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Anti-Inflammatory and Antioxidant Potential of *Ageratum conyzoides* Ethanol Extract Against Carbon Tetrachloride-Induced Renal Toxicity in Rats

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Kidney diseases remain a significant global health challenge, prompting researchers to explore the therapeutic potential of medicinal plants for treating various ailments, including renal disorders. This study aimed to evaluate the anti-inflammatory and antioxidant effects of the ethanolic extract of Ageratum conyzoides leaves (ESE) on kidney damage induced by carbon tetrachloride (CCl4) in a rat model. Haematological and biochemical indicators were used to evaluate the nephroprotective efficacy. Rat kidney homogenates were used to assess antioxidant and anti-inflammatory biomarkers. The histological data was used to evaluate the amount of kidney injury. Pro-inflammatory cytokine expression increases brought on by CCl4 insult were dramatically and dose-dependently inhibited by ESE treatment. A significant reduction in lipid peroxidation of kidney tissue brought the concentration of defensive antioxidant enzymes to near-normal levels. The beneficial activities of ESE on CCl4-induced hepatotoxicity damage were validated by histopathological analysis of the kidney; the maximum percentage of liver protection was observed when ESE was induced at a dosage of 500 mg/kg b.wt. According to the findings, ESE protects the kidneys against CCl4-induced toxicity through anti-inflammatory and anti-oxidative effects. This response possibly may be due to the presence of phytochemicals.

Keywords: Biochemical; Cytokines; Homogenates; Kidney; Peroxidation.

The kidney is the main metabolic organ responsible for removing waste products, undesirable substances, and potentially dangerous substances from the human system[1].

Its high blood circulation and intricate cellular transport networks cause these compounds to accumulate inside nephron epithelial cells, making them highly vulnerable to the harmful effects of drugs and environmental pollutants[2].It is necessary for animal tissues to constantly cope and deal with highly reactive oxygen species (ROS), which are generated during various metabolic physiological processes. Examples of these radicals include hydrogen peroxide, hydroxyl radicals,

superoxide anion, and others. It seems that life requires some free radical generation, however, the overproduction of free radicals could lead to oxidative stress. According to a study[3], oxidative pathways associated with the cytochrome P450 enzyme system, which are involved in the hepatic metabolism of pharmaceuticals, are responsible for almost half of all cases of acute liver failure in Ghana. In several sectors, carbon tetrachloride (CCl_4) is a common chemical solvent. Hira and colleagues[4] reported that kidney damage brought on by CCl_4 is similar to damage brought on by other common causes of renal deterioration, including alcohol, chemicals, viruses, and autoimmune illnesses. Kidney disease is a very prevalent and burdensome condition, and plant-based treatments have shown to be promising. It has been discovered that many plants and their extracts possess antioxidant properties, which can help mitigate kidney damage by preventing processes like inflammation, fibrosis, and oxidative stress from occurring[4].

Ageratum conyzoides (AC), is a persistent weed that is widespread in tropical and subtropical areas[5]. Despite its reputation as a weed, the nutritional and medicinal potential of *A. conyzoides* remains largely unexplored[6]. Analgesic, hemostatic, insecticidal, and antimicrobial properties have all been demonstrated for this Asteraceae family herbal plant[7]. In this study, we investigated the anti-inflammatory and antioxidant activities of *Ageratum conyzoides*ethanoic crude extract against carbon tetrachloride using a rat model.

MATERIALS AND METHODS

Plant Collection and authentication

The Northern Region of Ghana's Nyankpala Reserve Forest was the source of the hand-picked *A. conyzoides* leaves. The leaves were verified by a botanist and a voucher specimen (UDS/ HM57/ 2023 /L011) was deposited at the herbarium. They were properly cleaned with water three times, dry at room temperature, and ground to powdered form.

Preparation of extracts

One hundred grams of powdered sample of *A. conyzoides* leaves were macerated using 1000 ml of 99.99 % hydroethanolic solution. For 24 hours at 25°C, it was shaken continuously with a shaker (Gallenkamp, England). Centrifugation was employed to process the supernatant for 20 minutes at 1106 x g at ambient temperature. Following centrifugation, the supernatants were combined and concentrated using a rotary evaporator.

