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Extracts of Ageratum *Conyzoides* L. Protects against Carbon Tetrachloride – Induced Toxicity in Rats through Inhibiting Oxidative Stress

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study was aimed at investigating the hepatoprotective and nephroprotective activities of 50% hydroethanolic leave extracts of the *Ageratum conyzoides* and fractions on carbon tetrachloride - induced hepatotoxicity in rats.
Study Design: A total of 30 Sprague Dawley rats were used in this study with six groups of five animals each.
Place and Duration of Study: Department of Biochemistry and Biotechnology, College of Science, Kwame Nkrumah University of Science and Technology, between April 2016 and July 2017.
Methodology: Extracts were characterised by basic phytochemical screening, FTIR, GC-MS, DPPH and Folin-Ciocalteau assays. Hepatoprotective activities were assessed using the CCl_4 model (1 ml/kg) and extracts tested at 250 mg/kg bwt and Silymarin as standard drug (100 mg/kg bwt). Serum liver and kidney function, as well as antioxidant enzymes (SOD, CAT, GSH, MDA and MPO) in liver and kidney homogenate were assayed. Histological examinations were made on the livers and kidneys.

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Results: Results showed the extract treatments resulted in significant increase in SOD, CAT and GSH levels and a significant decrease in MDA and MPO level against CCl_4 both in liver and kidney as well as the restoration of kidney and liver function to near normal levels. Biochemical data was corroborated by histological observations.
Conclusion: The present investigation suggests that *A. conyzoides* crude extract possesses remarkable hepato- and nephroprotective properties and this can be attributed to the inhibitory effect on oxidative stress.

Keywords: Ageratum conyzoides; hepatoprotective; nephroprotective; SOD; MPO.

1. INTRODUCTION

Liver, an important organ in the human body is responsible for metabolism and excretion. It is responsible for the metabolism of carbohydrate, protein, fat and detoxification unwanted substance from the body [1]. Viral infection, immune system abnormality, and cancer are considered to directly or indirectly result in liver diseases. Free radicals, in the form of reactive oxygen species [2], appear to be one of the fundamental mechanisms underlying this disease [3]. According to the World Health Organization [4], 46% global diseases recorded are of liver origin and about 35 million people worldwide die of such diseases [5].

In recent years, liver diseases have increased rapidly [6]. In addition, its development has also become a global issue and their treatment and management mostly result in more economic problems, especially in the African population. Many side effects are also related with synthetic drugs used in the treatment of hepatic disorders. There is, therefore, the need to explore other natural products of plant origin for their hepatoprotective effect and further evaluate their modulatory effect on endogenous liver antioxidant enzymes. Traditional herbal plants have been used for various disease treatment for several years due to their low toxicity factor, thus resulting in a lower side effects. Many drugs currently available were derived from the medicinal plant either directly or indirectly. Global interest in natural therapies and alternative medicines has made scientists and researchers to pay much attention to traditional medicine, especially in Africa. Hence, it has become necessary to presently investigate *A. conyzoides* for its hepatoprotective, antioxidant and phytochemical constituents.

Ageratum conyzoides is herbal plant belonging to the Asteraceae family. It's described as an invasive weed in many regions and commonly known as billy goat weed. In Nigeria, especially among the Yoruba natives of south-western Nigeria, it is referred as 'imiesu' [6], in Brazil as 'catingo do bode' [7], and in Ghana as 'adwowakuro' by Akans, 'efoe momoe' by the Fantes, and 'gu-akuro' by the Asantes. Its antioxidant, insecticidal, anti-inflammatory, gastroprotective, haemostatic and analgesic properties have also been reported [8]. Studies on the plant specie from Ghana have been demonstrated to contain terpenoids, tannins and coumarins and exhibited significant inhibitory activity against leukemic (Jurkat) and prostate (LNCap) cell lines [9]. Many different bioactive principles have also been identified and isolated in *A. conyzoides* such as kaempferol and glycoside (rhamnoside), quercetin, scutellarein and ageratochromene derivatives [10]. A novel methoxy-flavone have also been isolated and demonstrate to induce caspase-3 and 7 activations in Jurkat cells [11].

In the present study, we assessed the *in vivo* protective activity of hydroethanolic crude extracts as well as solvent fractions of the leaves of *A. conyzoides* on both the liver and kidney tissues of rats.

2. MATERIALS AND METHODS

2.1 Reagent

Solvents of analytical grade were obtained from Merck and Aldrich. All chemicals for antioxidant studies including Silymarin were purchased from Sigma-Aldrich, Germany. Chemicals for biochemical determination were also purchased from Fortress Diagnostics, UK. All experiments were conducted at the Department of Biochemistry and Biotechnology, Central Laboratory, Department of Physiology, SMS all at KNUST, Kumasi-Ghana.

2.2 Plant Material

A. conyzoides leaves were handpicked from the land around Atwea Mountain, Ashanti region, Ghana. The plant samples were authenticated based on a voucher specimen from Acheampong

et al. [9] study (KNUST/HMI/2014/WP005) deposited at the herbarium of the Department of Pharmacognosy, KNUST, Kumasi. Preparation of the plant samples was done only on the leaves. They were washed with water three times and air-dried at room temperature for duration of two weeks. The dried samples were pulverised using a grinding mill (Christy Lab Mill, England).

