Journal of Traditional Medicine & Clinical Naturopathy

Antwi, et al., J Tradit Med Clin Natur 2025, 14:3

Research Article Open Access

Ethanol Extract of *Ageratum conyzoides* L. Protects Kidney against Carbon Tetrachloride–Induced Toxicity in Rats

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Abstract

Background: Globally, kidney illnesses continue to pose a significant health burden. Furthermore, the use of medicinal plants to treat a variety of illnesses, including kidney disease, has become a great concern to researchers. Therefore, the goal of the current investigation was to determine how well *Ageratum conyzoides* leaf extracts might reduce oxidative damage and kidney injury caused by CCl_4 in a rat model.

Methods: Haematological and biochemical indicators were used to evaluate the nephroprotective efficacy. Rat kidney homogenates were used to assess *in vivo* antioxidant activities, including TNF-α, TGF-β1, NF κβ1, and COX-2. The histological data were utilized to assess the amount of liver injury.

Results: Pro-inflammatory cytokine expression increases brought on by CCl_4 insult were dramatically and dose-dependently inhibited by ESE treatment. In addition, ESE significantly decreased the amount of lipid peroxidation in the kidney tissue and brought the levels of defensive antioxidant enzymes SOD, GSH, and CAT back to almost normal levels. The benefits of ESE against CCl_4 induced hepatotoxicity damage were validated by histopathological analysis of the kidney; the maximum percentage of liver protection was observed when ESE was administered at a dosage of 500 mg/kg b.wt.

Conclusion: According to the findings, ESE protects the kidneys against CCl_4-induced toxicity through anti-inflammatory and anti-oxidative effects. This effect may be partly attributed to the phytochemicals such as triterpene, alkaloids, phenols, flavonoids, and saponins present

Keywords: Anti-inflammatory; Carbon tetrachloride; Kidney; Nephroprotective

Introduction

The primary metabolic organ responsible for removing waste products, undesirable substances, and dangerous substances from the human system is the kidney [1]. The kidney is essential for the body's removal of toxic substances through filtration and excretion. 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. Animal tissues must constantly cope with highly Reactive Oxygen Species (ROS), which are created during various metabolic physiological activities. Examples of these radicals include hydrogen peroxide, hydroxyl radicals, superoxide anion, and others. Moderate levels of free radical generation appear to be important in life, but high production of Reactive Oxygen Species (ROS) causes oxidative stress. More than half of all instances of acute liver failure in Ghana are caused by oxidative pathways related to the cytochrome P450 enzyme system, which are involved in the hepatic metabolism of pharmaceuticals [2]. In several sectors, Carbon Tetrachloride (CCl_4) is a common chemical solvent. Hira and colleagues reported that kidney damage brought on by carbon tetrachloride is similar to damage brought on by other common causes of renal deterioration, including alcohol, chemicals, viruses, and autoimmune illnesses [3]. The herbal plant *Ageratum conyzoides* is a member of the Asteraceae family. It has also been shown to have antioxidant, insecticidal, anti-inflammatory, gastroprotective, hemostatic, and analgesic qualities [4]. Research on Ghanaian plant species has shown that they contain coumarins, tannins, and terpenoids and that they significantly inhibit leukemic (Jurkat) and prostate (LNCap) cell lines [5]. *A. conyzoides* has also been shown to contain a wide variety of bioactive compounds, including derivatives of ageratochromene, quercetin, scutellarein, kaempferol, and glycoside [6]. In the current investigation, we evaluated the ethanoic crude extract of A*geratum conyzoides* leaves *in vivo* nephroprotective efficacy against carbon tetrachloride using a rat model.

