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Cymbopogon citratus Leaf Extract Mitigates Hepatorenal Injury in Carbon Tetrachloride and Rifampicin-Exposed Rats

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Abstract

The rising trend in the global burden of liver and kidney diseases calls for concern. The present study therefore investigates the medicinal potentials of *C. citratus* in rat model of hepatorenal toxicity. Forty male albino rats were randomly grouped and exposed to carbon tetrachloride (3 ml/kg bw.) and rifampicin (250 mg/kg bw.) toxicity and post-treated with *C. citratus* leaf extract and silymarin (100 mg/kg bw) as standard drug according to experimental design. Specific biomarkers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), urea, uric acid, total bilirubin, total protein (TP) as well as lipid profile were determined. Exposure to toxicants caused a marked decrease in antioxidant enzymes and high-density lipoprotein (HDL) with concomitant increase in triglyceride, total cholesterol and low-density lipoprotein (LDL). Levels of AST, ALT, ALP, total bilirubin, urea and uric acid were markedly increased in animals exposed to toxicants without post treatment with the extract. Post-administration of *C. citratus* leaf extract caused a decrease in AST, ALT and ALP, TC, TG and LDL-c while there was significant increase in the level of HDL and antioxidant enzyme relative to the untreated group. Effect of *C. citratus* was comparable to the reference drug, silymarin (100 mg/kg) at the same dose.

The study showed the medicinal potentials of *C. citratus* as revealed in both biochemical and histopathogical parameters. Hence, it is a choice candidate that can be exploited in the treatment of liver and kidney diseases.

Keywords: *C. citratus*, silymarin, liver, kidney, hepatoprotective, nephroprotective, biomarkers

Introduction

Oxidative stress depicts the pathological effect of an unfavorable distortion in the delicate balance between free radicals and antioxidants in the biological system. Usually, it is caused by an increased level of free radicals over and above the endogenous antioxidative capacity of the physiological system (1). It has been suggested that oxidative stress is implied in the etiology of almost all known diseases, hence, the concept has attracted huge research attention in the last three decades (1). Specifically, oxidative stress has been linked to the onset and complications of diseases (2), non-ulcer dyspepsia (3), Parkinson's disease (4), Alzheimer's disease (5,6) Atherosclerosis (7) and major depression (8) among others. Under normal phys-

iological condition, free radicals are produced during metabolic activities such as in the electron transport chain and are important biochemical species for the movement of electron down the chain (1). Free radicals also play pivotal roles in apoptosis, phagocytosis, necrosis and destruction of pathogens (9). Chemically, free radicals are extremely reactive because they are unstable due to the presence of unpaired electron in their electronic shell. In an attempt to attain stability, free radicals interact with any reduced compounds such as the major macromolecules- proteins, carbohydrates, nucleic acids and lipids abstracting proton from them. This interaction is deleterious because due to the oxidation of critical compounds become oxidized ultimately leading to a compromise in their biological functions. This is the fundamental basis of biochemical havocs wrecked by radicals in the physiological system (9). Under normal physiological conditions, endogenous antioxidants are synthesized in the liver and kidney to mitigate against free radicals' assault on these critical organs (10). However, whenever there is a alteration that results in a surge in level of free radicals against antioxidants, these organs suffer oxidative attack resulting to several pathological conditions including fibrosis and inflammation (11).

Oxidative stress has been suggested as playing a critical role in the pathophysiology of renal dysfunction. Specifically, oxidative stress is involved in the onset, progression and complications of chronic kidney diseases (CKD). CKD is characterized by a gradual compromise of kidney function (12). At the moment, CKD constitutes a major public health challenge due to its annual increasing prevalence and its global consequence in terms of morbidity and mortality since it ranks second on the scale of significant risk factors for cardiovascular disease (CVD) (12). A high percentage of the 41 million annual global death has been linked to cardiovascular disorders largely caused by CKD (13). In fact, kidney diseases form a major threat to economic development on the global scale, considering the cost of treatment and its adverse effect on productivity (14). The burden of kidney disease rose from 31.7 % in 2015 to 41.5 % in 2017 and its projected to rise further to become the fifth most common cause of global death in the next two decades (12).

Liver disease can either present as acute or chronic with different mechanisms and etiologies (15). Globally, chronic liver disease (CLD) is approximately 19% prevalent causing about 2million death annually (15). Specifically, hepatocellular carcinoma (HCC) has been ranked the sixth most common type of cancer and is responsible for approximately 26,000 transplants annually (10). A number of etiologies have been identified for CLD which includes: hepatitis B and C virus infection constituting 67% while alcoholic liver disease and nonalcoholic steatohepatitis constitutes 33% (15).

Cymbopogon citratus (*C. citratus*) is a tropical plant with multiple names, perhaps due to its wide distribution and ability to thrive in different climates and soil types (16,17). Its leaves are rich in bioactive phytochemicals such as alkaloids, flavonoids, tannins phenolics and saponins with vast medicinal importance (18-20).

In folkloric medicines, *C. citratus* is used to aid digestion, weight loss as well as in the treatment of headache, malaria, hypertension, respiratory disorders and skin irritations (21-24). Leaves of *C. citratus* have been used for oral hygiene, management of menstrual disorders and as flavor enhancer in food and cosmetics (25-27). Other notable medicinal properties of *C. citratus* include: anti-carcinogenic (28), antibacterial (29), antiprotozoal (30), antifungal (31), anti-inflammatory (32-34), antioxidant (35), cardioprotective (36), antidiabetic(37,38) and anti-HIV(39).

In view of the versatile medicinal applications of *C. citratus* leaves coupled with the negative impact of CLD and CKD on public health and economic development, there is a dire need to identify a cheap, locally available

but potent therapeutic alternative to checkmate the menace of hepatorenal diseases. Hence, the need for this study.

