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Artemisia annua leaves extracts protect against Carbon Tetrachloride-Induced Hepatorenal injury in Rats: A Biochemical and Histopathological study

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Herbal remedies for treating ailments have numerous favor over drugs. *Artemisia annua* L. (*A. annua*) has been discovered to have antioxidant potential. The present study investigated the protective effect of aqueous and ethanolic extracts of *A. annua* leaves against carbon tetrachloride (CCl_4)-induced hepatorenal toxicity in rats. To induce hepatotoxicity, rats were injected intraperitoneally with a single dose of 50% CCl_4 in liquid paraffin (1 ml/kg). Both the aqueous and the alcoholic extracts of *A. annua* leaves were administered orally at 150 mg/kg doses for 21 days and one hour before the CCl_4 injection. CCl_4-induced hepatorenal damage was confirmed histopathologically by hepatic and kidney tissue degeneration, inflammation, damage, and necrosis. Besides the observed significant decrease in body weight gain percentages, plasma total protein, albumin (A), globulin (G), and A/G ratio. CCl_4 also increased plasma levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, uric acid, and creatinine. CCl_4-induced oxidative stress was confirmed by the increased plasma and liver contents of malondialdehyde and decreased glutathione and superoxide dismutase. Rats treated with aqueous and ethanolic extracts of *A. annua* showed significant protection in the liver and kidney histology, liver and kidney function markers, and oxidative stress measures compared to CCl_4-injected rats. The ethanolic extract of *A. annua* provided higher noticeable hepatorenal preservation. The results also documented higher total polyphenols and flavonoid levels in the alcoholic extract. In conclusion, *A. annua* extracts (aqueous and ethanolic) prevented CCl_4-induced hepatorenal damage through reduced oxidative stress and improved antioxidant status.

Keywords: *Artemisia annua*, Carbon tetrachloride, Hepatorenal toxicity, Histopathology, Antioxidant

INTRODUCTION

Hepatic injury is the term used to describe liver malfunction or damage caused by an excess of xenobiotics or medicines (Papay et al. 2009 and Yousefi-Manesh et al. 2020). Renal toxicity is a condition characteristic of the kidney in which the excretion process is impaired by dangerous compounds known as nephrotoxins (Basile et al. 2011). In general, the nephrotoxic chemicals destroy the kidneys and impair their essential functions of synthesizing erythropoietin, removing metabolic waste products, and maintaining electrolyte and fluid balance (Basile et al. 2011). The industrial chemical carbon tetrachloride (CCl_4) is employed routinely as a toxicant to cause poisoning in various organs in laboratory models, including hepatic, renal, cardio, lung, testicular, neuro, and blood toxicity (Ibrahim et al. 2020 and Yousefi-Manesh et al. 2020). CCl_4 causes organ toxicity by producing reactive oxygen species (ROS) and an oxidative stress environment (Alsheblak et al. 2016).

Herbs are rich in antioxidants that fight against various types of ROS (Iqbal et al. 2020). As a result, many researchers are now concentrating on finding and studying therapeutic plants that might protect against organ toxicity. The Asteraceae family's *Artemisia annua* L. (*A. annua*), sometimes called annual wormwood, sweet wormwood, or sweet annie, is a significant genus of aromatic and medicinal plants (Ferreira and Janick, 2009).

Although it originated in temperate Asia, particularly China, it has spread over the world and is now found in many nations, including Argentina, Bulgaria, France, Hungary, India, Italy, Romania, Spain, and the United States (Bora and Sharma, 2011). In Africa, it has been introduced in Cameroon, Ethiopia, Kenya, Tanzania, Uganda, Zambia, Ghana, Rwanda, and South Africa (Willcox et al. 2004).