Preliminary Phytochemical analysis

The phytochemical components of *A. conzyoides* leaves powder and ethanolic crude extracts (ESE) were identified by applying conventional techniques, as stated by Donkor and colleagues[8]. The phytochemicals that were investigated included triterpenes, alkaloids, flavonoids, saponins, polyphenols, and tannins.

Evaluation of Nephroprotective Activity

Forty-five (45) male rats, weighing between 120 and 150 g, were split up into 9 groups and treated for 7 days (n = 5). Table 1 shows the groups and treatments. The rats, which were acquired from the Noguchi Memorial Institute for Medical Research's Animal Experimentation Unit, were housed at room temperature (25 ±1°C) and humidity (50–60%) for five days before the experiment. They also had a 12-hour light/day cycle maintained. Agricare, Kumasi, Ghana, provided them with a standard laboratory rat diet, and they had unrestricted access to clean water. The study was approved by the University for Development Studies Institutional Review Board (UDSIRB) with an ethically approved number of UDSIRB/188/23.

The animals were given 50 mg/kg intraperitoneal pentobarbitone to induce anesthesia on the eighth day. Through cardiac puncture, blood was obtained into EDTA (ethylenediaminetetraacetic acid)-2K tubes for hematological examination. For biochemical analyses, portions of the blood were obtained into gel-separator tubes. Afterward, the rats were euthanized, and the kidneys were removed for examination by histopathologists. Antioxidant enzymes, oxidative stress indicators, and pro-inflammatory cytokines were all measured in kidney homogenates. The animals' carcasses were interred in a specific location following institutional ethical standards in the area.

Change in body weight of rats

The expression below was used to determine the body weights of rats after treatment:

$$\% \text{ Change in Body Weight} = (\text{Weight}_n - \text{Weight}_o) / \text{Weight}_o \times 100$$

Effect of extracts on rat kidney

The absolute organ weight (AOW) of the rat's kidney was calculated and used to determine the Relative Organ Weight (ROW) as shown below:

ROW= AOW/(Body Weight at Sacrifice)×100

Hematological and Biochemical analyses

The animals' hematological profiles were measured using an automated Sysmex XS-1000ianalyzer. Serum was extracted by centrifuging blood samples in activated gel tubes for 15 minutes at 1500x g after they had been allowed to clot. A completely automated Flexor E chemistry analyzer (VitalScientific, Japan) was used to carry out the biochemical analysis. Urea and creatinine were the twobiochemical markers that were examined. The following approach was used to calculate thepercentage of nephroprotection utilizing the primary kidney functional indicators, such as relativekidney weight, urea, and creatinine.

Percent Protection= (Value of Toxin control-Values of test group)/(Values of toxin control-Values of normal control)×100

Antioxidant and oxidative stress biomarkers

After homogenizing the liver (1.0 g) in 10 mL phosphate buffer (0.1 M, pH 7.4) and centrifuging at 10,000 rpm for 20 min at 4°C, the post-mitochondrial fraction was extracted (PMF). The supernatant was collected and utilized for antioxidant assays such as reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and myeloperoxidase (MPO) using methods as stated by Sarfo-Antwi and colleagues[6].

Inflammatory cytokine assay

Using homogenized liver samples, measurement of anti-inflammatory proteins of specific cytokines, namely Tumour Necrosis Factor-alpha (TNF-á), Transforming Growth Factor-Beta1 (TGF-â1), Nuclear Factor Kappa B (NFk-â), and Cyclo-oxygenase 2 (COX-2), were tested using appropriate ELISA kits. TNF-á, NFkâ, and COX-2 ELISA kits were obtained from RayBio – tech, Inc. (Norcross, GA, USA) and TGF-â1 kits were from Aviscera Bioscience, Inc. (Santa Clara, CA, USA).

Histopathological studies

The kidney sections were embedded in paraffin, fixed in 10% buffered formalin, and then cut into 4 m sections from each block. For histological analysis, the paraffin-embedded slices were stained with hematoxylin and eosin. Light microscopy (Olympus Manual System Microscope BX43) was used to conduct blinded examinations of each sample from each group while being seen at 100x and 400x magnification.

Statistical Analysis

Multiple comparison tests using Tukey were performed after a one-way ANOVA with GraphPad Prism. Statistical significance was established at the 5% level ($p < 0.05$), and the results were displayed as mean ± SEM.