Materials and Methods

Plant materials

Fresh *C. citratus* leaves were obtained within a local farm washed and air-dried. The air-dried leaves were then powdered, weighed and stored in an airtight container.

Reagents and chemicals

All reagents and chemicals were of high analytical grade obtained from standard commercial suppliers. All biochemical parameters were determined using Randox kit.

Extraction of the extract

C. citratus leaves were air-dried and ground to fine powder using a blender. Thirty percent (30%, w/v) of the powdered leaves was prepared in 80% ethanol and l eft for 72 h allow for extraction. The mixture was then filtered using cheese cloth to obtain a filtrate which was tightly covered with an insect-proof net and allowed for evaporation to dryness, and a residue which was discarded. After evaporation to dryness at room temperature, the crude extract obtained was weighed and kept refrigerated in an airtight glass petri-dish. The extract was then reconstituted with distilled water and administered to experimental animals.

Animals protocol

Forty albino rats weighing 150 g – 170 g were obtained from a reputable animal house facility. The experimental rats were housed in a clean, well aerated and spacious plastic cages under standard conditions for laboratory animal research. They were given unrestricted access to their feed and water *ad libitum*. Animal beddings rat beddings were turned over on a daily basis while the study lasted. A single intraperitoneal exposure to carbon tetrachloride (3 ml/kg bw) used to induce liver damage while 250 mg/kg bw of rifampin was administered orally depending on animal grouping as indicated in Table 1.0

Table 1.0: Experimental Design

Group	Treatment
I (Negative Control)	Oral administration of distilled water only
II (Positive control)	Single intraperitoneal injection of 3 ml/kg CCl_4 alone
III (Treated)	Treated with 50 mg/kg bw *C. citratus* after exposure to CCl_4
IV Treated)	Treated with 100 mg/kg bw *C. citratus* after exposure to CCl_4
V (Standard)	Treated with 100 mg/kg bw silymarin
VI (Positive Control 2)	Exposed to 250 mg/kg bw. rifampicin only
VII (Treated)	Treated with 50 mg/kg bw *C. citratus* after exposure to rifampicin
VIII (Treated)	Treated with 100 mg/kg bw *C. citratus* after exposure to rifampicin

Preparation of organs homogenate

Fourteen (14) days after the commencement of the experiment, experimental rats were fasted for 24 h prior to decapitation under very mild ether anesthesia. Dissection was rapidly performed following established protocols to obtain the liver, kidney and whole blood. The organs (liver and kidney) obtained were trimmed of fatty tissue, washed in saline, blotted with filter paper and weighed. Ten (10) percent homogenates of the tissues were prepared in 50 mM potassium phosphate buffer pH 7.4 in a Teflon homogenizer. The homogenates obtained were centrifuged at 3000 rpm for 30 min at 4 °C, to obtain a supernatant which was carefully decanted and stored at 4 °C for biochemical analyses.

Preparation of serum

Whole blood was collected by cardiac puncture and allowed to coagulate at room temperature. The coagulated blood was then centrifuged at 3000 rpm for 15 min to obtain a supernatant (serum) safely decanted, kept on ice and subsequently used for estimating selected biochemical parameters.

Assay for creatine kinase (Ck-Mb) activity

The method described by Mattenheimer (40) was adopted in the estimation of creatine kinase. Absorbance of the resulting mixture at 340 nm was monitored for 5 min at 30 sec interval.

Determination of aspartate aminotransferase (AST) activity

Aspartate aminotransferase activity was assayed as described by Reitman and Frankel (41). Absorbance of the resulting mixture was taken at 546 nm against the reagent blank. Enzymatic activity of AST was then obtained by interpolation.

Determination of alanine amino transferase (ALT) activity

The method previously described by Reitman and Frankel (41) was adopted for the determination of ALT activity using Randox kit. Absorbance of the solution was then taken at 546 nm. Activity of ALT activity was obtained by interpolation.

Determination of alkaline phosphatase (ALP) activity

Enzymatic activity of ALP was measured according to the method of Englehardt (42). Activity of ALP was then obtained by interpolation according to manufacturer's instructions.

Estimation of total cholesterol level

Total cholesterol level was measured based on established method of Trinder (43). Amount of cholesterol was determined as indicted below:

$$\text{Cholesterol concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Concentration of standard}$$

Evaluation of concentration of triglyceride

Amount of triglycerides was determined as described by Tietz (44). Absorbance was measured at 546 nm against the blank, while amount of triglyceride was determined as indicated below:

$$\text{Triglyceride concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Concentration of standard}$$

High density lipoprotein (HDL-c)-cholesterol assay

Amount of HDL-cholesterol was measured according to Grove (45).

Low density lipoprotein (LDL) - cholesterol determination

Level of low-density lipoprotein in the samples (serum, liver and kidney homogenates) was calculated according to Friedwald *et al.* (46):

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

Determination of catalase activity

Activity of catalase activity was determined according to Sinha (47). Hydrogen peroxide content of the withdrawn sample was determined as described below.

$$\text{Catalase activity} = \frac{(\text{H2O2 Consumed})}{(\text{mg protein})}$$

H_2O_2 consumed = 800 – Concentration of H_2O_2 remaining

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was estimated according to the method of Misra and Fridovich (48). Absorbance of the resulting solution was monitored at 480 nm for 150 s at 30 s interval.

Determination of reduced glutathione (GSH) level

Reduced glutathione level was estimated as described by Beutler and Kelly (49). Absorbance of the resulting solution was then taken at 412 nm against reagent blank.

Determination of total protein (TP) in serum

Total protein content of samples (serum, liver and kidney homogenates) was determined as described by Weichselbaum (50).