2.3 Extraction and Fractionation of the Plant Material

One hundred gram of powdered sample of *A. conyzoides* leaves were macerated using 1000 ml of 50% hydroethanolic solution. It was shaken continuously with a shaker (Gallenkamp, England) for 24 hours at 25°C. The supernatant was filtered by centrifugation for 20 minutes at a speed of 1106 x g at room temperature. The residue was reextracted and supernatants were pooled together and concentrated at 50°C using a rotary evaporator (Buchi R- 205, Switzerland). Extract was freeze dried (Labconco, England) and designated as *A. conyzoides* crude extract.

A mass of 15 g of hydroethanolic crude leaf extract was dissolved in 150 ml of ethanol: Water (50:50) mixture and successively partitioned with ethyl acetate and finally with methanol. For each fractionation step, extraction was performed twice with 300 ml of the solvents. The remaining portion was designated as hydro (water) fraction. The fractions were then concentrated under reduced pressure using a rotary evaporator (at temperatures of 69°C, 77°C and 79°C respectively). Ethyl acetate and methanol fractions were air-dried at a temperature of 25°C while the hydro fraction was freeze dried.

2.4 Qualitative Phytochemical Screening

Methods described by Ayoola et al. [12] was used in determining the phytochemicals of the crude extract and it fractions with a slight modification. Phytochemicals analysed included terpenoids, saponins, tannins, alkaloids, flavonoids, glycosides, and phytosterols.

2.4 *In vitro* Antioxidant Activity

The free radical scavenging activity was assessed using the DPPH assay as described by Brand-Williams et al. [13]. Extracts were prepared by dissolving 10 mg in 1 ml of respective solvents followed by serial dilution to obtain concentration range of 0.156–10 mg/ml. Also, stock solution of 10 mM ascorbic acid was prepared by dissolving 2.2 mg in 1 mL distilled water while 0.5 mM DPPH was prepared using 3 mg in 15 mL absolute methanol. Prepared solutions were vortexed to ensure complete dissolution. DPPH solution was kept in a dark immediately to prevent photo-bleaching in light.

The reaction was made in a 96 well plate and made up of 100 μL of 0.5 mM DPPH, and 100 μL of each concentration of the test sample. The standard, ascorbic acid, was used at a concentration range of 0.156–10 mM in distilled water. The solvents, methanol, ethyl acetate, and distilled water were used as blanks. Triplicate experiments were performed. The plates were covered with aluminium foil, shaken gently and kept in the dark for 20 minutes after which the absorbance was read on a Tecan-PC infinite M200 Pro Plate reader (Austria), at a wavelength of 517 nm. Percentage scavenging activity was determined. The mean percentage antioxidant activity for the triplicate experiment was plotted for the standard and samples and their effective concentration at 50% (EC_{50}) values calculated.

The total phenolic content was assayed using modified method previously described [14]. Absorbance was read at 750 nm using microplate spectrophotometer. The total phenolic content of each extract of *A. conyzoides* was calculated from the calibration curve of the gallic acid ($y = 0.0134x-0.0003$, $R^2 = 0.9986$) and expressed as gallic acid equivalents (GAE).

2.5 FT-IR Analysis

Fourier transform infrared spectrophotometer (FTIR, PerkinElmer, USA) was used to identify the various chemical bonds available in the compounds. 10 mg each of dried extracts were encapsulated in 100 mg of KBr pellet, to prepare translucent sample disc. Using a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}, the powdered sample of each extract was loaded in FTIR spectroscope and readings taken.

2.6 GC-MS Analysis

GC-MS analysis on fractions was performed using a PerkinElmer GC Clarus 580 and a Gas Chromatograph interfaced to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused capillary column (30.0 m × 250 µm). For GC-MS detection, an

electron ionisation system was operated in electron impact mode with ionisation energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed. The injector temperature was maintained at 250 °C and the ion-source temperature was 150°C [15]. The oven temperature had an initial temperature of 40°C for 3 minutes followed by an increase of 5°C/minute to 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 minutes, and the total GC/MS running time was 51 minutes. The mass-detector used in this analysis was Turbo-Mass, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass version 6.1.0. Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns [16].

2.7 Evaluation of Hepatoprotective Activity

A total of 30 animals were used in this study with six groups (five animals in each group) using methods previously described by Arthur *et al*. [17]. Animals in Group I served as the control and received 1 ml/kg normal saline throughout the study. CCl_4 was administered to animals in Group II to Group VI on the 2nd and 3rd day of experiment. Group II animals were maintained as CCl_4 control without any treatment, while group III animals were pre-treated with 100 mg/kg of silymarin. Group IV, V and VI animals were pre-treated with 250 mg/kg each with the crude extracts, methanol and hydro fraction extract respectively. Treatment was performed through oral administration from day 1 to 7 with a concurrent administration of CCl_4 (1 ml/kg b.wt) on the 2nd and 3rd day. The doses for the extract was selected based on subchronic toxicity assessment of crude extract of *A. conyzoides* that established 250 mg/kg as the safest dose [18].

2.7.1 Assessment of hepatoprotective activity

All animals were sacrificed on the 8th day following an overnight fasting. Animals were exposed to ether anaesthesia. Incisions were quickly made in the neck region of the animals and blood samples collected from the heart into gel activated tubes for biochemical analyses which included ALT, AST, ALP, GGT, bilirubin (total and direct), total cholesterol, triglycerides, urea and creatinine. All biochemical analyses were performed using Fortress Diagnostics Reagents (UK) following manufacturer's instructions. All measurements were performed using Response 920 fully Automated Biochemistry Analyser. Livers and kidneys of sacrificed animals were excised, washed in buffered saline, blotted dry and weighed to obtain the absolute organ weights (AOW). The relative weights (ROW) were calculated using the formula:

$$ROW = 100\% \times \frac{AOW}{Weight\ at\ Sacrifice}$$

2.7.2 Histopathology analysis

A piece of liver and kidney tissues were preserved for 24 hours in a 10% formalin solution. Livers were processed for histological examination and stained with haematoxylin and eosin. Histographs were taken and analysed by a pathologist. Scoring of specimens were done after evaluating five microscopic areas of the tissue. Liver and kidney injuries were classified based on the following criteria: Vascular congestion, lobular inflammation, glomerulosclerosis, tubular hyalinisation, cellular degeneration and tubular necrosis and fibrosis.