RESULTS

Preliminary Phytochemical screening

Table 2 below shows the phytochemicals present in the extract and raw powder. Alkaloids and tannins were the most abundant phytochemicals present in the extract.

Body weight after Treatment

Rats treated with CCl_4-treated only, observed a substantive reduction in weight as shown in Table 3.

Relative Weight of Organs Following Therapy

Figure 1 illustrates that in comparison to the normal group, the relative organ weight of animals treated with CCl_4 only increased significantly (p 0.05).

The impact of therapy on hematological parameters

The hematological indices were measured to assess the effect of the CCl_4, silymarin, and ESE on the rats. In general, therapy had no significant effect on the rats' hematological indicators, as indicated in Table 4.

The impact of remedies on serum urea and creatinine

Figure 2 shows that rats administered CCl_4 alone had substantially higher creatinine and urea levels than the normal group.

Impact of therapy on percentage protection

Figure 3 depicts the kidney-protective impact of Silymarin and ESE at 100, 250, and 500 mg/kg b.wt. against CCl_4induction. ESE at 500

mg/kg b.wt provided the most effective therapy against CCl4 at 96%, surpassing standard silymarin at 93%.

Treatments' effects on antioxidant indicators

Table 4 presents the effects of therapy on oxidative stressors and renal antioxidants. When

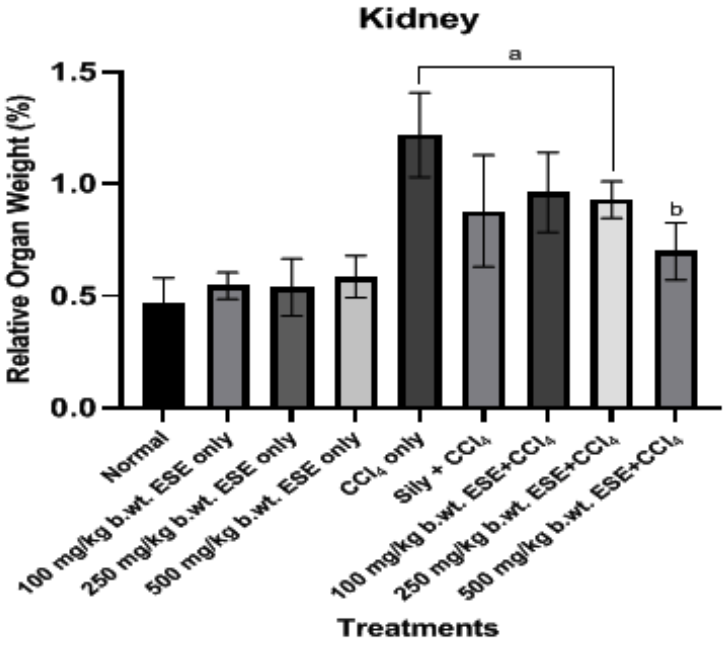

Fig. 1. Treatment's Impact on Relative Organ Weight. The results are presented as mean ± SEM (n=5). Statistical significance "a" between 0.05 and 0.001 in comparison to Normal; "b" between 0.05 and 0.001 in comparison to the CCl_4-treated group only

Table 1. Animal Groups and Treatments

S/N.	Group	Treatment
1	Normal (control)	Potable water p.o (1 ml/kg b.wt)
Extracts Only		
2	100 mg ESE only	100 mg/kg ESE only (per day, p.o.) for 7 consecutive days
3	250 mg ESE only	250 mg/kg ESE only (per day, p.o.) for 7 consecutive days
4	500 mg ESE only	500 mg/kg ESE only (per day, p.o.) only for 7 consecutive days
Toxicant Induced		
5	CCl_4	CCl_4 i.p (1 ml/kg b.wt, 1:1 v/v olive oil) received on 2nd and 3rd day.
6	CCl_4 +Sily	Silymarin p.o (100 mg/kg per day) received for seven days plus a single dose of CCl_4 in olive oil (1:1 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day
7	100 mg ESE + CCl_4	100 mg/kg ESE (per day, p.o.) for seven days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.
8	250 mg ESE + CCl_4	250 mg/kg ESE (per day, p.o.) for seven days for seven consecutive days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.
9	500 mg ESE + CCl_4	500 mg/kg ESE (per day, p.o.) for seven days plus a single dose of CCl_4 in olive oil (1:0 v/v, 1.0 ml/kg, i.p.) on the 2nd and 3rd day.

comparing the CCl_4-treated group to the Normal group, there was a notable rise in the oxidative stress biomarker (MDA) and a substantial decrease in the antioxidant assay (CAT, SOD, and GSH) following CCl_4 administration. Comparing the CCl_4 group to the co-treated groups with ESE at all dosages, however, revealed substantial ($p<0.05$) differences in antioxidant and oxidative stress biomarker levels.