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Received: 21-November-2023, Manuscript No. JHAM-23-120618; **Editor assigned:** 23-November-2023, PreQC No. JHAM-23-120618 (PQ); **Reviewed:** 07-December-2023, QC No. JHAM-23-120618; **Revised:** 02-June-2025, Manuscript No. JHAM-23-120618 (R); **Published:** 09-June-2025, DOI: 10.4172/2573-4555.1000497

Citation: Antwi FS, Lansah R, Larbie C (2025) Ethanol Extract of *Ageratum conyzoides* L. Protects Kidney against Carbon Tetrachloride–Induced Toxicity in Rats. J Tradit Med Clin Natur 14: 497.

Materials and Methods

Chemicals and reagents

The supplier of 99.9% ethanol was Changshu Hongsheng Fine Chemicals Co. Ltd. in China. The investigation only employed analytical-grade materials that were sourced from reliable vendors. Every experiment was conducted at the central laboratory of the school of medicine at the university for development studies in Tamale, Ghana.

Collection and authentication of plant materials

Leaves of *A. conyzoides* were handpicked from the Nyankpala reserve forest of the Northern Region of Ghana. A voucher specimen (UDS-SPPS/DP1/2023/L010) was placed in the herbarium after they were authenticated by a botanist in the university for development studies, school of pharmacy and pharmaceutical sciences, department of pharmacognosy and herbal medicine. The plant samples were prepared using only the leaves. They spent two weeks being air-dried at room temperature after being thoroughly cleaned with water three times. A grinding mill (Christy Lab Mill, England) was used to crush the dry materials.

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 used to filter the supernatant for 20 minutes at a speed of 1106 × g at room temperature. Subsequently, the supernatants were pooled together and concentrated using a rotary evaporator (Buchi R-205, Switzerland). The ethanolic crude extracts of *A. conzyoides* leaves (ESE) were frozen at -20°C freeze-dried and stored until use.

Preliminary phytochemical analysis

The phytochemical components of *A. conzyoides* leaf raw powder and ethanolic crude extracts (ESE) were identified by applying conventional techniques, as stated by Donkor and colleagues [7]. 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. Before the initial oral dosage was administered, all animals were fasted for a duration of 12 hours. During the experiment, they were allowed unlimited access to food and purified water. On the seventh day, all of the rats were sacrificed following an overnight fast under cervical decapitation. Blood samples were extracted from the animals' necks and put in gel-activated tubes and EDTA tubes for biochemical analysis and hematological testing. Kidney homogenates were used to investigate pro-inflammatory cytokines, antioxidant enzymes, and oxidative stress indicators.

Table 1: Animal groups and treatments.

S. no	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.

Effect of extracts on body weight of rats

The following formula was used to calculate the body weights of all animals on the first (D_1) and seventh (D_7) days of treatment.

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

Where weight n is the weight on day 7 and weight o is the weight on day 1.

Effect of extracts on organ weights of rats

To determine the Absolute Organ Weight (AOW), the kidneys of the rats were removed, cleaned in a buffered saline solution, dried on tissue paper, and visually examined. The Relative to each organ was calculated using the procedure below. To calculate the Absolute Organ Weight (AOW), the kidneys of the rats were removed, cleaned in a buffered saline solution, dried on tissue paper, and visually examined. The Relative Organ Weight (ROW) of each organ was calculated using the formula below:

$$\text{ROW} = (\text{AOW} \times 100)/\text{Body weight at sacrifice}$$

Hematological and biochemical analyses

An automated hematological analyzer called Sysmex XS-1000i was used to measure the animals' hematological profiles. The parameters tested include Hemoglobin (HGB), Platelets (PLT), Red Blood Cells (RBC), White Blood Cells (WBC), and Platelets (PLT).