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) content in the samples (serum, liver and kidney homogenates) were estimated as described by Okhawa *et al*. (51) using Randox kits. Absorbance of the supernatant 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

Data are expressed as mean ± SEM. 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 and Discussion

Exposure of experimental animals to CCl_4 and rifampicin in this study (Table 1) resulted to a significant increase in both renal and hepatic cholesterol relative to the negative control animals (Table 2). A similar trend of result was obtained for triglyceride and LDL relative to the negative control (Table 2). On the other hand, there was a significant depletion in hepatic and renal HDL in rats exposed to both toxicants without treatment relative to the negative control. Post administration of *C. citratus* leaf extract restored the distorted lipid profile in the liver and kidney to level comparable with animals that were not exposed to toxicants at all, howbeit, in a dose-dependent manner.

Hepatic catalase and superoxide activities were significantly inhibited by CCl_4 and rifampicin in the liver and kidney of exposed animals relative to negative control rats. Treatment by oral administration of *C. citratus* extract relieved the inhibition imposed in a comparable manner to animals treated with silymarin (Table 3).

Activities of aspartate aminotransferase (AST), alanine amino transferase (ALT) in the serum, liver and kidney of animals exposed to CCl_4 and rifampicin were significantly increased relative to negative control (Table 4). Oral administration of *C. citratus* extract at 50 and 100 mg/kg bw restored hepatorenal level AST, ALT and ALP in a manner similar to animals treated with silymarin (Table 4).

Urea, uric acid and creatine kinase levels were markedly increased in the serum and kidney of experimental rats exposed to CCl_4 and rifampicin (Table 5). Treatment with *C. citratus* extract showed a restorative trend in urea, uric acid and creatine kinase levels comparable to exposed animals that were treated with silymarin-the standard drug (Table 5). There was a surge in lipid peroxidation as measured by MDA level in the liver, kidney and serum of animals exposed to CCl_4 and rifampicin toxicity relative to negative control (Figure 1 A and B). Post administration of *C. citratus* extract significantly inhibited lipid peroxidation in a dose dependent manner comparable to animals treated with silymarin. Reduced glutathione (GSH) was significantly diminished in the serum of rats exposed to toxicants without treatment with extract (Figure 2A and B). Treatment with *C. citratus* extract restored hepatic GSH in a dose dependent fashion relative to negative control. Figures 3(A-D) show the hepatic tissue slices of experimental albino rats showing histomorphological manifestation of the hepatocytes, density of hepatocytes (black arrow head), distribution of hepatocytes, staining intensity of hepato-

cytes, size of central veins, content of central veins and expression of large vacuolations (dotted black circles). Figures 3(E-G) show the photomicrographs of the kidney tissue slice of rats under high power magnification (x400 mag) of renal corpuscle (black outline) which houses the glomerulus within the urinary space that is supplied by the afferent arteriole and drained by the efferent arteriole. The histomorphology presents with the convoluted tubule (CT), glomerular capillaries (GC) and inherent cells which include the intraglomerular podocytes (P) as well as the juxtaglomerular cells and macula densa cells in the vascular poles of the renal corpuscles. The urinary pole continues out as the proximal convoluted tubules.

Table 2: Effects of *C. citratus* extract on hepatic and renal lipid profile of CCl_4 and rifampicin-exposed rats

Lipid Profile	Tissues	Normal control	CCl_4 (3 ml/kg bw) only	CCl_4 + *C. citratus* (50 mg/kg bw)	CCl_4 + *C. citratus* (100 mg/kg bw)	CCl_4 + silymarin (100 mg/kg bw)	Rifampicin (250 mg/kg bw) only	Rifampicin + *C. citratus* (50 mg/kg bw)	Rifampicin + *C. citratus* (100 mg/kg bw)
T.C. (mg/dl)	Liver	56.08± 2.09[a]	89.12 ± 1.73	80.47±2.12[a]	67.29±3.06[a]	52.33±1.34[a]	97.23±1.42	82.23±2.10[a]	62.51±2.02[a]
	Kidney	30.07± 2.18[a]	41.86 ± 2.09	33.22±2.03[a]	27.63±1.97[a]	30.07± 1.05	53.24±1.03	40.36±1.11[a]	33.56±0.94[a]
	Serum	52.16± 2.19[a]	72.44 ± 1.86	61.32±1.36[a]	56.82±1.45[a]	58.27±1.56[a]	87.46±1.38	64.03±1.02[a]	59.34±2.16[a]
TG (mg/dl)	Liver	41.33±1.07[a]	70.25± 1.24	61.26±1.76[a]	52.45±1.90[a]	39.43±1.13[a]	86.50±0.42	77.49±1.13[a]	68.24±0.93[a]
	Kidney	11.82±0.34[a]	19.47± 0.42	14.06±0.77[a]	12.93±0.64[a]	13.43±0.29[a]	26.48±0.33	21.17±0.64	13.49±0.73[a]
	Serum	37.51±1.39[a]	61.41± 1.28	50.14±1.07[a]	42.66±1.33[a]	40.14±1.72[a]	70.39±1.45	58.20±1.68[a]	44.56±1.49[a]
HDL (mg/dl)	Liver	24.72± 0.44[a]	46.07 ± 0.53	24.48±0.86[a]	31.33±1.43[a]	26.31±0.59[a]	18.26±0.24	19.60±0.43	20.41±0.54[a]
	Kidney	8.26 ± 0.17[a]	5.31 ± 0.87	7.63±0.42[a]	8.02±0.53[a]	8.42±0.68[a]	6.08±0.63	6.39±0.59	6.82±0.43[a]
	Serum	9.42 ± 0.31[a]	6.37 ± 0.43	6.89±0.27	7.03±0.20[a]	8.79±0.29[a]	5.10±0.07	6.93±0.05[a]	7.42±1.03[a]
LDL (mg/dl)	Liver	33.76±1.27[a]	41.27 ± 1.53	37.04±1.39[a]	35.09±1.27[a]	33.04±0.62[a]	57.81±0.11	46.17±0.21[a]	41.21±0.14[a]
	Kidney	13.26± 0.73[a]	21.60 ± 0.82	14.32±0.50[a]	12.16±0.80[a]	12.13±0.48[a]	31.27±0.57	23.04±0.31[a]	18.13±0.36[a]
	Serum	22.36± 0.32[a]	34.26 ± 0.53	32.46±0.55	30.36±0.32[a]	24.67± 0.72[a]	39.53±0.68	23.04±0.31[a]	18.13±0.31[a]

Data show mean ± SEM of three experiments performed in triplicate.