Treatment impact on inflammatory markers

Inflammatory markers were substantially higher in the group treated with only CCl_4 than in the normal group ($p<0.05$-0.001).

Impact of Therapy on Liver Histology

The kidneys of the normal group recorded no undesired morphological changes; nevertheless, the group receiving CCl_4only had significant diffuse tubular ectasia with coagulation necrosis. Animals who received 500 mg/kg bwt of ESE recovered almost normal architecture in place of these abnormalities as shown in Figure 4.

Table 2. Phytochemical composition of raw powder and ESE

Phytochemicals	ESE	Raw Powder
Alkaloids	+	+
Phenols	+	+
Flavonoids	+	+
Triterpene	+	+
Tannins	+	+
Saponins	+	+

Key: Present (+); Absent (-)

DISCUSSION

Numerous articles have shown that a variety of mutagens and carcinogens can create peroxide radicals which can initiate the formation of cancer and other health disorders[9-12].

The current study sought to assess any potential safeguards provided by ESE against CCl_4-induced oxidative stress, which causes damage to

Table 3. Variation in Weight of Rats

Treatment	% Change in Body Weight, D7
Normal	3.11 ± 1.02^{b}
Extracts only	
100 mg/kg b.wt. ESE only	1.18 ± 0.12^{a}
250 mg/kg b.wt. ESE only	2.35 ± 0.25^{b}
500 mg/kg b.wt. ESE only	3.23 ± 1.31^{b}
CCl_4only	-2.32 ± 1.04^{a}
Sily+CCl_4	2.75 ± 0.15^{b}
100 mg/kg b.wt. ESE +CCl_4	0.21 ± 0.47^{ab}
250 mg/kg b.wt. ESE +CCl_4	0.57 ± 0.05^{ab}
500 mg/kg b.wt. ESE +CCl_4	1.25 ± 0.11^{ab}

Mean ± SEM (n=5) is used to express values. The significance level is "a" at $p<0.05$-0.001 relative to the normal group and "b" at $p<0.05$-0.001 when relative to the CCl_4-treated group alone.

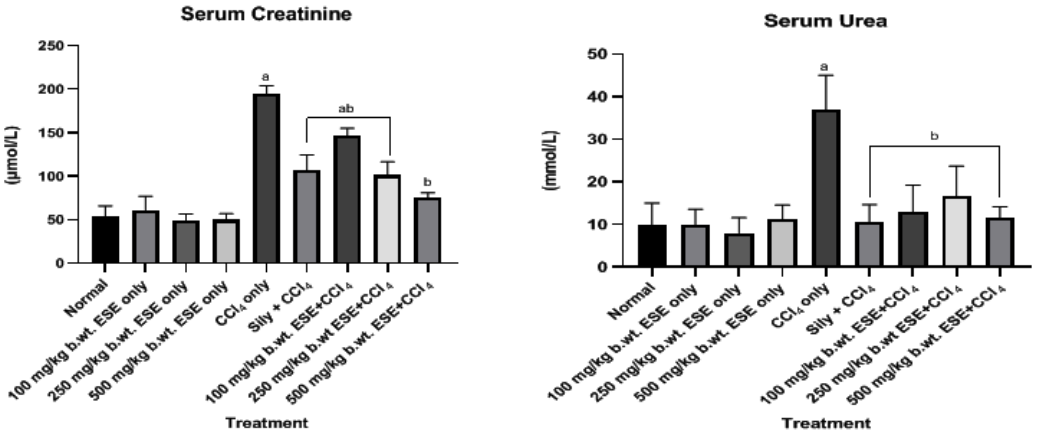

Fig. 2. Effects of treatments on serum creatinine and urea. The findings (n=5) are shown as mean ± SEM. Statistical significance: "a" compares to Normal at $p<0.05$-0.001, and "b" compares to the group that received only CCl_4 at $p<0.05$-0.001