Blood samples were put in activated gel tubes, allowed to coagulate, and then centrifuged at 1500 × g for 15 minutes to collect serum. A completely automated Flexor E chemistry analyzer (Vital Scientific, Japan) was used to carry out the biochemical analysis. Urea and creatinine were the two biochemical markers that were examined. The main kidney functional indicators, such as relative kidney weight, urea, and creatinine, were used to compute the percentage of nephroprotection using the method below;

Percent protection=((Value of toxin controlValue of test group) × 100)/(Value of toxin controlValue of normal control)

Histology and antioxidant assays of the kidney

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. A pathologist identified the amount of the injury after observing the changes in the kidney architecture. Independently, the kidney tissues (0.3 g) were homogenized in 3 ml of ice-cold, 100 mM KH_2PO_4 buffer containing 1 mM EDTA, pH 7.4, and centrifuged at 12,000 rpm for 30 min at 4°C. The supernatant was taken and used to determine the level of kidney antioxidant assays including Malondialdehyde (MDA), Catalase (CAT), reduced glutathione (GSH), and Superoxide Dismutase (SOD) [8].

Statistical analysis

Data were processed using GraphPad Prism 9.0 (USA). One-way ANOVA was used to examine the data, and Tukey's multiple comparison tests were then performed. The results were reported as mean ± SEM. Statistical significance was set at 5% (p 0.05).

Results

Preliminary phytochemical screening

As shown in Table 2, preliminary phytochemical screening of raw powder and ESE revealed the presence of major phytochemical groups.

Table 2: Phytochemical constituents of raw powder and ESE.

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

Note: Present in low concentration (+); Present in moderate concentration (++); Present in high concentration (+++).

Effect of the treatments on rat body weight

Treatments generally resulted in positive increases in body weight except in the CCl_4 treated group only, which observed a significant reduction in body weight as shown in Table 2.

Effect of treatment on relative organ weight

As shown in Figure 1, rats treated with CCl_4 had a rise in relative kidney weight compared to the control group, which was statistically significant (p 0.05), however co-treatment with ESE at 500 mg/kg b.wt. lowered the RKW.

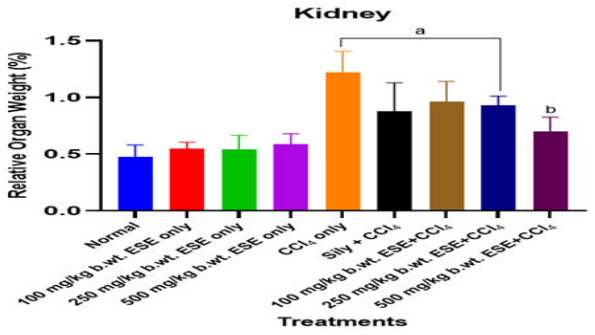

Figure 1: Effect of treatment on relative organ weight. Values are expressed as mean ± SEM (n=5). Statistical significance "a" at p<0.05-0.001 compared to Normal; "b" at p<0.05-0.001 when compared to the CCl_4 treated group only.

Effect of treatment on hematological parameters

The effect of ESE and MSE treatment on hematological indices of CCl_4 intoxicated rats is shown in Table 3. The hematological indices were measured to assess the effect of the CCl_4, silymarin, and ESE on the rats. Generally, the treatments did not cause significant changes to the hematological indices of the rats.

Table 3: Effect of treatment on percent change in body weight of rats.

Treatment	% Change in body weight, D_7
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_4 treatment	
CCl_4 only	-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}

Note: Values are expressed as mean ± SEM (n=5). Statistical significance "a" at p<0.05-0.001 compared to Normal; "b" at p<0.05-0.001 when compared to the CCl_4 treated group only.

Effects of treatments on serum creatinine and urea

Animals treated with CCl_4 significantly increased creatinine and urea levels compared to the normal group (Figure 2).

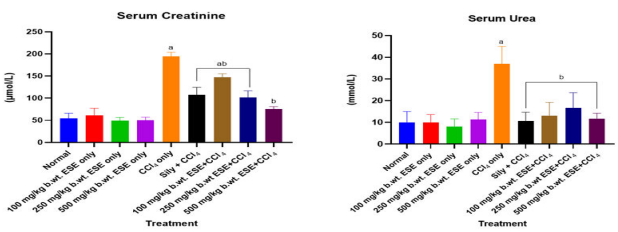

Figure 2: Effects of treatments on serum creatinine and urea. Values are expressed as mean ± SEM (n=5). Statistical significance "a" at p<0.05-0.001 compared to Normal; "b" at p<0.05-0.001 when compared to the CCl_4 treated group only.