2.8 Antioxidant Enzyme in Liver and Kidney Samples

4.5 ml of TNG buffer at pH of 7.4 was added to 0.5 g organ sample and homogenised. The homogenised sample was then centrifuged at 4000 x g for 20 minutes in a refrigerated centrifuge and supernatant preserved for antioxidant enzyme analysis. Microtiter plate reading (Synergy H1 Reader, USA) was performed thrice for each sample.

Malondialdehyde (MDA) is mostly involved in thiobarbituric acid (TBA) assay to indicate the presence of PUFA oxidative damage. Method described by Hegde and Joshi [19] was used to determine the levels of MDA formed. Catalase (CAT) has the potential to hydrolyse H_2O_2 and hence prevent the formation of chromate acetate by H_2O_2. CAT was determined by method described by Sinha [20]. Superoxide dismutase (SOD) prevents the autoxidation of adrenaline to adrenochrome. Activity of SOD was also measured as unit activity of SOD per mg protein by methods described by Misra and Fridovich [21]. Methods described by Elman [22] was used to determine the level of reduced glutathione

(GSH). GSH concentration was determined using the curve y=0.0004 x+0.0026, where x is the absorbance at 412 nm and y the concentration of GSH at µmol/mg protein. O-dianisidine method by Şenoğlu et al. [23] was used to determine the MPO enzyme concentration and absorbance read at 460 nm. Method described by Lowry et al. [24] was slightly modified for the determination of total protein content in the samples using bovine serum albumin standard curve.

2.7 Statistical Analysis

GraphPad Prism 5 for Windows was used to analyse the data. Results were expressed as the mean ± standard error mean (SEM). Data were assessed by one- way analysis of variance followed by Newman–Keuls multiple comparison test. Values for which $P < 0.05$ were considered as statistically significant. The percent protection was calculate using the principal indices of liver protection (ALT, ALP, GGT, Bilirubin, ROW) and kidney protection (Urea, Creatinine and ROW) using the formula:

$$\%\ Protection = \frac{Value\ of\ CCl_4 - Value\ for\ Test}{Value\ of\ CCl_4 - Value\ for\ Silymarin}$$

3. RESULTS

3.1 Qualitative Phytochemical Constituent of A. Conyzoides Crude Extract and Fractions

The presence of various phytochemicals was determined in the 50% hydroethanolic crude extracts of *A. conyzoides* and its fractions. Most phytochemicals were found to be abundant in the crude extract with the ethyl acetate fraction having the least phytochemicals. Table 1 shows the phytochemicals constituents in the extracts.

3.2 In vitro DPPH Scavenging Activity

Table 2 shows the results of the DPPH scavenging assay. The crude extract scavenged DPPH radicals more strongly with EC_{50} value of 0.14 mg/ml, followed by the hydro fraction (0.20 mg/ml). Ethyl acetate fractions had the weakest activity.

3.3 Total Phenol Content

Table 3 shows the total phenol contents expressed as gallic acid equivalent (mg/g GAE).

3.4 FTIR Spectroscopic Analysis

When the samples were passed into the FTIR, the functional groups of compounds present were separated based on their peak ratio. The identified functional groups include alcohol, phenol, aldehydes, aromatics, alkyl halide, alkanes, carbonyls, carboxylic acids, alkenes and alkynes.

3.5 GC-MS Spectra Analysis of Extracts

Various compounds were found in the crude extract and its hydro and methanolic fractions. The presence of these compounds was seen to play key roles in the pharmacological functions of the plants. These compounds were determined based on the NIST database which displayed all the suggested compounds present in the plant. The identified compounds included phenol, dodecanol, oleic acid, hexadecanoic acid, dimethyl sulfoxide, cholest-7-en-3-one, erucic acid, glycidol stearate and 2-pentadecanone.

3.6 Effect of Treatment on Organ Weight

Fig. 1 shows the absolute and relative weights of liver and kidneys of sacrificed animals. CCl_4 treatment resulted in significant increases in absolute liver and kidney weight. Crude extract prevented this increase and restored organ weights to near normal levels.

3.7 Effect of Treatment on Serum Biochemistry

The biochemical profiles of the treated animals were determined as presented in Fig. 2 and 3. The levels of liver biomarkers (AST, ALT, GGT, ALP and Bilirubin) of treated animals were determined. The extent of kidney damage of treated animals were determining by measuring the levels of Creatinine and Urea. CCl_4 treatment resulted in significant increase ($p<0.001$) in ALP, GGT and Bilirubin levels against the normal. Extract treatment results in decreases in these parameters to near normal. The increase in creatinine levels due to CCl_4 treatment were also restored to near normal ranges. Total cholesterol levels in CCl_4 and methanol groups were elevated while crude and hydro fraction and silymarin had no effect.

3.8 Percentage Protection of Silymarin and *A. conyzoides* Extracts

Fig. 4 shows the percent protection of silymarin, crude extract, hydro and methanol fractions of *A.*

conyzoides. These data were calculated using the principal indices of liver protection (relative liver weight, ALT, ALP, GGT and Bilirubin) and kidney protection (relative kidney weight, Creat and Urea). Crude extract offered better protection to CCl_4 induced liver damage comparable to standard silymarin. Methanol extract did not offer any significant protection.