the kidneys in rats. Phytochemical analysis of ESE and raw powder revealed the existence of alkaloids, phenols, flavonoids, triterpenes, tannins, as well as saponins. CCl_4 is a widely used, trusted, and well-known chemical that can harm the liver and kidney tissue[13-15]. As expected, the CCl_4-treated group had a significant reduction in body weight at termination because it interfered with the animal's normal metabolism of fat, protein, and carbohydrates. Additionally, a decrease in appetite directly affects body weight reduction. However, co-treatment with ESE at all dosages resulted in a significant increase in body weight. Figure 1 illustrates how co-treatment with extracts returned kidney weight to a nearly normal range, despite the possibility that the negative effects of CCl_4 caused the rats' kidneys to enlarge and allow materials like fatty acids and glycerol to enter the kidney cells. According to past research, CCl_4 treatment increased several organs, including the kidney and liver[16-18].

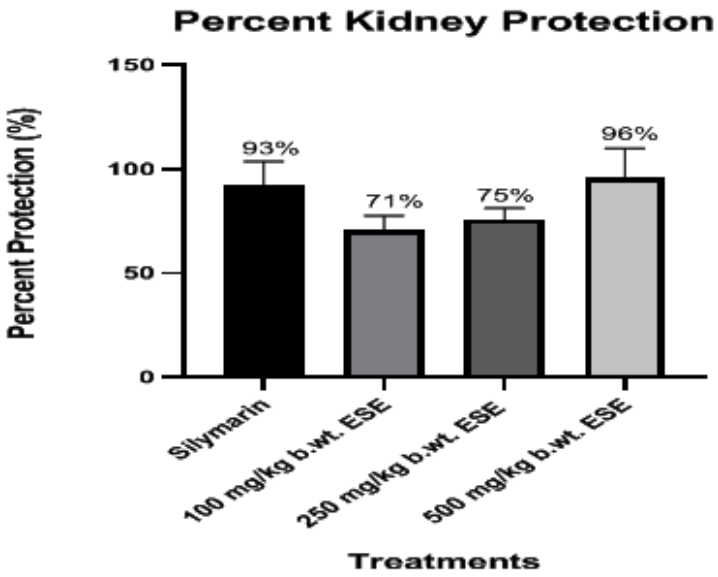

Fig. 3. Shows the percentage of protection provided by silymarin and all doses of ESE against carbon tetrachloride. The individual bar represents the average percentage protection of the kidney biomarkers.

Table 4. Treatment's Impact on Haematological Markers

Parameter	HGB (g/dL)	WBC (10^3/µL)	RBC (10^6/µL)	PLT (10^3/µL)
Normal	11.43 ± 1.32	7.13 ± 1.21	5.83 ± 2.20	713.34 ± 32.12
Extract Only				
100 mg/kg b.wt. ESE only	12.11 ± 2.34	9.51 ± 2.32	5.62 ± 1.31	764.85 ± 43.65
250 mg/kg b.wt. ESE only	11.34 ± 1.52	12.21 ± 1.26	5.22 ±1.12	843.10 ± 38.47
500 mg/kg b.wt. ESE only	11.10 ± 1.61	8.30 ± 1.42	5.95 ± 1.45	821.83 ± 54.23
CCl_4 only	7.11 ± 1.43[a]	5.02 ± 1.17[a]	3.02 ± 0.35[a]	1165.54 ± 70.23[a]
Silymarin + CCl_4	11.07 ± 1.33[b]	8.33 ± 1.25[ab]	3.61 ± 1.24[a]	965.12 ± 36.16[ab]
100 mg/kgb.wt.ESE+ CCl_4	9.43 ± 1.42[ab]	6.76 ± 2.12	3.98 ± 1.11[a]	1012.43 ± 50.87[a]
250 mg/kgb.wt.ESE+ CCl_4	9.51 ± 0.85[ab]	6.26 ± 1.64	3.82 ± 1.32[a]	1000.97 ± 67.82[a]
500 mg/kgb.wt.ESE + CCl_4	10.86 ± 1.19[b]	7.42 ± 1.32[b]	4.17 ± 1.73	865.74 ± 43.73[b]

The data are shown as mean±SEM (n=5). Significant differences between the Normal and CCl_4-treated groups were statistically indicated by "a" at $p<0.05$-0.001 and "b" at $p<0.05$-0.001 respectively.

