.26± 2.01^{b*}	43.17± 1.33^{b*}

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls - ((PC I(CCl_4) 'a' and PC II (rifampicin)) 'b' respectively ; NC- negative control; SA- *S. acuta*

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls – ((PC I(CCl4) 'a' and PC II (rifampicin)) 'b' respectively; NC- negative control; SA- *S. acuta*

Low density lipoprotein (LDL) - cholesterol determination

The concentration of low-density lipoprotein in the serum was calculated using the formula of Friedwald et al. [14] as given below:

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

Very low density lipoprotein (VLDL) - cholesterol determination

The concentration of very low-density lipoprotein in the serum was calculated using the formula of Friedwald et al. [14] as given below:

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{\text{S}}$$

Antioxidant assay

Determination of catalase activity

This experiment was carried out following the method described by Sinha [59]. Two hundred microliter each of serum, liver and kidney was mixed separately with 0.8 ml distilled H_2O to give 1 in 5 dilution of the sample. The assay mixture contained 2 ml of solution (800 μmol) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. Properly diluted enzyme preparation (0.5 ml) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1 ml portion of the reaction mixture was withdrawn and blown into 1 ml dichromate/acetic acid reagent at 60 s intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described below.

$$\text{Catalase activity} = \frac{\text{H}_2\text{0}_2 \text{ Consumed}}{\text{mg protein}}$$

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

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

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by the method of Misra and Fridovich [37]. An aliquot of 10% dilution of serum, liver and kidney homogenates was added separately to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate

Table 6 Effect of *S. acuta* extract on lipid peroxidation in rats exposed to CCl_4 and rifampicin

Parameter	Tissues	NC	PC I	PCI + SA (50 mg/kg bw)	PCI +SA (100mg/kg bw)	PCI + silymarin (100mg/kg bw)	PCII	PCII + SA (50 mg/kg bw)	PCII + SA (100 mg/kg bw)
Malonidialdehyde (MDA) (mM/g tissue)	Liver	0.23±0.01^{a*}	0.42±0.01^{a}	0.25±0.02^{a*}	0.16±0.01^{a*}	0.20±0.01^{a*}	0.58±0.01^{b}	0.20±0.0^{b*}	0.09±0.01^{b*}
	Kidney	0.89±0.01^{a*}	1.76±0.03^{a}	1.03±0.04^{a*}	0.84±0.02^{a*}	1.02±0.01^{a*}	0.94±0.01^{b}	0.48±0.01^{b*}	0.42±0.01^{b*}
	Serum	0.64±0.02^{a*}	2.55±0.01^{a}	0.70±0.02^{a*}	0.60±0.07^{a*}	0.59±0.04^{a*}	1.84±0.09^{b}	0.65±0.01^{b*}	0.48±0.01^{b*}
Total Protein (mg/g tissue)	Liver	2.64±0.16^{a*}	1.47±0.19^{a}	2.54±0.19^{a*}	2.76±0.26^{a*}	2.64±0.16^{a*}	1.04±0.09^{b}	2.03±0.12^{b*}	2.71±0.17^{b*}
	Kidney	1.73±0.020^{a*}	0.76±0.06^{a}	1.07±0.04^{a*}	1.81±0.08^{a*}	1.44±0.07^{a*}	1.06±0.01^{b}	1.13±0.01^{b*}	1.24±0.03^{b*}
	Serum	2.18±0.08^{a*}	1.16±0.06^{a}	1.78±0.58^{a*}	1.96±0.08^{a*}	2.17±0.08^{a*}	1.02±0.18^{b}	1.43±0.10^{a}	1.95±0.06^{b*}

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls - ((PC I(CCl_4) 'a' and PC II (rifampicin)) 'b' respectively ; NC- negative control; SA- *S. acuta*

Data indicates mean ± SEM values of four independent experiments performed in triplicate. 'a*' and 'b*' represent significant difference ($p<0.05$) from the positive controls – ((PC I(CCl4) 'a' and PC II (rifampicin)) 'b' respectively; NC- negative control; SA- *S. acuta*

(adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

$$\text{Increase in absorbance per minute} = \frac{A3 - A0}{2.5}$$

Where;

A_0 = absorbance at time zero

A_3 = absorbance after 150 s

$$\%\text{inhibition} = \frac{\text{change in absorbance for substrate}}{\text{Change in absorbamce of blank}} \times 100$$

1 unit of SOD activity was given as the amount of SOD required to cause 50% inhibition of the oxidation of adrenaline to adrenochrome per min.