Table 3: Effects of *C. citratus* extract on selected antioxidant enzymes (superoxide dismutase and Catalase) in the liver and kidney of CCl_4 and rifampicin -exposed rats

Enzyme marker	Tissues	Normal control	CCl_4 (3 ml/kg bw) only	CCl_4 + *C. citratus* (50 mg/kg bw)	CCl_4 + *C. citratus* (100 mg/kg bw)	CCl_4 + silymarin (100 mg/kg bw)	Rifampicin (250 mg/kg bw) only	Rifampicin + *C. citratus* (50 mg/kg bw)	Rifampicin + *C. citratus* (100 mg/kg bw)
SOD (U/mg protein)	Liver	6.14±0.31[a]	4.53±0.22	5.13±0.16	5.31±0.25[a]	6.20±1.23[a]	2.36±0.19	2.47±0.10	3.62±0.21[a]
	Kidney	3.48±1.03[a]	2.46±0.75	2.51±0.62	2.53±0.49	3.15±0.53[a]	1.93±0.19	2.02±0.13	2.41±0.20[a]
	Serum	4.29±0.19[a]	3.17±0.10	3.29±0.12	3.53±0.10[a]	4.34±0.21[a]	2.42±0.07	2.76±0.06	3.11±0.10[a]
Catalase (U/mg protein)	Liver	4.70±0.56[a]	2.89±0.21	2.96±0.30	3.04±0.41[a]	4.45±1.20[a]	1.63±0.16	1.88±0.20	2.76±0.20[a]
	Kidney	2.77±0.15[a]	2.02±0.18	2.32±0.20[a]	2.47±0.11[a]	2.58±0.20[a]	1.88±0.26	2.16±0.12[a]	2.53±0.10[a]
	Serum	3.06±0.11[a]	2.38±0.10	2.44±0.21	2.91±0.17[a]	2.93±0.20[a]	1.76±0.14	1.91±0.10	2.18±0.08[a]

Data show mean ± SEM of three experiments performed in triplicate.

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Table 4: Effects of *C. citratus* extract on selected biomarkers (AST, ALT, ALP and T. BIL.) in the liver and kidney of CCl4 and rifampicin - rats

Enzyme marker	Tissues	Normal control	CCl_4 (3 ml/ kg bw) only	CCl_4 + *C. citratus* (50 mg/kg bw)	CCl_4 + *C. citratus* (100 mg/kg bw)	CCl_4 + silymarin (100 mg/kg bw)	Rifampicin (250 mg/kg bw) only	Rifampicin + *C. citratus* (50 mg/kg bw)	Rifampicin + *C. citratus* (100 mg/kg bw)
AST (IU/l)	Liver	54.33±2.31[a]	82.31±2.47	77.76±1.65	56.27±1.48[a]	57.23±1.72[a]	96.14±2.41	69.10±1.1[5]	54.27±2.06[a]
	Kidney	21.60±2.07[a]	37.81± 1.02	34.71±1.01	30.37±1.00[a]	25.06±1.25[a]	56.82±1.73	38.16±1.29[a]	22.63±1.31[a]
	Serum	71.26±1.71[a]	115.63± 1.67	106.24±2.17	82.49±1.51[a]	75.22±1.56[a]	127.13±2.60	93.71±1.24	68.14±1.38[a]
ALT (IU/l)	Liver	40.71± 1.32[a]	64.21 ± 1.43	62.17±1.23	55.09±1.33[a]	51.33±1.27[a]	77.23±1.42	58.08±1.24[a]	40.13±1.18[a]
	Kidney	17.27± 0.25[a]	30.44 ± 0.97	26.29±0.82	25.32±1.04[a]	23.63±1.03[a]	40.29±1.77	29.55±0.65[a]	18.24±0.57[a]
	Serum	56.17± 1.98[a]	92.76 ± 1.23	84.80±0.92	76.31±0.81[a]	59.33±1.34[a]	88.45±2.43	62.34±2.70[a]	59.16±2.03[a]
ALP (IU/l)	Liver	47.25 ± 1.92[a]	58.78±1.63	51.34±1.09[a]	48.98±2.17	46.88±1.39[a]	73.84±0.81	65.60±0.66	45.00±0.60[a]
	Kidney	31.21 ±1.25[a]	48.17 ± 0.84	42.34±0.76	37.72±0.81[a]	29.32±0.90[a]	53.66±1.98	38.40±0.83[a]	31.66±1.05[a]
	Serum	62.13 ± 3.12[a]	102.43 ± 4.20	93.41±2.08	86.01±1.79[a]	67.11±2.09[a]	108.52±1.32	70.23±1.21[a]	63.56±1.14[a]

Data show mean ± SEM of three experiments performed in triplicate.