The current investigation found that serum creatinine and urea levels were considerably higher in rats who received CCl_4 induction as shown in Figure 2. Lipid peroxidation caused a loss in membrane integrity and the accumulation of indicators like creatinine and urea in damaged kidneys brought on by CCl_4 intoxication. However, pre-treatment with silymarin and ESE at all doses resulted in a significant reduction in these markers.

This was confirmed by the histological observation, which revealed widespread tubular ectasia in the CCl_4 alone group together with coagulation necrosis of epithelium and kidney interstitial inflammation as shown in Figure 5. As seen in Figure 3, these results indicate that ESE and silymarin can protect nephrocytes from CCl_4-induced injury. The maximum protection effect of the kidney was achieved with pretreatment ESE at 500 mg/kg b.wt. Erythropoietin synthesis by the kidney is disrupted by CCl_4, which also alters erythrocyte viability and morphology[19].In this study, the reduction of hemoglobin synthesis and the reduction of erythrocyte life may have contributed to the anemia associated with CCl_4 as seen in Table 4. Similar findings postulated that CCl_4 direct detrimental effects on the cell membrane were the reason for the rats' shortened life span[20-23].

Additionally, the capacity of iron to generate hemoglobin in the mitochondria is inhibited by CCl_4[24-25]. Free radical processes are known to be involved in the kidney damage caused by CCl_4[25]. A significant rise in MDA levels and a significant reduction in antioxidant makers were seen in rats exposed to CCl_4, however, as Table 5 illustrates, rats who received both CCl_4

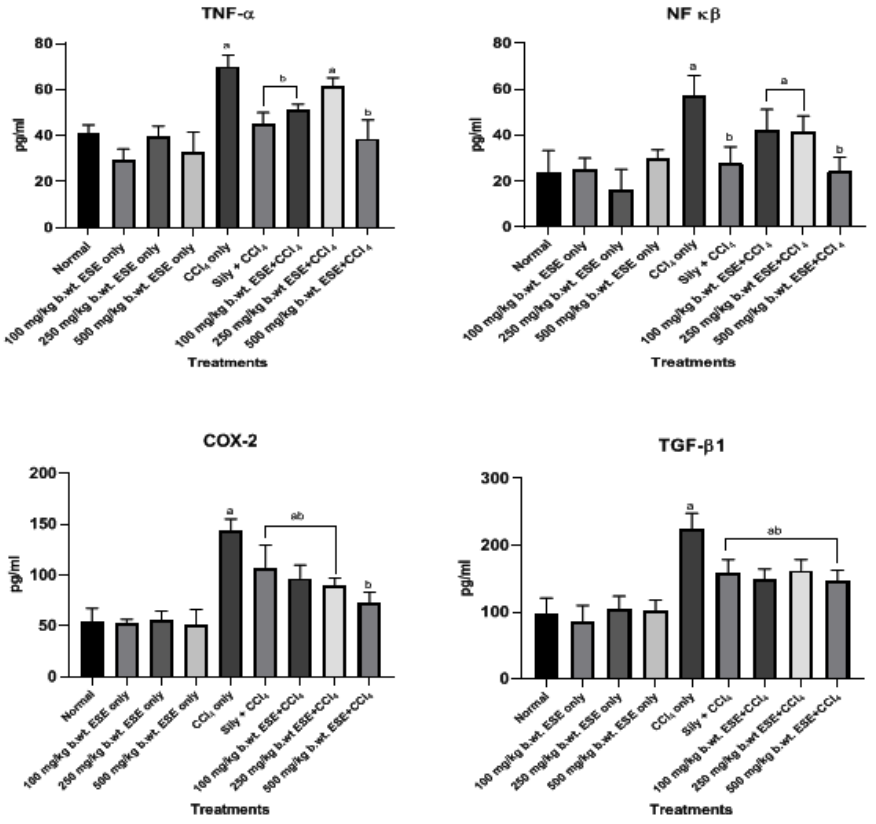

Fig. 4. Depicts the impact of therapy on inflammatory biomarkers. Data are presented as mean ± SEM (n=5). "a" indicates a significant distinction from the normal group ($P < 0.05$ - 0.001), while "b" indicates a significant variance from the CCl_4-treated group.