3.9 Histopathology Examination

Animals in the normal group showed regular cellular structure of the liver when observed under microscope. CCl_4 treated group showed ballooning of hepatocytes, moderate congestion and moderate necroinflammation of the liver. Fibrosis with architectural distortion of the liver was also observed in this group. Mild lobular active inflammation was observed in the liver of animals with Silymarin treatment. Animals treated with *A. conyzoides* crude extract showed congestion of portal tract with no significant inflammation in the liver. Group treated with hydro fraction of *A. conyzoides* also showed lobular inflammation of the liver with focal interface hepatitis, however, no fibrosis was observed. Methanol fraction treated group showed mild portal congestion of the liver without evidence of necrosis (Fig. 5 A-F).

For histological observation of kidney cells, the normal group showed no pathological changes. CCl_4 treated group showed tubular hyalinisation, glomerulosclerosis, with interstitial inflammation of the kidney. Congestion and mild interstitial inflammation of the kidney was observed in animals with Silymarin treated group. Animals treated with CCl_4 and crude extract group showed no inflammation or congestion in their kidney. Mild interstitial inflammation was observed in the kidneys of rats treated with hydoethanolic and methanol fractions (Fig. 6 A-F).

3.10 Effect of Treatment on Liver and Kidney Antioxidant Enzymes

Antioxidant enzymes serves as biomarkers in monitoring the levels of ROS presence in the body. Essential antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) were determined in the study. Also, the levels of peroxidative products, myeloperoxidases (MPO) and malondialdehyde (MDA) were also assessed. CCl_4 administration resulted in significant decreases ($p<0.001$) in CAT, SOD and GSH levels and increases in MPO and MDA levels. Silymarin and extract treatment restored these to near normal levels. The crude *A. conyzoides* was observed to be most protective (Table 4).

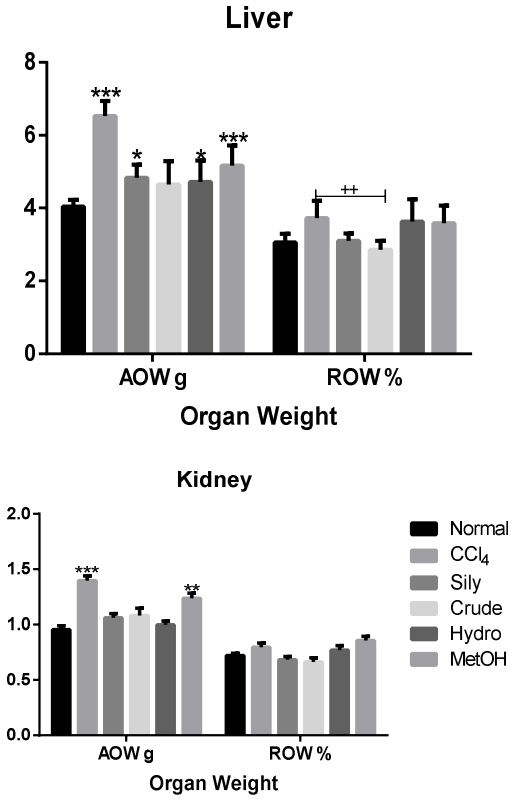

Fig. 1. Effect of treatment on liver and kidney weight. Each column represents a mean±SEM of 5 animals

**p<0.05, **p<0.01, ***p<0.001 against normal; ++p<0.001 against CCl_4*

4. DISCUSSION

Activity of antioxidants were expressed in EC_{50}, which measures the concentration (µg/ml) of extracts that could scavenge 50 % of DPPH radicals. The EC_{50} of crude extract of *A. conyzoides* was found to be 0.14±0.010 mg/ml, which is almost the same as ascorbic acid (0.18±0.06, Table 2). Phenolic compounds were present in the crude extract and all the fractions, with the highest TPC reported in the crude extract and lowest recorded in ethyl acetate fraction. In this study, the antioxidant activity of *A. conyzoides* crude extract and its fractions correlated partly with the total phenolic contents.

Table 1. Phytochemical constituents of A. conyzoides crude extract and fractions

Phytochemicals	Crude extract	Ethyl acetate	Methanol	Water
Alkaloid	+	-	+	+
Flavonoid	+	-	+	+
Terpenoid	+	-	+	+
Tannins	+	+	-	-
Saponin	+	+	+	+
Phytosterol	+	+	-	+
Glycosides	+	+	+	+

(+) presence; (–) absent

The high phenolic content supported the antioxidant properties of the extracts, its ability to neutralise and scavenge free radicals. This is confirmed by observations of Acheampong *et al*. [9] who reported the antioxidant properties of hydroethanolic extract of *A. conyzoides* and suggested phenol and flavonoids to be responsible for such effect. This was further confirmed by FTIR analysis that showed the presence of phenols, alcohols and aromatics. These compounds are known scavengers of free radicals with various associated medicinal benefits including anticancer [25], enhancing male fertility [26] and improving cardiovascular health [27].