Determination of reduced glutathione (GSH) level

The method of Beutler et al. [6] was followed in estimating the amount of GSH in the serum and tissue homogenates. Exactly 0.2 ml of supernatant was added to 1.8 ml of distilled water followed by the addition of 3 ml of the precipitating solution and then shaken thoroughly. The mixture was then allowed to stand for approximately 5 min and then filtered. 1 ml of filtrate was added of 4 ml of 0.1 M phosphate buffer pH 7.4. Finally, 0.5 ml of the Ellman reagent was added. A blank was prepared with 4 ml of the 0.1 M phosphate buffer, 1 ml of diluted precipitating solution and 0.5 ml of Ellman reagent. The absorbance was measured at 412 nm against reagent blank. Amount of GSH in the serum was obtained from the standard curve.

Determination of Total protein (TP) in serum

The Biuret method described by Weichselbaum [70] was employed in the determination of total protein in the serum using commercially available kits (Randox laboratories, UK). One milliliter of Reagent R1 containing sodium hydroxide (100 mmol/l), Na-K-tartrate (18 mmol/l), potassium iodide (15 mmol/l) and cupric sulphate (6 mmol/l) was added to 0.02 ml of the serum sample, the mixture was incubated at 25 °C and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

$$\text{Total Protein Concentration} = \frac{A_{\text{sample}} \text{ X standard concentration}}{A_{\text{standard}}}$$

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) content in the serum, liver and kidney homogenates were

measured as described by Ohkawa et al. [46] using Randox kits. One hundred microliters (100 μl) each of serum and organs homogenates were mixed separately with 2.5 ml reaction buffer and boiled for 1 h. The resulting mixture was centrifuged at 3000 rpm for 10 min. Absorbance of the supernatant obtained for each tube was then measured at 532 nm. Malonidialdehyde (MDA) level in the supernatant was expressed as μmole MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric chromophore (1.56×10^5/M/cm).

Statistical analysis

All values are expressed as mean ± SD. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 11.09 for windows. The significance level was set at $p < 0.05$.

Results

Marked derangement in serum lipid profile was observed following the administration of CCl_4 and rifampicin respectively. This derangement was however restored to normal values comparable to the negative control and animals treated with the standard drug, following treatment with graded doses of the *S. acuta* leaf extract. The trend was same regardless of the tissue involved (Table 2). Serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were markedly increased following intraperitoneal injection of CCl_4 and oral administration of rifampicin relative to negative control. Treatment with *S. acuta* leaf extract resulted in the reversal of the toxic effects as the values of these biomarkers were comparable to the negative control and animals treated with silymarin (Table 3). Kidney biomarkers in serum and kidney homogenates (urea, uric acid, creatine kinase, bilirubin and total protein) which were significantly elevated when animals were exposure to CCl_4 and rifampicin toxicity were brought back to normal values comparable with the negative control (Table 4). Activity of antioxidant enzymes: SOD and CAT were markedly inhibited following exposure to CCl_4 and rifampicin (Table 5), while malonidialdehyde (MDA) level in the serum and tissues homogenates was significantly elevated relative to the negative control (Table 6). Treatment with *S. acuta* leaves extract however restore activity of antioxidant enzymes and inhibited lipid peroxidation in a dose-dependent manner (Tables 5 and 6).

Discussion

The rising global burden of liver and kidney diseases calls for concern [33, 39]. Since the origin of all known diseases has been linked to oxidative stress, deliberate research effort geared towards identifying medicinal plants with potent antioxidant and therapeutic properties should be emphasized. The liver and kidney are critical to biotransformation and ultrafiltration respectively. Hence, oxidative assault on hepatic and renal cells could result in diseases due to accumulation of toxic waste products [36, 72]. It has been suggested that rifampicin, a first line antibiotic used globally for the treatment of tuberculosis is a culprit in renal damage [12].