and ESE concurrently showed a considerable drop in MDA and an increase in antioxidant enzymes. Histological studies have demonstrated that low levels of antioxidant enzymes could cause oxidative stress and lipid peroxidation. In this study, pre-treatment with ESE at all dosages

Table 5. Effect of Treatment on Antioxidant Makers

Parameter	GSH (µmol/mg protein)	CAT (U/mg Protein)	SOD (U/mg Protein)	MDA (mmol/mg protein)
Normal	187.75 ± 9.23	50.42 ± 8.12	3.12 ± 0.75	10.83 ± 2.98
Treatment (without CCl_4*)*				
100 mg/kg b.wt. ESE only	180.76 ± 4.34	54.24 ± 9.11	2.98 ± 0.43	8.34 ± 2.41
250 mg/kg b.wt. ESE only	192.18 ± 8.54	52.71 ± 4.26	3.40 ±1.05	9.45 ± 1.48
500 mg/kg b.wt. ESE only	188.23 ± 6.07	55.42 ± 7.93	3.21 ± 1.11	7.98 ± 1.23
CCl_4only	84.73 ± 3.54[a]	14.39 ± 5.36[a]	0.83 ± 0.10[a]	98.63 ± 7.62[a]
Silymarin + CCl_4	172.41 ± 5.33[b]	30.58 ± 6.04[ab]	3.21 ± 0.35[b]	20.11 ± 6.16[ab]
100 mg/kg b.wt. ESE + CCl_4	102.06 ± 6.87[ab]	26.92 ± 3.53[ab]	2.04 ± 0.36[ab]	46.42 ± 8.25[ab]
250 mg/kg b.wt. ESE+ CCl_4	131.52 ± 4.82[ab]	33.17 ± 4.02[ab]	2.75 ± 0.66[b]	35.19 ± 5.32[ab]
500 mg/kg b.wt. ESE+ CCl_4	162.75 ± 8.22[ab]	46.11 ± 5.32[b]	3.54 ± 0.73[b]	26.24 ± 5.03[ab]

The results are shown as mean ± SEM (n=5). "a" represents the statistical significance when compared to the Normal group, and "b" represents the statistical significance when compared to the CCl_4-treated group only.