Additionally, *A. annua* was successfully cultivated in Egypt with high artemisinin contents (El-Askary et al. 2004). *A. annua* is considered a "generally regarded as safe" (GRAS) plant suitable for human consumption (Garcia, 2015). It has long been used in traditional Asian medicine to treat and prevent fevers and chills (Cheng et al. 2011). In China, aqueous formulations of dried *A. annua* plants are recommended for treating fever, malaria, skin diseases, jaundice, cancer-induced stomatitis, and hemorrhoids (Li, 2012). In Pakistan, a decoction of this plant is utilized to treat malaria, although the leaves are employed to treat fevers, coughs, colds, and diarrhea (Hayat et al. 2009).

Some populations in Uganda consume *A. annua* as a once-weekly herbal tea to treat HIV/AIDS and other ailments, including malaria (Ogwang et al. 2011). The Chinese Pharmacopeia recommends drinking tea from dried *A. annua* leaves immersed in hot water (Suberu et al. 2013). In addition, certain Asian nations and the United States consume *A. annua* leaves in salads. *Numerous companies offer A. annua leaves and their extracts as nutritional supplement*s (El-Askarya et al. 2020).

A. annua is now an important source of the antimalarial drug artemisinin (Feng et al. 2020). Various malignancies, schistosomiasis, inflammatory illnesses, viral infections, and fungi have all been treated with artemisinin (P'eterfi and Domokos, 2018). Recent studies have demonstrated that artemisinin and its derivatives can be active against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus at various doses, making them a top option for anti-SARS-CoV-2 medication discovery and development (Cao et al. 2020 and Gilmore et al. 2021).

A. annua has garnered much interest due to its broad biological effects, including antioxidant, immunomodulatory, and anticancer properties *(*Alesaeidi and Miraj 2016 and Septembre-Malaterre et al. 2020). Additionally, the polar fraction of the alcoholic *A. annua* leaf extract showed hepatoprotective and antihyperglycemic properties (El-Askarya et al. 2020).

This work aimed to examine and contrast the protective effects of aqueous and alcoholic extracts of *A. annua* leaves against CCl_4-induced hepatorenal toxicity in rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of *A. annua* L. were collected from the Experimental Station of Medicinal, Aromatic, and Poisonous Plants, Faculty of Pharmacy, Cairo University, Giza. Leaves were identified by Flora & Phytotaxonomy Researchers Department, belonging to Horticultural Research Institute, Agricultural Research Center, Cairo, Egypt.

Chemicals and kits

This experiment employed only analytical-grade chemicals. CCL_4 was brought from Sigma Chemical Co. (St. Louis, Mo, USA). Silymarin (SIL), Legalon® capsules, containing (140 mg), Sedico, Egypt was utilized in this study. The kits used were purchased from Biodiagnostic Egypt. Dokki, Giza, Egypt.

Animals

Forty adults male Sprague Dawley rats weighing 170 -200 g were purchased from the animal unit of the National Research Center (NCR), Dokki, Egypt. Animals were housed in well-aerated cages (4 rats in each) under standard laboratory conditions that include all hygienic measures with constant illumination and ventilation, temperature, and humidity.

The experiment was done at the NCR, Dokki, Egypt animal unit. Animals were given a regular diet and unrestricted access to water. They were given a week to adapt before the experiment began.

Preparation of *A. annua* aqueous extract

Leaves of *A. annua* were washed, air-dried, and then ground into fine powder. 200g of the powdered dried leaves was soaked in boiled distilled water (1g: 25 ml), filtered using a clean cotton cloth, and subsequently with Whatman No.1 (Shiwei et al. 2020). Water was evaporated by freeze-drying (Labconco freeze dryer, Console, 12L, -50°C, Stoppering Tray Dryer, Free. Zone, 240 V, Catalog No. 7754030, Serial No. 100931482 D, USA). The extract was kept at -20 °C in a closed, dark glass container until it was used.

Preparation of *A. annua* alcoholic extract

The powdered dried leaves of *A. annua* (200 g) was subjected to extraction with ethanol (70%). After filtration, the extract was evaporated by a rotary evaporator Heidolph (under vacuum at 40 °C) and then lyophilized (Ghanbari and Sadeghimahalli, 2022). The extract was kept at -20 °C till used.