Table 5: Effects of *C. citratus* extract on selected biomarkers (urea, uric acid, bilirubin and creatine kinase in the liver and kidney of rats after exposure to CCl_4 and rifampicin toxicity

Biomarker	Tissues	Normal control	CCl_4 (3 ml/kg bw) only	CCl_4 + *C. citratus* (50 mg/kg bw)	CCl_4 + *C. citratus* (100 mg/kg bw)	CCl_4 + silymarin (100 mg/kg bw)	Rifampicin (250 mg/kg bw) only	Rifampicin + *C. citratus* (50 mg/kg bw)	Rifampicin + *C. citratus* (100 mg/kg bw)
Urea (mg/ dl)	Kidney	47.82± 0.93[a]	76.32 ± 0.88	63.08±0.67	58.12±1.31[a]	53.76±1.61[a]	70.06±0.83	64.07±1.19	55.38±0.85[a]
	Serum	38.77 ± 0.83[a]	77.64 ± 1.36	62.72±1.57	51.26±0.89[a]	45.26 ± 0.76[a]	64.39±0.62	53.39±0.62	37.08±0.37[a]
Uric acid (mg/dl)	Kidney	29.54± 1.52[a]	42.65± 0.82	34.09±1.0[8]	33.56±0.96[a]	32.89± 1.62	54.23±0.78	34.42±0.67[a]	30.56±0.48[a]
	Serum	19.17 ± 0.24[a]	33.51 ± 0.52	30.20±0.67	27.68±0.59[a]	21.17 ± 0.68[a]	35.43±0.52	29.54±0.30	22.71±0.41[a]
Creatine kinase (IU/L)	Kidney	27.61± 1.07[a]	38.44± 0.87	32.63±1.41[a]	29.60±0.90[a]	25.73± 0.85[a]	49.17±0.94	33.65±0.84[a]	28.06±0.79[a]
	Serum	20.33 ± 0.30[a]	31.70 ± 0.29	26.17±0.63	24.00±0.51[a]	23.66 ± 0.22[a]	44.58±0.44	29.20±1.20[a]	19.17±0.60[a]
T. bilirubin (mg/dl)	Liver Serum	31.18±4.04[a] 42.25±1.43[a]	44.37± 3.54 72.18 ± 1.54	38.61±2.00 64.06±1.07[a]	35.14±2.08 69.08±1.08	31.72±3.82 45.37±1.84[a]	56.18±0.54 69.30±1.02	40.53±0.52[a] 51.53±0.59[a]	34.71±0.83[a] 41.05±0.81[a]

Data show mean ± SEM of three experiments performed in triplicate.

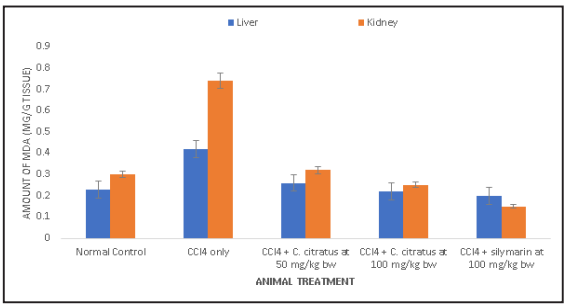


Figure 1A: Effect of *C. citratus* leaf extract on the MDA level in the liver and kidney of CCl_4-exposed rats. Data show mean ± SEM of three experiments performed in triplicate

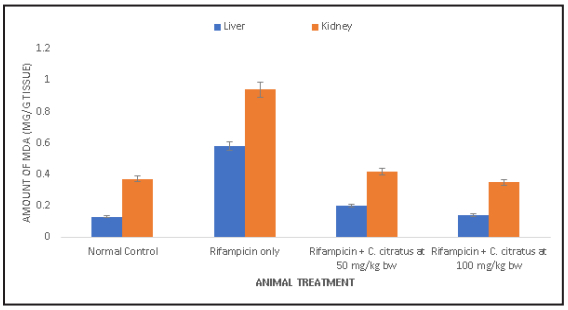


Figure 1B: Effect of *C. citratus* leaf extract on the MDA level in the liver and kidney of rifampicin- exposed rats. Data show mean ± SEM of three experiments performed in triplicate

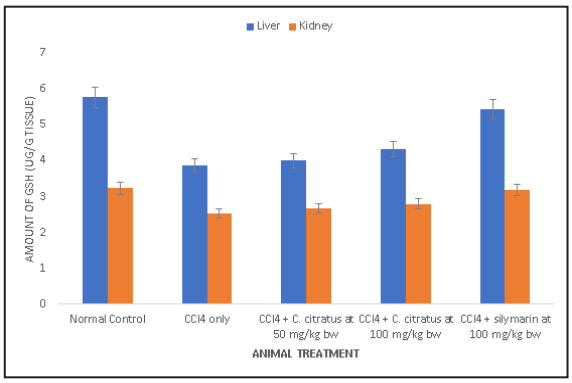


Figure 2A: Effect of *C. citratus* leaf extract on amount of GSH in the liver and kidney of CCl4-exposed rats. Data show mean ± SEM of three experiments performed in triplicate.