Table 2. DPPH scavenging activity (EC_{50}) values of standard ascorbic acid and extracts

Sample	EC_{50} (mg/ml) n = 3	P value
Crude	0.14 ± 0.01*	0.00
Hydro	0.20 ± 0.04*	
Methanol	3.56 ± 2.67**	
Ethyl acetate	> 5**	
Ascorbic acid	0.18 ± 0.06*	

Values having different superscript (**) other than (*) in a column are statistically differ significantly p < 0.00

Table 3. Total phenolic content of standard and extracts

Sample/ standard	TPC (mg/g GAE) n = 3	P value
Crude	7845.59 ± 17.33*	0.00
Hydro	7605.63 ± 10.22*	
Ethyl acetate	6009.39 ± 9.73**	
Methanol	5842.46 ± 11.17***	

*Values having different superscripts (**, ***) other than (*) in a column are statistically differ significantly p < 0.00*

In the present studies pre-administration of animals with *A. conyzoide* before CCl_4-induced toxicity resulted in a significant protection against elevated marker enzymes. Elevation in serum liver marker enzymes and bilirubin following CCl_4 administration is indication of liver damage [28]. Administration of CCl_4 to rats in the studies caused significant increase in the weight of the liver and kidney significantly (Fig. 1). In most cases, liver and kidney weight are expected to increase due to hypertrophy of the tissues following CCl_4 treatment [29]. The cellular integrity and normal functioning of the liver was determined by the concentration of ALT, AST, GGT, ALP and bilirubin in the blood serum [30,31]. The levels of serum liver enzymes (ALT, AST and GGT) and bilirubin increased significantly in the group treated with CCl_4, thus the presence of CCl_4 was suggested to damage the liver causing these liver enzymes to leak into the blood serum [32]. This agrees with findings by LeSage *et al*. [33] in a similar investigation. Serum transaminases level of rat treated with crude extract and fractions after CCl_4 induction were observed to have significantly reduced. The pretreatment of animals with extracts was purposely to condition the hepatocytes to accelerate the regeneration of parenchymal cells, hence avoiding or decreasing the leaking of liver enzymes into blood serum through the protection of membrane. High liver protection was seen in the group treated with the crude extract (Fig. 4). The two fractions, hydro and methanolic, also demonstrated hepatoprotective effect against CCl_4.

Silymarin is a popular drug for patients known to have hepatotherapeutic and anti-fibrotic properties [34]. In many research, it has been proven to have a low bioavailability [35]. The hepatoprotective potential demonstrated by the crude extract and hydro fraction at 250 mg/kg dose respectively were comparable to silymarin on CCl_4-induced liver injury rats with altered levels of biochemical indicators returning to near normal levels.

Table 4. Effect of treatment on liver and kidney antioxidant enzymes and lipid peroxidation

	SOD (U/mg protein	CAT (U/mg protein)	GSH (µmol/mg protein)	MPO (U/mg Protein)	MDA (mmol/mg protein)
Liver					
Normal	4.04±0.27	68.53±1.18	382.7±2.68	126.4±2.48	183.1±4.64
CCl_4	1.65±0.10***	28.12±0.26***	213.0±1.28***	474.1±6.64***	268.7±5.32***
Silymarin	3.59±0.17^{+++}	72.81±1.44^{+++}	358.4±1.99^{+++}	348.1±7.08^{+++}	196.0±1.73^{+++}
Crude	4.12±0.05^{+++}	62.82±0.38^{+++}	378.4±3.81^{+++}	298.0±13.91^{+++}	185.9±8.47^{+++}
HE	3.71±0.19^{+++}	53.27±1.51^{+++}	267.9±0.38^{+++}	438.3±5.45***	248.3±6.36***
MetOH	1.64±0.17***	62.01±0.76^{+++}	304.0±1.10^{+++}	386.1±6.43^{+++}	243.2±4.57***
Kidney					
Normal	3.74±0.01	33.98±0.56	96.12±0.52	50.29±1.55	12.17±0.93
CCl_4	1.59±0.13***	14.15±0.58***	46.90±0.81***	120.8±6.25***	50.88±2.25***
Silymarin	3.56±0.00^{+++}	33.45±1.23^{+++}	85.97±2.28^{+++}	81.29±2.94^{++}	6.42±1.88^{+++}
Crude	2.97±0.01^{++}	34.16±0.27^{+++}	115.4±0.54^{+++}	90.72±1.71^{++}	13.51±1.21^{+++}
HE	2.31±0.15^{++}	17.67±0.34^{+}	72.83±0.49^{+++}	114.1±5.34***	40.40±1.48***
MetOH	2.31±0.02^{++}	15.26±0.34	56.10±0.12***	86.75±2.20^{++}	47.14±0.40***

*Each value represents a mean of 5 animals. *p<0.05, **p<0.01, ***p<0.001 against normal; +p<0.05, ++p<0.01, +++p<0.001 against CCl_4 group*

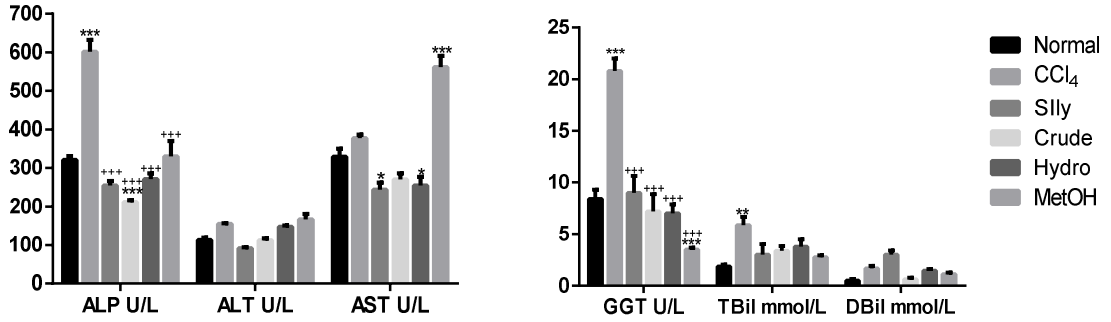

Fig. 2. Effect of treatment on liver markers of rats. Each column represents a mean±SEM of 5 animals