Determination of total polyphenols and flavonoids content of *A. annua* extracts

Total phenolic content was assessed as described in the Folin-Ciocalteu procedure. It was calculated by a calibration curve prepared with gallic acid and expressed as mg of gallic acid equivalent (mg GAE) / ml sample**.** Total flavonoid content was assessed using aluminum chloride ($AlCl_3$) colorimetric assay. It was determined using a catechin-based calibration curve and represented

as mg of catechin equivalent (mg CE) per milliliter of sample (Zilic et al. 2012).

Induction of hepatotoxicity

Overnight fasted rats were injected intraperitoneally (i.p.) with a single dose of CCl_4 (1 ml/kg of 50% CCl_4 solution in liquid paraffin) (Feng et al. 2010).

Experimental protocol

After the period of adaptation, rats were randomly divided into five groups of 8 rats each as the following scheme:

GR1: Control group.

GR2: Hepatorenal toxicity group, rats were injected i.p. with CCl_4.

GR3: SIL+CCl_4 group; rats were orally ingested SIL (100 mg/kg) as a standard drug for 21 consecutive days (Yuvaraj and Subramoniam, 2009) before CCl_4 injection.

GR4: *A. annua* aqueous extract + CCl_4 group; rats were orally ingested *A. annua* aqueous extract (150 mg/kg) daily for 21 days (Baek et al. 2015) before CCl_4 injection.

GR5: *A. annua* alcoholic extract + CCl_4 group; rats were orally ingested *A. annua* alcoholic extract (150 mg/kg) daily for 21 days (Baek et al. 2015) before CCl_4 injection.

In G3, G4, and GR5, CCl_4 was injected one hour after the last administration of SIL, *A. annua* aqueous extract, and *A. annua* alcoholic extract, respectively.

Assessment of biological evaluation

The rats were weighed at the beginning of the trial (initial weight, IW), weekly, and at the end (final weight, FW). Body weight gain percentage (BWG %) was calculated using the following equation: BWG % = [(FW-IW)/IW] X 100

Samples collection

After 24 hours of CCl_4 injection, all rats were anesthetized. Blood samples were taken from the retro-orbital plexus, and plasma was separated by centrifuging them at 3000 rpm for 15 minutes and kept at -20°C until analysis. Livers and kidneys were gathered from each rat, and portions of the liver and kidneys were fixed in 10% formalin for the histopathological examination. Other liver portions were homogenized in 5 ml cold buffer/1g tissue, centrifuged at 4000 rpm for 15 min, then the supernatant samples were kept at -20°C.

Assessment of hepatic and renal functions

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (A), globulin (G), total protein (TP), urea, creatinine, and uric acid were determined in plasma as described in the kits' procedure.

Assessment of oxidative stress/ antioxidants indices

Plasma and liver lipid peroxide contents measured as malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) contents were quantified using ELISA kits.

Histopathological examination

The formalin-fixed liver and kidney sections were prepared. Hematoxylin and eosin (H&E) staining was applied, and a histopathologist performed a blind examination under a light microscope.

Statistical analysis

The results were reported as mean ± standard error (SE). SPSS software (version 27) was used to do the statistics analysis (one-way analysis of variance (ANOVA) followed by LSD test). The significance level was set at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

Total phenols and total flavonoids contents determined in *A. annua* leaves ethanolic and aqueous extracts

The findings of the current work showed that when *A. annua* leaves were extracted using ethanol, the amounts of total phenols and total flavonoids were significantly higher than when the leaves were extracted using water ($p \leq 0.01$). Both the ethanolic and aqueous extracts from *A. annua* leaves contained 2.21± 0.10 and 1.75 ± 0.08 mg GAE/ml of total phenols, respectively. Additionally, the quantity of total flavonoids in the ethanolic and aqueous extracts of *A. annua* leaves were 0.71 ± 0.06 and 0.51 ± 0.06 mg CE/ml, respectively (Table 1).