Effect of treatment on percentage protection

Figure 3 shows the comparative analysis of the nephroprotective effect of silymarin and ESE at 100, 250, and 500 mg/kg b.wt against CCl_4 administration. ESE at 500 mg/kg b.wt offered the best treatment against CCl_4 at 96% when compared to the standard silymarin at 93%.

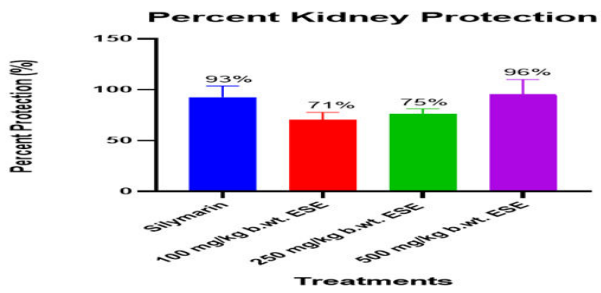

Figure 3: Percentage protection by silymarin, 100 mg/kg b.wt., 250 mg/kg b.wt, and 500 mg/kg b.wt of ESE against Carbon tetrachloride (CCl_4). Each bar is a mean of percentage protection of principal indicators of kidney protection.

Effect of treatments on kidney antioxidants and oxidative stress markers

Table 4 presents the effects of various dosages of ESE on oxidative stressors and renal antioxidants. When 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.

Table 4: Effect of treatment on haematological parameters.

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
Treatment with CCl_4				
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 ± 36.16a^b
100 mg/kg b.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/kg b.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/kg b.wt. ESE+CCl_4	10.86 ± 1.19^b	7.42 ± 1.32^b	4.17 ± 1.73	865.74 ± 43.73^b

Note: Values are expressed as mean ±SEM (n=5). Statistical significance "a" at p<0.05-0.001 compared to Normal; "b" at p<0.05-0.001 when compared to the CCl_4-treated group only.

Treatment effect on inflammation

The effects of treatments on inflammatory cytokines are shown in Figure 4. In the CCl_4 group, TNF-α, TGF-β1, NF κβ, and COX-2 levels were significantly higher ($p<0.05$-0.001) than normal control. Groups treated with extracts only at all doses recorded no significant changes in the inflammatory cytokines.

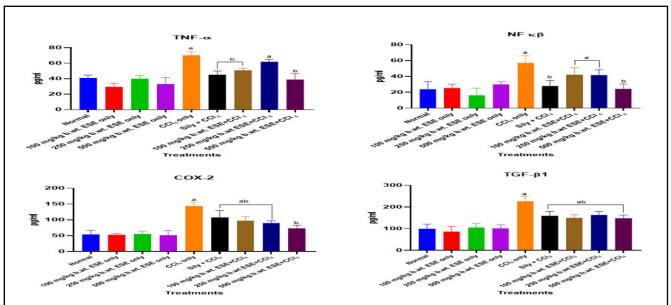

Figure 4: Effect of treatment on inflammatory biomarker. Values are expressed as mean ± SEM (n=5). "a" represents a significant difference from Normal at P<0.05–0.001; "b" represents a significant difference from the CCl_4 treated group at P<0.05–0.001.

Treatment effect on liver histology

The kidneys of the normal groups in Figure 5 display micrographs without any undesired morphological changes; nevertheless, the group receiving CCl_4 alone 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.