In the present study, lipid profile was significantly deranged in the serum and organs homogenates of experimental animals following exposure to CCl_4 and rifampicin respectively. This implies a disruption in membrane lipids eventually triggering membrane dysfunction as a result of compromise in membrane integrity. A noticeable surge in total cholesterol and triglycerides following exposure to toxicant might be due to an up-regulation in cholesterol biosynthesis, a major risk factor for atherosclerosis. Oral treatment with *S. acuta* extract however restored cholesterol and triglycerides level to values comparable with animals treated with silymarin. This restoration is probably due to a down-regulation in cholesterol biosynthesis, which can be attributed to antioxidant phytochemicals in the extract. This observation implies that *S. acuta* leaves extract can be employed in the management of several cardiovascular diseases whose etiology is related to hypercholesterolemia.

Reports have suggested an inverse relationship between HDL concentration and atherosclerosis [11, 50]. This might not be unconnected to its pivotal role in reverse cholesterol transport, a process that involves the movement of cholesterol from the wall of arteries to the liver where it is excreted [54, 60]. Since treatment with *S.acuta* leaves extract caused an increase in HDL level, it could suggest that the plant is a potential agent in the management of atherosclerosis. Rifampicin and CCl_4 exposure resulted in marked depletion in HDL-C relative to the negative control animals. This depletion in HDL-C level was however reversed by treatment with graded doses of *S. acuta* leaves extract in a manner comparable to negative control animals. This is a strong indication that *S. acuta* leaves is a potential therapeutic agent in the management of renal and hepatic disorders.

High serum LDL has been used to predict the risk of atherosclerosis [49]. Low density lipoprotein (LDL)-cholesterol was markedly elevated in the serum and organ homogenates of animals administered with CCl_4 and rifampicin only, suggesting these two compounds are toxic. Perhaps, free radicals generated by the two toxicants halt the uptake of LDL by the liver, by altering the native conformation of LDL-receptor in the liver, modifying it structurally, such that it cannot bind LDL [9, 67]. Consequently, hypercholesterolemia results with

markedly increased LDL cholesterol levels. Treatment with *S. acuta* leaves extract caused a depletion in LDL-C level and consequent restoration of LDL to its basal. Perhaps, this is attributable to the ability of notable antioxidants (polyphenols) present in the extract to reverse the conformational alteration in hepatic LDL-receptor, thereby allowing LDL cholesterol to bind to its receptor in the liver for its uptake. This may probably explain why LDL-cholesterol in the serum and tissue homogenates became depleted [9, 67].

Routinely, the health status of specific organs such as the liver and kidney is usually monitored by assaying for their marker enzymes [74]. In healthy cells, marker enzymes such as AST, ALT, and ALP are kept solely within the cellular compartments; hence, their serum levels are usually low. However, in the event of an oxidative attack, the compartmental barrier is damaged thereby causing leakage of these enzymes into the serum resulting in an elevation in their levels. In the present study, there was a significant increase in AST, ALT and ALP levels in the serum and organs homogenates following exposure to CCl_4 and rifampicin respectively. Assuredly, the increase was as a result of damage in the hepatic and renal cells resulting in the leakage of ALP, AST and ALT which are considered the primary and specific markers of hepatic and renal injury [4, 68]. This observation is in agreement with the earlier reports of Mosa and Khalil [40] and Chen et al. [8]. Prevention and/or restoration of deranged biochemical parameters is one of the ways of assessing the antioxidant potential of compounds [48]. Significant restoration of AST, ALT and ALP levels, in animals treated with silymarin and *S. acuta* compared to animals exposed to toxicants without treatment indicates that the plant exhibits both hepato- and nephroprotective activity. These observations are consistent with earlier reports [18, 25, 47, 73].

Lipid peroxidation has been suggested as an index of toxicity. It measures deterioration of polyunsaturated lipids as a result of an interaction with free radicals. This mechanism has been linked to several pathological conditions including neurodegenerative diseases [32]. Administration of CCl_4 and rifampicin respectively, triggered a surge in lipid peroxidation as measured by the malonidialdehyde (MDA) content in the serum and tissue homogenates of experimental animals. This explains the leakage earlier described in respect of ALT, AST and ALP. Treatment with *S. acuta* leaves extract significantly inhibited lipid peroxidation in the liver, kidney and serum, in a manner comparable to the negative control and animals treated with standard drug. This observation suggests that *S. acuta* is a potential antioxidant plant that can be exploited in the management of liver and kidney diseases.