Table 1: Total phenols and total flavonoids contents quantified in *A. annua* leaves ethanolic and aqueous extracts

Extract type	Total phenols (mg GAE/ml)	Total flavonoids (mg CE/ml)
***A. annua* aqueous extract**	1.75 ± 0.08	0.51 ± 0.06
***A. annua* ethanolic extract**	2.21 ± 0.10 [a**]	0.71 ± 0.06 [a**]

Values were presented as the mean of 3 replicates ± SE. [a]Significantly differ compared to the aqueous *A. annua* extract values at ($p \leq 0.01$).

Effects of *A. annua* leaves ethanolic and aqueous extracts on IBW, FBG, and BWG% quantified in CCl_4-induced hepatorenal toxicity in rats

Table 2 showed the IW, FW, and BWG% of the control (GR1), CCl_4 (GR2), SIL (GR3), aqueous *A. annua* leaves extract (GR4), and ethanolic *A. annua* leaves extract (GR5). There was no significant difference in IW among all rat groups. Rats in GR2 had significantly lower FW and BWG% values than those in GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly higher FW and BWG% values compared to GR2 ($p \leq 0.01$). Compared to GR4 and GR5, FW and BWG% of GR3 were significantly higher ($p \leq 0.01$ and $p \leq 0.05$, respectively).

Additionally, GR5 showed statistically significant increases in FW and BWG% compared to GR4 ($p \leq 0.05$).

Table 2: Effects of *A. annua* leaves ethanolic and aqueous extracts on IBW, FBG, and BWG% quantified in CCl_4-induced hepatorenal toxicity in rats.

Groups	IW(g)	FW(g)	BWG%
GR1	180.63 ± 3.39	226.32 ± 1.76	25.29 ± 0.99
GR2	184.25 ± 2.15	195.63 ± 2.08 ^{a**}	6.18 ± 0.48 ^{a**}
GR3	183.88 ± 2.42	220.25 ± 2.29 ^{b**}	19.78 ± 0.84 ^{b**}
GR4	182.38 ± 2.15	206.13 ± 2.69 ^{b**,c**}	13.02 ± 0.87 ^{b**,c**}
GR5	184.00 ± 2.52	213.88 ± 1.48 ^{b**,c*,d*}	16.24 ± 1.47 ^{b**,c*,d*}

Values were presented as mean ± SE (n=8). ^{a} Significantly differ compared to GR1, ^{b} significantly differ compared to GR2. ^{c} significantly differ from GR3, ^{d} significantly differ from GR4 ($^{*}p \leq 0.05$ and $^{**}p \leq 0.01$).
IW: Initial weight; FW: Final weight; BWG%: Body weight gain percentage.
GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

Effects of *A. annua* leaves ethanolic and aqueous extracts on hepatic functions quantified in CCl_4-induced hepatorenal toxicity in rats

Table 3 showed the markers of hepatic function ALT, AST, and ALP of the control (GR1), CCl_4 (GR2), SIL (GR3), aqueous *A. annua* leaves extract (GR4), and ethanolic *A. annua* leaves extract (GR5). Rats in GR2 had significantly higher plasma ALT, AST, and ALP levels than those in GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly lower plasma ALT, AST, and ALP levels compared to GR2 ($p \leq 0.01$). Compared to GR4 and GR5, plasma ALT, AST, and ALP levels of GR3 significantly decreased ($p \leq 0.01$ and $p \leq 0.05$, respectively). Additionally, GR5 showed statistically significant decreases in plasma ALT, AST, and ALP levels compared to GR4 ($p \leq 0.05$).