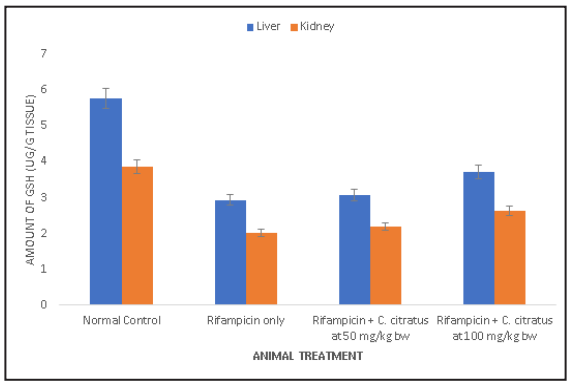
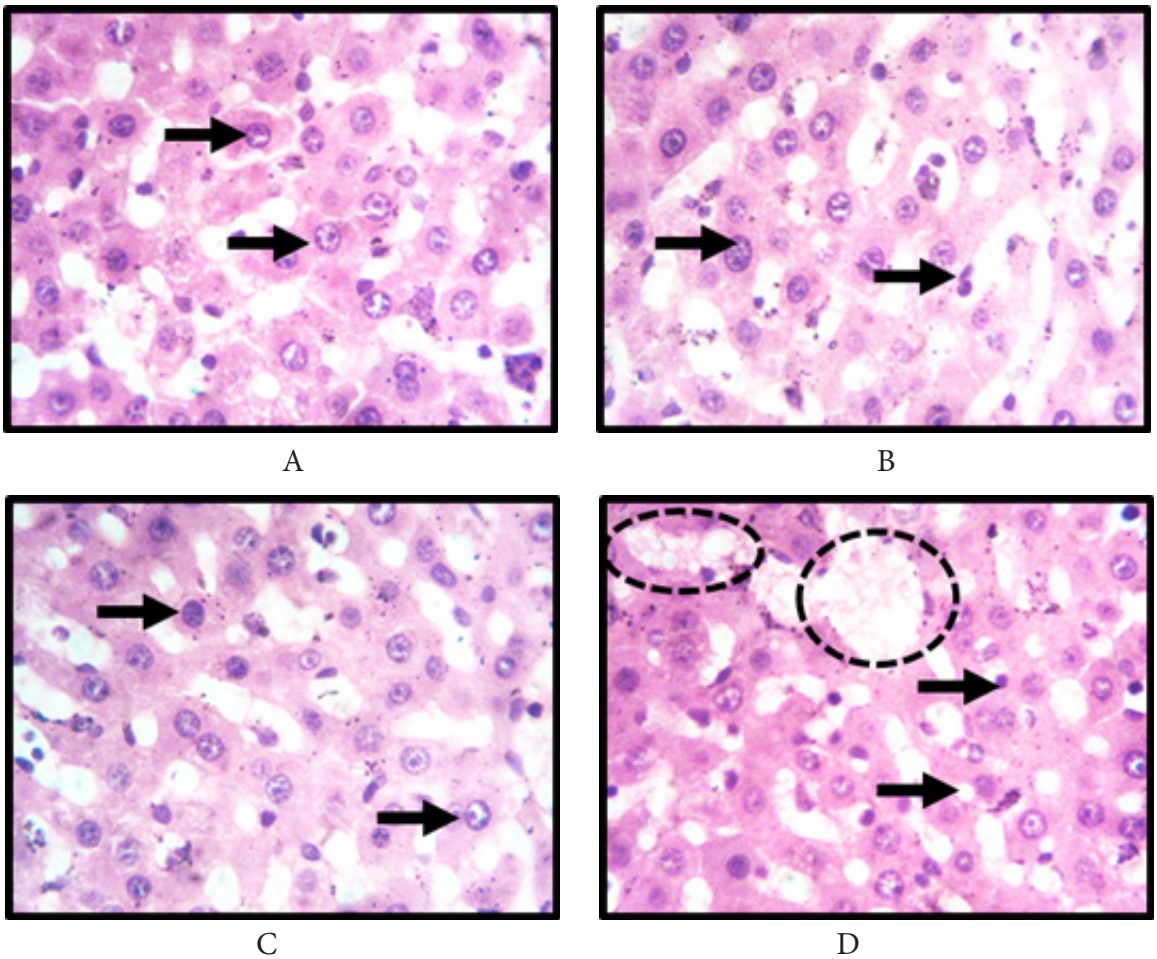
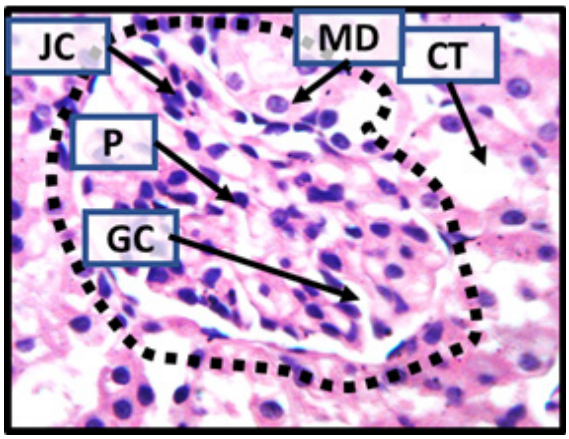


Figure 2B: Effect of *C. citratus* leaf extract on amount of GSH in the liver and kidney of rifampicin- exposed rats. Data show mean ± SEM of three experiments performed in triplicate.



Cymbopogon citratus leaf extract mitigates hepatorenal injury in carbon tetrachloride and rifampicin-exposed rats



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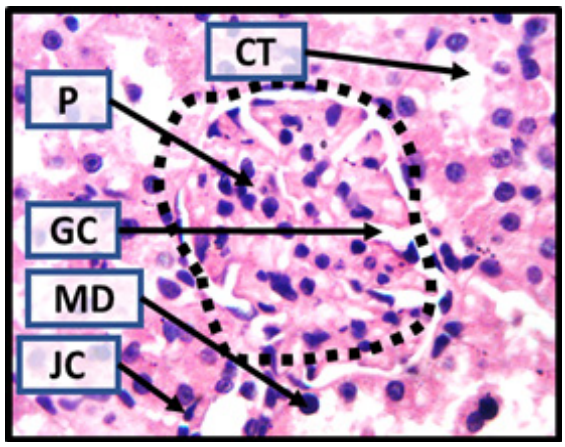

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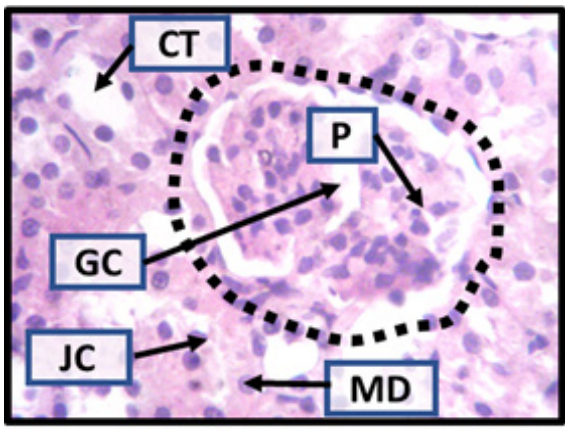

G

A- liver slice of animals fed with animal feed and distilled water only; The nuclei of hepatocytes are distinctively stained and properly disposed within their respective cytoplasm. There are no histopathological alterations in the histological presentation of these tissues.