**p<0.05, **p<0.01, ***p<0.001 against normal; $^{+++}$p<0.001 against CCl_4*

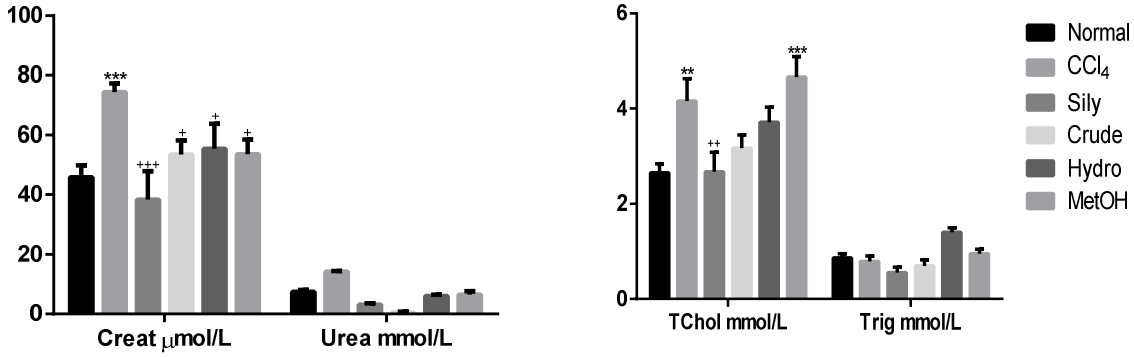

Fig. 3. Effect of treatment on kidney (Creatinine and Urea) and Lipid profiles (TChol and Trig) of rats. Each column represents a mean±SEM of 5 animals