Effects of *A. annua* leaves ethanolic and aqueous extracts on renal function quantified in CCl_4-induced hepatorenal toxicity in rats

Table 4 showed the markers of renal function urea, uric acid, and creatinine of the control (GR1), CCl_4 (GR2), SIL (GR3), aqueous *A. annua* leaves extract (GR4), and ethanolic *A. annua* leaves extract (GR5). Rats in GR2 had significantly higher plasma urea, uric acid, and creatinine levels than those in GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly lower plasma urea, uric acid, and creatinine levels compared to GR2 ($p \leq 0.01$). Compared to GR4 and GR5, plasma urea, uric acid, and creatinine levels of GR3 were significantly decreased ($p \leq 0.01$ and $p \leq 0.05$, respectively). Additionally, GR5 showed statistically significant decreases in plasma urea, uric acid, and creatinine levels compared to GR4 ($p \leq 0.05$).

Table 3: Effects of *A. annua* leaves ethanolic and aqueous extracts on hepatic functions quantified in CCl_4-induced hepatorenal toxicity in rats.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
GR1	85.60 ± 1.51	19.83 ± 1.51	160.64 ± 5.90
GR2	166.99 ± 5.66 ^{a**}	107.37 ± 3.58 ^{a**}	321.56 ± 12.35 ^{a**}
GR3	94.05 ± 2.26 ^{b**}	32.03 ± 2.19 ^{b**}	205.16 ± 9.25 ^{b**}
GR4	114.16 ± 2.35 $^{b**, c**}$	56.63 ± 4.56 $^{b**, c**}$	279.70 ± 13.73 $^{b**, c**}$
GR5	103.99 ± 3.69 $^{b**, c*, d*}$	45.12 ± 4.05 $^{b**, c*, d*}$	246.21 ± 10.99 $^{b**, c*, d*}$

Values were presented as mean ± SE (n=8). ^{a} Significantly differ compared to GR1, ^{b} significantly differ compared to GR2. ^{c} significantly differ from GR3, ^{d} significantly differ from GR4 ($^{*}p \leq 0.05$ and $^{**}p \leq 0.01$).
ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.
GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

Table 4; Effects of *A. annua* leaves ethanolic and aqueous extracts on renal functions determined in CCl_4-induced hepatorenal toxicity in rats.

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
GR1	25.98 ± 1.61	1.61 ± 0.20	0.235 ± 0.01
GR2	63.27 ± 2.44 ^{a**}	6.66 ± 0.26 ^{a**}	0.719 ± 0.05 ^{a**}
GR3	30.65 ± 1.63 ^{b**}	2.14 ± 0.18 ^{b**}	0.302 ± 0.01 ^{b**}
GR4	43.77 ± 1.71 ^{b**,c**}	3.47 ± 0.13 ^{b**,c**}	0.449 ± 0.02^{b**,c**}
GR5	36.74 ± 1.78 $^{b**, c*, d*}$	2.87 ± 0.20 $^{b**, c*, d*}$	0.366 ± 0.03$^{b**, c*, d*}$

Values were presented as mean ± SE (n=8). [a] Significantly differ compared to GR1, [b] significantly differ compared to GR2. [c] significantly differ from GR3, [d] significantly differ from GR4 ($^{*}p \leq 0.05$ and $^{**}p \leq 0.01$).
GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

Effects of *A. annua* leaves ethanolic and aqueous extracts on plasma TP, albumin (A), globulin (G), and A/G ratio quantified in CCl_4-induced hepatorenal toxicity in rats

Table 5 showed the plasma levels of TP, albumin (A), globulin (G), and A/G ratio of the control (GR1), CCl_4 (GR2), SIL (GR3), aqueous *A. annua* leaves extract (GR4), and ethanolic *A. annua* leaves extract (GR5). Rats in GR2 had significantly lower plasma TP, A, G, and A/G ratio than those in GR1 ($p \leq 0.01$). Rats in GR3 showed significantly higher plasma TP, A, G, and A/G ratio compared to GR2 ($p \leq 0.01$). Compared to GR4, plasma TP, A, and G levels of GR3 were significantly higher ($p \leq 0.01$, ≤ 0.01, and ≤ 0.05, respectively). Additionally, GR5 showed statistically significant increases in plasma TP and A compared to GR4 ($p \leq 0.05$).