B- liver slice of animals administered with 3ml/kg CCl_4, without treatment; it shows fatty liver with cholestasis C- liver slice of animals induced with 3ml/kg CCl_4 and treated with 100mg/kg C. citratus leaf extract; It shows normal histomorphology of liver tissue presenting with typical cellular density and cellular distribution. The nuclei of hepatocytes are distinctively stained and properly disposed within their respective cytoplasm. There are no histopathological alterations in the histological presentation of these tissues.

D - liver slice of animals administered with 3ml/kg CCl_4 and treated with 100mg/kg silymarin; It shows normal histomorphology of liver tissue presenting with typical cellular density and cellular distribution. The nuclei of hepatocytes are distinctively stained and properly disposed within their respective cytoplasm. There are no histopathological alterations in the histological presentation of these tissues

E - kidney slice of animals administered with distilled water only; it shows normal renal corpuscle with typical cellular delineation, distribution, density and staining intensity. No apparent histopathological alteration

F - kidney slice of animals administered with 250mg/kg rifampicin only without treatment; Distortion of the renal architecture and atrophy of glomeruli was observed. The renal tubules were degenerated and showed intraluminal exfoliation with granular cast formation as well as pyknosis of the nuclei.

G- kidney slice of animals administered with 250mg/kg rifampicin and treated with 100 mg/kg bw of C. citratus leaf extract. It shows normal renal corpuscle with typical cellular delineation, distribution, density and staining intensity. No

apparent histopathological alteration

Discussion

Oxidative stress has been identified as the major culprit with established intrinsic relationship to the onset and complications of almost all known diseases. Hence, several animal models of existing pathological conditions have been created to enhance robust research investigation and possible non-conventional therapeutic intervention for the management of those conditions (52). Considering the critical role of membrane integrity in biochemical homeostasis, the profile of lipids in the physiological system must be sustained, since a distortion can result in the compromise of membrane function. In the present study (Table 1), total cholesterol, triglycerides and LDL were markedly elevated while HDL level was significantly depleted relative to compared to negative control animals (Table 2). This result agrees with Abu *et al*. (53) and suggests the hepatorenal toxicity of CCl_4 and rifampicin. Carbon tetrachloride is metabolized in the liver into a reactive metabolite, causing the activation of nuclear factor kappa B (NF-kB), which in turn triggers the expression of specific proinflammatory cytokines in the liver thereby wrecking havocs (54). The noticeable distortion in lipid profile in the present study can be linked to free radical induced oxidative stress caused by exposure to CCl_4 and rifampicin. Treatment with C. citratus extract, caused a significant restoration of lipid profile in the liver and kidney of exposed rats (Table 2). This result agrees with Jeonghyeon *et al*. (54) and possibly suggests the potential usefulness of C. citratus leaf extract in the management of hepatorenal diseases. This effect is also a function of the phytoconstituents of the leaf extract es earlier identified by Kiani *et al*. (55).

Detailed bioactive constituents of ethanolic extract of C. citratus leaf has been reported (55) . Several reports have linked hypolipidemic potentials of plant to the avalanche of bioactive phytochemicals present in their leaf extracts (55). Generally, flavonoids regulate the level of cholesterol and triglycerides in the blood thereby reducing the risk of cardiovascular diseases including atherosclerosis (55) . Perhaps, the presence of flavonoids in C. citratus leaf extract can be attributed to its cholesterol-lowering potential observed in the study.

Moreover, other reports have suggested the anti-inflammatory potentials of flavonoids and other polyphenols (56). Flavonoids cause a down regulation in the expression of inflammatory transcription factors by activating transcription factor-3, activator protein-1 and CREB binding proteins, which collectively arrest the expression of NF-kB thereby inhibiting inflammation subsequently averting hepatorenal injury. Since, inflammation is involved in the hepatorenal assault, we can imply that the presence of polyphenols in C. citratus extract is key to its restorative potentials in intoxicated animals as earlier suggested (55). On the other hand, the toxicity of rifampicin is thought to occur by lipid peroxidation and inhibition of key phase I and II detoxification enzymes. Rifampicin is bioactivated in the liver to deacetyl rifampicin, an intermediate with strong covalent affinity for critical macromolecules in the nephrons and hepatocytes (57). Treatment with C. citratus extract reversed the derangement in renal lipid profile in a dose dependent manner in a manner (Table 2). This result agrees with the report of Samvedana (58) and can be linked to the presence of substantial amount of polyphenols with potent antioxidant properties in its leaf extract (56).

Antioxidant enzymes such as superoxide dismutase and catalase help in scavenging free radicals thereby preserving critical macromolecules in their biologically useful form. In the present study, animals exposed to CCl_4 and rifampicin without post treatment with C. citratus extract showed significant inhibition of SOD and catalase relative to those that were not exposed to the toxicants at all (Table 3). Obviously, the inhibition was caused by the deleterious interaction between reactive intermediates of CCl_4 and rifampicin metabolism and critical functional groups at the active sites of these enzymes

resulting in inactivation of the enzymes (57). Treatment of exposed animals with C. citratus extract restored the activity of these enzymes (Table 3). This restoration can be linked to the presence of polyphenols present in the extract as earlier reported by Kiani *et al.* (55).