****p<0.001 against normal; $^{+}$p<0.05, $^{+++}$p<0.001 against CCl_4*

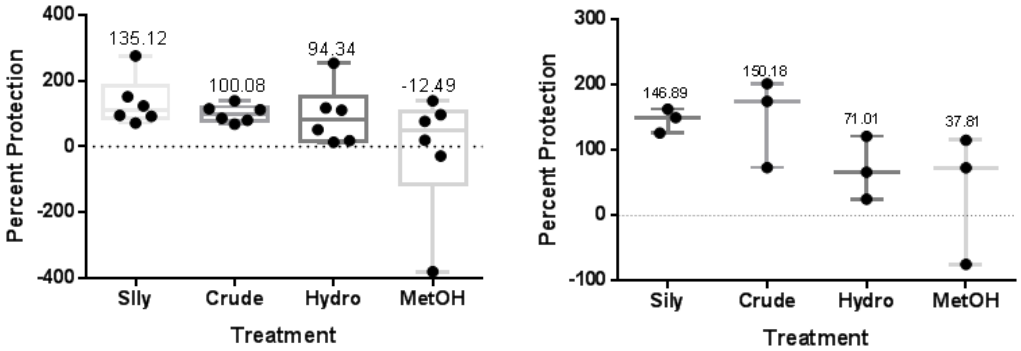

Fig. 4. Percent Liver Protection of Silymarin and *A. conyzoides*

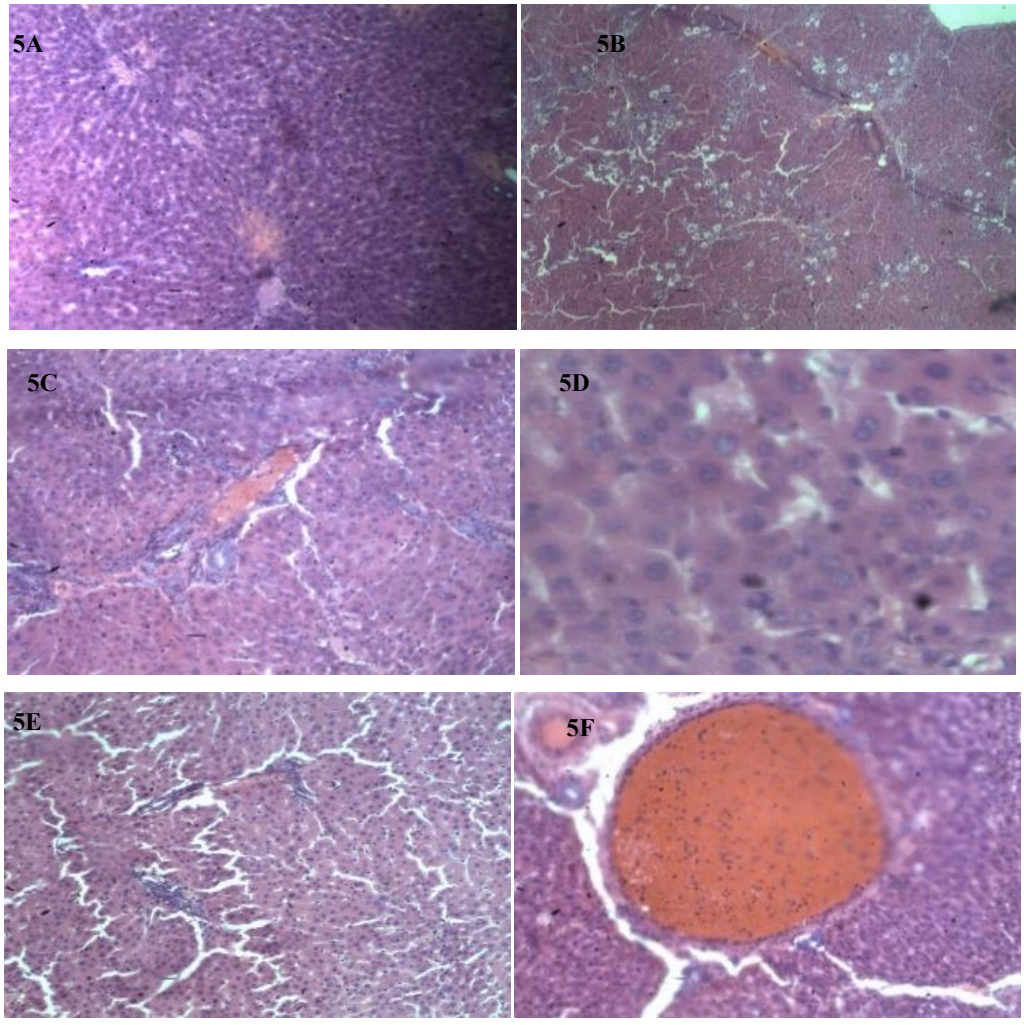

Fig. 5 (5A – 5F)). Effect of treatment on liver histology. 5A- Normal group showing no morphological changes; 5B-CCl_4 group showing ballooning of hepatocytes, moderate congestion, and moderate necroinflammation with bridging fibrosis; 5C-Silymarin group showing mild lobular active inflammation; 5D-Crude extract group showing congestion of portal tract, no significant inflammation; 5E-Hydro fraction treated group showing Lobular inflammation with focal interface hepatitis / Stage 1 fibrosis; 5F-Methanolic fraction treated group showing mild congestion, no inflammation

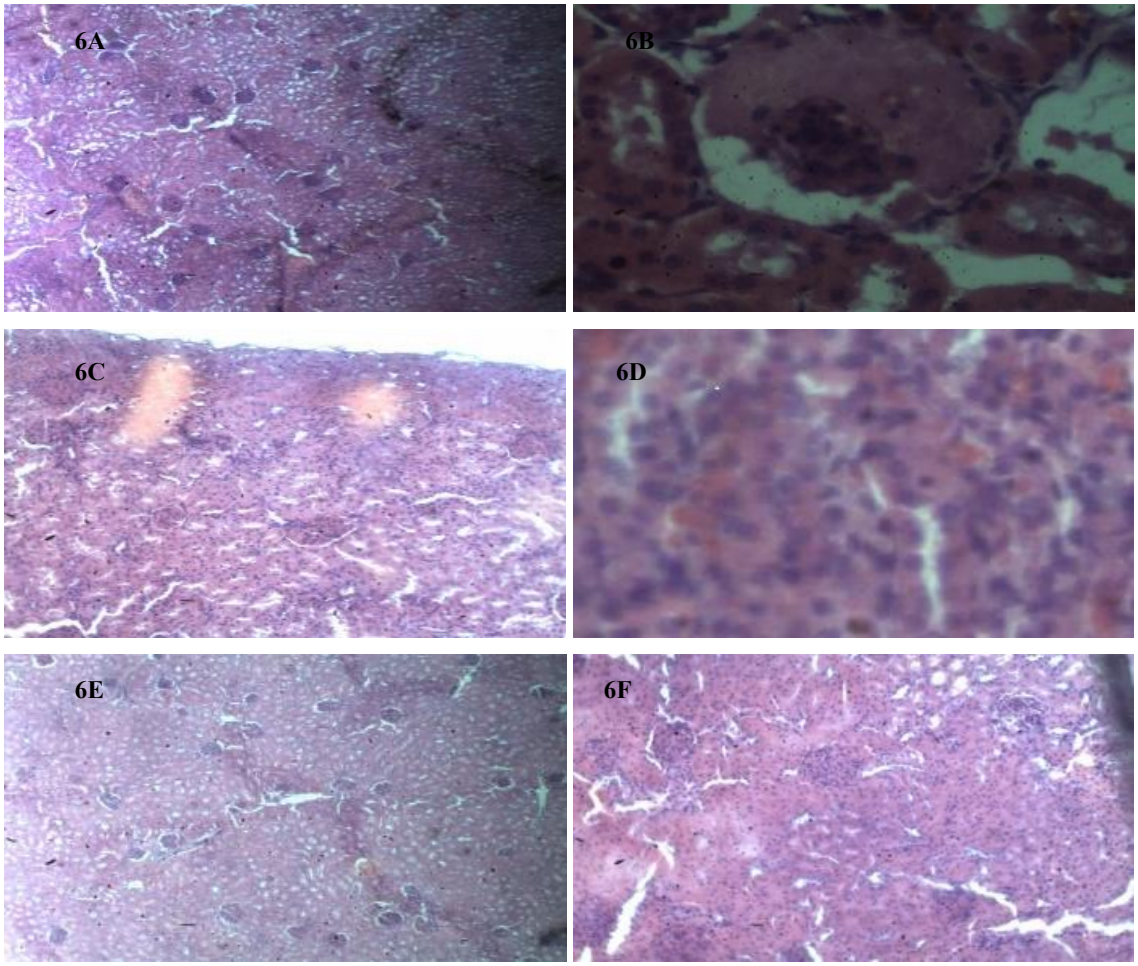

Fig. 6 (6A-6F). Effect of treatment on kidney histology. 6A-Normal group showing no morphological changes; 6B-CCl_4 group showing Tubular hyalinisation. Glomerulosclerosis. Interstitial inflammation; 6C-Silymarin group showing Congestion, mild interstitial inflammation; 6D-Crude extract group showing no inflammation or congestion; 6E-Hydro fraction treated group showing mild interstitial inflammation; 6F-Methanolic treated group showing no congestion or inflammation

Liver cirrhosis is characterised by abnormal changes in the liver lobular structure, occurrence of fibrosis and hepatic parenchyma lesion [36]. Results from histological findings proved that crude extract of *A. conyzoides* positively affected the liver's structural recovery in CCl_4-induced liver cirrhosis in rats (Fig. 5). No fibrosis with architectural distortion of the liver was significantly observed in the group treated with hydro fraction of the plant extract. The severe changes caused by CCl_4 in the liver of rats were eventually treated by *A. conyzoides* which corroborated with biochemical observations.