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) as well as the non-protein thiol-reduced glutathione (GSH) are routine parameters used to assess the antioxidant status of laboratory animals [57]. There was a significant depletion in these parameters in the serum, kidney and liver homogenates of animals exposed to CCl_4 and rifampicin without treatment relative to the negative control animals. Oral administration of *S. acuta* extract caused a dose-dependent restoration of these parameters to level similar to positive control and animals treated with the standard drug. This restorative potential of the plant can be attributed to the presence of antioxidant phytochemicals with potent therapeutic ability.

Serum bilirubin was significantly elevated in animals challenged with CCl_4 and rifampicin respectively without treatment. Bilirubin is a toxic degradation product of hemoglobin, which is enzymatically conjugated to UDP by the enzyme, UDP-glucuronyltransferase, and subsequently excreted in bile [16]. It suggests that the increase in bilirubin level following exposure of animals to the toxicants can be attributed to inhibition of the enzyme required for conjugating bilirubin for easy excretion. The fact that bilirubin level was brought back to that of the negative control animals after treatment with *S. acuta* indicates a relieve of the inhibition earlier imposed on UDP-glucuronyltransferase by the toxicant, thereby allowing rapid conjugation of bilirubin, consequently restoring normalcy to the treated animals.

Functionality of the kidney is routinely monitored by the level of urea in the serum [3, 43, 65, 69]. Marked elevation in serum urea following CCl_4 and rifampicin respectively suggest their nephrotoxic potentials. However, the dose-dependent reversal of the toxicity imposed by the toxicants following oral treatment with *S. acuta* is indicative of the fact that the extract enhanced ultrafiltration in the kidney. A plausible explanation is that treatment with the extract restored the histoarchitecture of the kidney which has been earlier distorted by the toxicant.

Uric acid is another parameter often used to measure kidney efficiency. Specifically, it has been intrinsically linked to the risk of developing gout in humans (Lehninger et al., 1993). Several degenerative diseases such as hypertension [7], diabetes and obesity (Safi et al., 2004) have been linked to elevated uric acid. In the present study, there was a significantly higher uric acid in the positive control relative to negative control animals. Treatment with *S. acuta* extract caused a dose-dependent inhibition of uric acid synthesis. This observation can be attributed to the antioxidative constituents of the extract which acted by down-regulating purine metabolism or enhancing uric acid excretion.

Histopathological examination revealed a fatty liver with cholestasis coupled with a distorted histoarchitecture following exposure to CCl_4 (Fig. 1). This observation is in agreement with the previous report of Mahli et al. [34] and Huda and Mosaddik [23]. Treatment with *S. acuta* extract restored the normal histoarchitecture of the liver (Fig. 1), establishing the ameliorative potential of the plant against hepatotoxicity as earlier reported in other medicinal plant [34]. On the other hand, rifampicin, a known bactericidal antibiotic, normally administered for treating tuberculosis, has been known to trigger free radicals-induced renal failure [12, 26, 75]. Rifampicin administration caused severe distortion in renal histoarchitecture and glomerular atrophy (Fig. 1). However, treatment with *S. acuta* extract resulted in the restoration of normal renal architecture (Fig. 1), suggesting that the plant has constituents with nephroprotective potentials that can be harnessed for the management of kidney diseases. This observation agrees with earlier reports on the use of antioxidants for treating kidney diseases [15, 62].

In summary, ethanolic extract of *S. acuta* leaves exhibited potent ameliorative ability against renal and hepatic damage. It can therefore compete favorably with conventional drugs currently in use for the management of these diseases.

Acknowledgements
Authors hereby acknowledge the support of Department of Medical Biochemistry, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria.

Authors' contributions
Ogunmoyole T, conceived and designed the work, interpreted the data, wrote and edited the manuscript. Onaopemiposi O. Falusi and Funmilayo Oderinde, performed the experiment and analyzed the data. The author(s) read and approved the final manuscript.

Funding
This work did not receive any funding from any source, public or private.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The present study was done in accordance with the guidelines for the care and use of experimental animals as approved by the Committee on the Use and Care of Experimental Animals, Ekiti state University, Ado-Ekiti, Ekiti State, Nigeria.

Consent for publication
Not applicable.

Competing interests
Authors declare that there is no conflict of interest of any kind.

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Received: 22 March 2021 Accepted: 30 December 2021
Published online: 24 January 2022

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