Exposure to CCl_4 and rifampicin caused a significant increase in the level of some biomarkers of kidney function such as ALT, AST and ALP in the liver and kidney of experimental animals relative to the control (Table 4). Under normal condition, the level of AST, ALT and ALP are very low in the blood. This is because these enzymes are compartmentalized within the liver hepatocytes. However, when exposed to free radical assault, membranes of these cell are damaged, leading to their leakage to the blood resulting in their elevation. This observation suggests that an injury in the liver and kidney of animals exposed to the toxicant without post treatment with the extract. When treated with *C. citratus* extract after initial exposure, levels of these biomarker enzymes returned to a level similar to animals that were not exposed to the toxicants at all (Table 4). This finding agrees with Samvedana (58) and can possibly be attributed to the presence of myriads of phytochemicals, particularly flavonoids, that have been earlier reported in the leaf extract of *C. citratus* (55)

The deleterious effect of lipid peroxidation on human health and its intricate link with several pathological conditions has been reported (54). In the present study, there was a significant increase in MDA level in the serum, liver and kidney homogenates of animals exposed to toxicants without post treatment with extract of *C. citratus* leaf (Figure 1A and B). This observation was an indication that the reactive intermediates of CCl_4 and rifampicin metabolism exhibits their toxic effects via lipid peroxidation (57). Post treatment of exposed animals with extract of *C. citratus* leaf extract restored MDA level to that comparable with animals that were not exposed at all (Figure 1). This suggests that one of the mechanisms of antioxidative effect of *C. citratus* leaf is via inhibition of lipid peroxidation and this can partly be attributed to antioxidant phytochemicals present in the extract.

The health status of an organism can be determined by the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) in its physiological system. The higher the ratio the healthier an organism all things being equal. In the present study, exposure to toxicants resulted in marked depletion in GSH, suggesting vulnerability to oxidative stress (Figure 2A and B). However, treatment of exposed rats with extract of *C. citratus* caused a restoration of GSH in a manner comparable to animals treated with silymarin. This suggests that specific phytochemicals present in the extract trigger the expression of GSH in response to free radical's onslaught caused by the toxicants.

Bilirubin level can provide useful clinical information about the health status of patients with liver disease. Reports have suggested a strong correlation between serum bilirubin and chronic liver disease (59,60). In the present study, exposure of animals to CCl_4 and rifampicin respectively resulted in a marked elevation in serum bilirubin (Table 5), which suggests a possible hepatocellular a damage. However, treatment with *C citratus* extract resulted in a dose-dependent restoration of bilirubin in a manner comparable to animals treated with silymarin after initial exposure to toxicants (Table 5). This observation agrees with and can be attributed to an avalanche of antioxidant phytochemicals present *C. citratus* leaf extract.

Urea has been suggested as a useful marker and diagnostic tool of kidney's integrity or compromise (61). Exposure to CCl_4 and rifampicin caused a marked increase in serum urea level of animals exposed to toxicants relative to negative control (Table 5). This is perhaps a free radical-induced assault on the glomerulus which possibly affects its integrity culminating in urea retention. *C citratus* treatment restored the serum urea level in a dose-dependent manner similar to normal animals. This observation agrees with earlier reports (59,60) and suggests

the potential of *C. citratus* leaf extract in the management of hepatorenal diseases.

Uric acid is another versatile diagnostic tool for the measurement of kidney function considering its pivotal role in the onset of gout, diabetes and renal failure (62). Animals exposed to CCl_4 intoxication exhibited significantly higher serum uric acid relative to animals that were not exposed at all (Table 5). This observation is possibly due to free radical induced upregulation in the purine metabolism. Post treatment with *C. citratus* leaf extract restored uric acid level in the serum and kidney homogenates to a level comparable to animals that were not exposed to the toxicants at all (Table 5). The restorative ability of *C. citratus* extract can possibly be linked to an inhibition of excessive purine degradation probably due to the presence of polyphenols in the extract.

One of the biomarkers of cardiac injury is creatine kinase. Its serum level is usually elevated in myocardial necrosis. Exposure to CCl_4 caused a marked elevation in serum creatine kinase, relative to animals that were not exposed to the toxicants (Table 5). In the present study, serum creatine kinase was significantly increased in animals exposed to toxicants, suggesting that the toxicants are also toxic to the heart. *C. citratus* extract restored the creatine kinase activity in a manner similar to the negative control and intoxicated animals treated with silymarin. Probably, this effect is due to a myriad of phytochemicals present in *C. citratus* extract.

Histopathological findings from the liver and kidney slices (Figures 3A-G) exhibited marked distortion in hepato- renal histoarchitecture coupled with severe necrosis, inflammation and central vein congestion. vein in animals exposed to CCl_4 and rifampicin without post treatment with *C. citratus* leaf extract. These notable histoarchitectural distortion is probably due to free-radical induced hepatic and renal damage. These findings are in agreement with previous report (55). Post administration of *C. citratus* leaf extract to intoxicated animals reversed the histoarchitectural distortions, thereby restoring normal liver and kidney histoarchitecture. This suggests that extract of *C. citratus* leaf contains phytochemicals that could reverse pathological injury on the liver and kidney.

Conclusion

C. citratus leaf extract relieved the inhibition imposed on antioxidant enzymes, restored distorted liver and kidney biomarkers as well as ameliorated deranged lipid profile. Distorted histoarchitecture of the liver and kidney slices was reversed by the administration of *C. citratus* extract. There is a strong correlation between biochemical and histopathological findings, giving credence to the potential of *C. citratus* leaf extract in the management of liver and kidney diseases. Hence, the plant can be used as a potent, locally available and cheap source for the management of liver and kidney diseases.

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