Furthermore, nephroprotective activities of crude extracts of *A. conyzoides* and its fractions were determined by measuring serum levels of creatinine and urea (Fig. 3). Usually, increase in these markers are possible indication for renal injury [37]. In the current study, significant increases in creatinine and urea levels were observed in the CCl_4 only group as compared to the normal group (Fig. 3). These were corroborated by the histological finding which showed tubular hyalinisation, glomerusclerosis, with interstitial inflammation of kidney in the CCl_4 only group (Fig. 6B). However, daily pre-treatment of rats with crude extract of *A. conyzoides* for 7 days showed the nephroprotective activity against CCl_4 renal injury, with histology showing no inflammation or congestion, with a significant decreased in

creatinine and urea levels. The two fractions were also seen to have moderate nephroprotective effect against CCl_4 renal injury rat.

In the present investigation, significant changes were observed in SOD, CAT, GSH, MPO and MDA after treatment with CCl_4, thus suggesting high oxidative damage in the liver. SOD was reported by Rajkapoor et al. [38] as an important enzyme in the enzymatic antioxidant defence system. Report from Palanivel et al. [39] suggested that *P. aculeate* caused a significant increase of SOD activity in the hepatic cells and this resulted in a reduction of reactive free radical and thus oxidative injury to the liver. In the current study, enzyme activity of SOD was significantly reduced in both the liver (1.65 ± 0.14 U/mg) and kidney (1.59 ± 0.16 U/mg) of CCl_4 only group compared with normal (liver: 4.04 ± 0.23 U/mg; kidney: 3.74 ± 0.04 U/mg; Table 4). However, treatment with the crude extract of *A. conyzoides* normalised the SOD enzyme activity, thus showing no significant difference in both organs when compared to normal group. The hydro and methanol fractions also showed significant increases in SOD enzyme activity, however no significant change was observed in liver with methanolic fraction treatment.

Administration of 250 mg/kg of crude extract and fractions of *A. conyzoides* increased the level of CAT similar to that produced by the standard silymarin (Table 4). Treatment with CCl_4 resulted in a significant reduction in CAT activity for both tissues. In the liver, treatment with crude extract of *A. conyzoides* and fractions reported a significant increase in CAT enzymatic activity when compared to CCl_4 only group, however in the kidney, only the crude extract recorded a significant increase in CAT activity.

Administration of crude extract of *A. conyzoides* resulted in significant ($P < 0.001$) increase GSH level in both liver and kidney, however decreased GSH level in CCl_4 only group was associated with an enhanced lipid peroxidation (Table 4). In the study, rats in CCl_4 only group were seen to have elevated levels of MPO activity (Table 4), an index of hepatic infiltration [40]. Pre-treatment with *A. conyzoides* crude extract significantly reduced the level of hepatic MPO activity in both the kidney and liver induced by CCl_4, thus preventing neutrophils infiltration into both tissues. Elevated level of MDA was observed in the rats intoxicated with CCl_4, however treatment with *A. conyzoides* crude extract significantly reduced the levels (Table 4). Its free radical scavenging property was suggested to be the allotting factor for MDA level reduction [41].

It is suggested that the observed hepato- and nephroprotective effect of *A. conyzoides* against CCl_4 induced damage in rats is due to its *in vitro* and *in vivo* antioxidant properties as corroborated by the EC_{50} values and TPC as well as the improved antioxidant enzymes (SOD, CAT, MPO, and GSH). This confirms the reports from literature showing protective effects of medicinal plants mediated through their antioxidants properties due to the high levels of flavonoids and alkaloid [42]. According to Khan et al. [43], saponins have also been recorded to protect the kidney and liver against CCl_4 intoxication. Hydroethanolic crude extract of *A. conyzoides* has also been reported to contain alkaloids, saponins, flavonoids and other active phytochemicals [9]. Based on the reported facts, it is possible the alkaloid, flavonoid and saponin phytochemicals present could be responsible for the hepato- and nephron-protective effect of *A. conyzoides*.

5. CONCLUSION

Data from this study suggested that crude ethanolic leaves extract of *A. conyzoides*, as well as, its hydro fractions had hepato- and nephron-protective properties, increasing the level SOD, CAT and GSH enzyme activity after treatment with CCl_4 which had been recorded to cause damage to tissue after exposure. Phytochemicals such as alkaloid, phenolic compounds and flavonoids were suggested to partly contribute to the plant's hepatoprotective and nephro-protective effects.

The plants could therefore be considered as an alternative source for management of liver diseases usually caused by free radicals. Further investigation is needed to identify the most active components allotting to the hepatoprotective effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Study was approved by the Animal Research Ethics Committee of KNUST, Kumasi, Ghana. All

animal experiments were conducted according to internationally approved protocols.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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