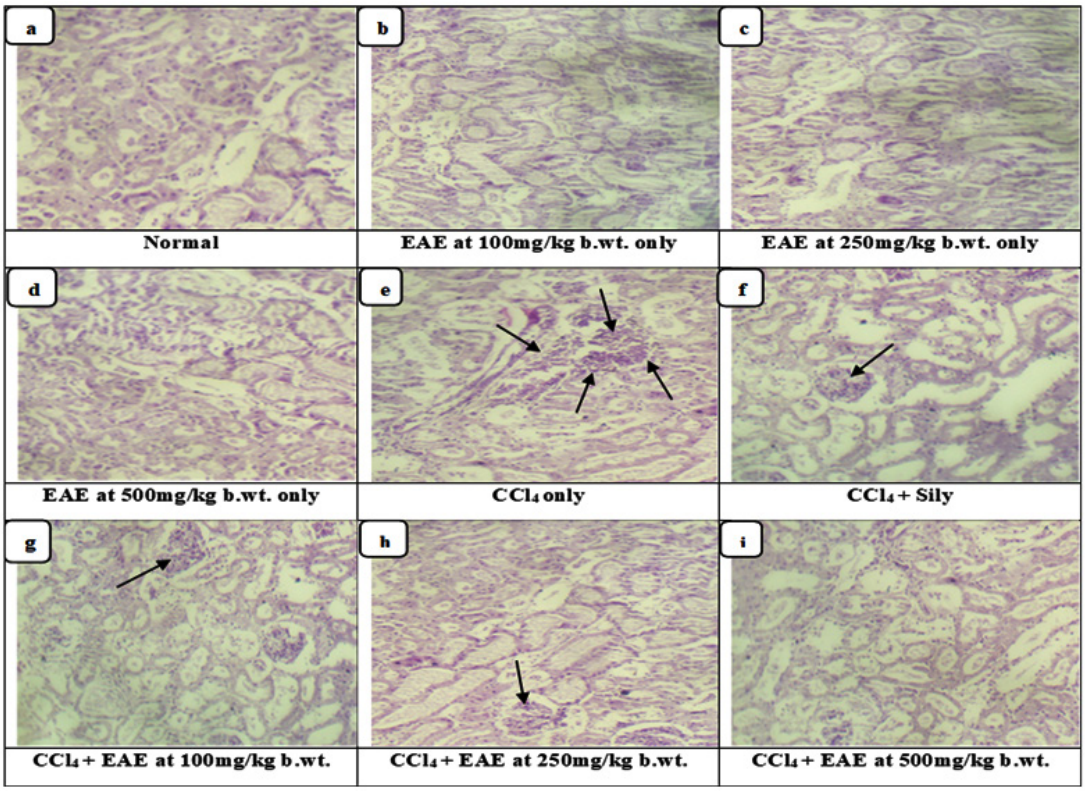

Fig. 5. Depicts the impact of therapy on kidney histology. (a) - (d) show no visible damage; (e) indicate diffuse tubular ectasia with epithelial coagulation necrosis; (f) - (h) show mild coagulation necrosis; (j) display no observable lesion

restored normal levels of TNF-á, TGF-â1, NF êâ1, and COX-2, which were significantly elevated owing to CCl_4 toxicity. These outcomes align with a previous investigation on CCl_4 poisoning, whereby molecular discoveries demonstrated increased quantities of TNF-á, TGF-â1, COX-2, IL-17, and IL-23, in addition to overexpression of NF êâ in the hepatic tissues[26].

Pro-inflammatory cytokines including TNF-á, TGF-â1, COX-2, IL-17, and IL-23 are produced through the mitogen-activated protein kinase (MAPK) pathway, which is reported to be activated by CCl_4[27-29].Furthermore, CCl_4 participates in the activation of NF-êâ, which regulates the production of several genes linked to inflammation, including adhesion molecules, chemokines, and pro-inflammatory cytokines[30]. Researchers in this study discovered that lowering the amounts of pro-inflammatory cytokines in kidney homogenates after ESE treatment at all dosages also reduced inflammatory responses. The anti-inflammatory mechanism of ESE therapies is suggested by their reported down-regulation of NF-êâ expression along with associated cytokines and chemokines, hence providing a nephroprotective effect. The protective impact of kidneys through inflammation suppression is indicated by positive regulation of TNF-á production by ESE. Nephrocytes are known to express cyclooxygenase 2 (COX-2), which both attenuates and stimulates cell proliferation in acute kidney damage and only occurs under conditions of prolonged aggressiveness. Furthermore, inflammatory neurotoxicity has been linked to COX-2 activation after lead exposure[31-32].

According to the current study, CCl_4 causes over-production of COX-2 in kidney tissues, which in turn causes excessive inflammation (together with NF-êâ and TNF-á). However, COX-2 expression was downregulated when co-treated with ESE and the standard drug, silymarin. This provides more proof of the extract's anti-proliferative ability in nephroprotection. The study's findings demonstrated that ESE had a protective effect at all dosages, with the highest protection occurring at 500 mg/kg b.wt. The biochemical and histological data suggest that ESE is nephroprotective, which is likely due to the phytochemicals present.

CONCLUSION

Our findings validated the antioxidant characteristics of ESE and showed, for the first time, that these benefits are linked to kidney protection through the suppression of anti-inflammatory cytokines. These findings corroborate previous research that suggested medicinal plants were a good substitute for kidney protection. We concluded that ESE could be essential for its economic use and perhaps further study could look into separating the pure bioactive chemicals that give its medicinal effect.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethical statements

The study was approved by the University for Development Studies Institutional Review Board (UDSIRB) with an ethically approved number of UDSIRB/188/23

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Authors' Contributions

Conceptualization and methodology: Frederick Sarfo-Antwi, Christopher Larbie, and Abdul-Rashid Lansah Adam. Formal analysis and investigation: Frederick Sarfo-Antwi, Benjamin Obukowho Emikpe, Christopher Larbie and Abdul-Rashid Lansah Adam. Writing of the original draft, review, and editing: Frederick Sarfo-Antwi, Rashid

Lansah Adam and Jennifer Suurbaar. All authors approved the final manuscript.

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