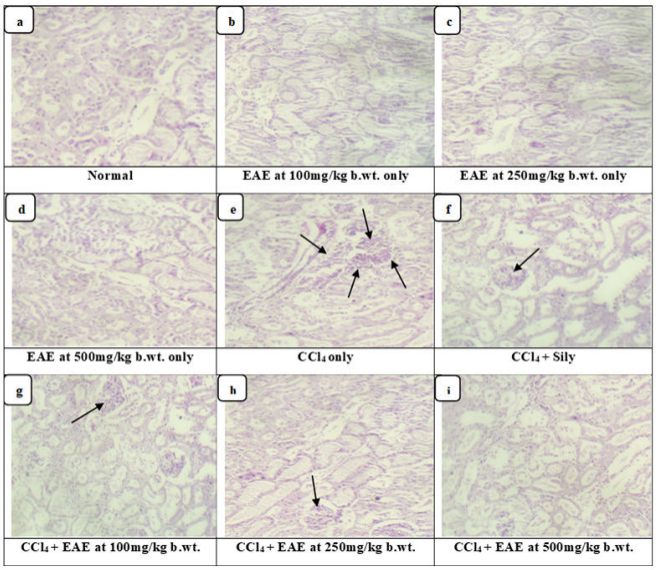

Figure 5: Effect of treatment on kidney histology. (a–d) shows no observable lesion; (e) shows diffuse tubular ectasia with coagulation necrosis of epithelium; (f–h) shows mild coagulation necrosis; (j) shows no observable lesion. CCl_4-Carbon tetrachloride; ESE–Ethanol extract of *A. conzyoides* leaves (HandE × 400).

Discussion

Numerous articles have shown that a variety of mutagens and carcinogens can create peroxide radicals, which are known to play a major role in the formation of cancer and other health disorders [9-11].

The current investigation set out to evaluate any possible protection offered by ESE against carbon tetrachloride-induced oxidative stress and kidney injury in rats. The presence of alkaloids, phenols, flavonoids, triterpenes, tannins, and saponins was shown by phytochemical studies conducted on ESE and raw powder. CCl_4 is a widely used, trusted, and well-known chemical that can harm to the liver and kidney tissue [12-14]. 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 [15-17].

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 4. 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 [18]. 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 [19,20]. Additionally, the capacity of iron to generate hemoglobin in the mitochondria is inhibited by CCl_4. Free radical processes are known to be involved in the kidney damage caused by CCl_4.

A significant rise in MDA levels and a significant reduction in antioxidant enzymes including Catalase (CAT), Superoxide Dismutase (SOD), and reduced Glutathione (GSH) were seen in rats exposed to CCl_4, however, as Table 5 illustrates, rats who received both CCl_4 and ESE concurrently showed a considerable drop in MDA and an increase in antioxidant enzymes. Histological studies demonstrated that reduced levels of antioxidant enzymes caused oxidative stress and lipid peroxidation. In this study, pre-treatment with ESE at all dosages restored normal levels of TNF-α, TGF-β1, NF κβ1, 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-α, TGF-β1, COX-2, IL-17, and IL-23, in addition to overexpression of NF κβ in the hepatic tissues.

Table 5: Effect of treatment on kidney oxidative stress parameters.

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
Treatment (with CCl_4)				
CCl_4 only	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^{a}b
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}
Note: Values are expressed as mean ± SEM (n=5). Statistical significance “a” at p<0.05-0.001 compared to Normal; “b” at p<0.05-0.001 when compared to the CCl_4 treated group only.				

The Mitogen-Activated Protein Kinase (MAPK) pathway, which is known to be triggered by CCl_4, is essential for the production of pro-inflammatory cytokines such as TNF-α, TGF-β1, COX-2, IL-17, and IL-23. Furthermore, CCl_4 participates in the activation of NF-κβ, which regulates the production of several genes linked to inflammation, including adhesion molecules, chemokines, and pro-inflammatory cytokines. 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-κβ 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-α 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.

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-κβ and TNF-α). 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.

Data Availability

The data used to support the findings of this study are available within the article.

Acknowledgment

The authors are grateful to the university for development studies for providing the laboratory to support this study.

Authors’ Contributions

All authors contributed equally to this work.

Funding

No funding was received for this work.

Disclosure

The authors report no conflicts of interest for this work.

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