Table 5: Effects of *A. annua* leaves ethanolic and aqueous extracts on plasma total protein (TP), albumin (A), globulin (G), and A/G ratio determined in CCl_4-induced hepatorenal toxicity in rats

Groups	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
GR1	5.95 ± 0.14	3.19 ± 0.13	2.76 ± 0.07	1.16 ± 0.019
GR2	3.18 ± 0.10 [a**]	1.58 ± 0.10 [a**]	1.59± 0.10 [a**]	0.99 ± 0.053 [a**]
GR3	4.77 ± 0.15 [b**]	2.69 ± 0.10 [b**]	2.08 ± 0.15[b**]	1.29 ± 0.051 [b**]
GR4	3.79 ± 0.12 [b**,c**]	2.08 ±0.03 [b**,c**]	1.71± 0.08 [c*]	1.22 ± 0.047 [b**]
GR5	4.28 ± 0.16 [b**, c*, d*]	2.36 ±0.08[b**, c*, d*]	1.92 ± 0.09 [b]	1.23 ± 0.036 [b**]

Values were presented as mean ± SE (n=8). [a] Significantly differ compared to GR1, [b] significantly differs compared to GR2. [c] significantly differs from GR3, [d] significantly differs from GR4 ($^{*}p \leq 0.05$ and $^{**}p \leq 0.01$).

GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

Effects of *A. annua* leaves ethanolic and aqueous extracts on plasma oxidative stress/ antioxidants indices quantified in CCl_4-induced hepatorenal toxicity in rats

Figure 1 showed the plasma concentrations of oxidative stress marker MDA, as well as the antioxidants markers GSH and SOD of the control (GR1), CCl_4 (GR2), SIL (GR3), aqueous *A. annua* leaves extract (GR4), and ethanolic *A. annua* leaves extract (GR5).

Rats in GR2 showed significantly higher plasma MDA concentration than that in GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly lower plasma MDA concentrations compared to GR2 ($p \leq 0.01$). Compared to GR4, the plasma MDA concentration of GR3 was significantly lower ($p \leq 0.05$). Additionally, GR5 showed a statistically significant decrease in plasma MDA concentration compared to GR4 ($p \leq 0.05$).

Conversely, rats in GR2 showed significantly lower plasma GSH and SOD concentrations than GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly higher plasma GSH and SOD concentrations compared to GR2 ($p \leq 0.01$). Compared to GR4, plasma GSH and SOD concentrations of GR3 were significantly higher ($p \leq 0.05$). Additionally, GR5 showed statistically significant increases in plasma GSH and SOD concentrations compared to GR4 ($p \leq 0.05$).

Effects of *A. annua* leaves ethanolic and aqueous extracts on hepatic oxidative stress/ antioxidants indices quantified in CCl_4-induced hepatorenal toxicity in rats

Figure 2 showed the hepatic concentrations of oxidative stress marker MDA, as well as the antioxidants markers GSH and SOD of the control (GR1), CCl_4 (GR2), SIL (GR3), aqueous *A. annua* leaves extract (GR4), and ethanolic *A. annua* leaves extract (GR5).

Rats in GR2 showed significantly higher hepatic MDA concentration than that in GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly lower hepatic MDA concentration compared to GR2 ($p \leq 0.01$). Compared to GR4, hepatic MDA concentration of GR3 were significantly lower ($p \leq 0.05$). Additionally, GR5 showed statistically significant decrease in hepatic MDA concentration compared to GR4 ($p \leq 0.05$).

Conversely, rats in GR2 showed significantly lower hepatic GSH and SOD concentrations than those in GR1 ($p \leq 0.01$). Rats in GR3, GR4, and GR5 showed significantly higher hepatic GSH and SOD concentrations compared to GR2 ($p \leq 0.01$). Compared to GR4, hepatic GSH and SOD concentrations of GR3 were significantly higher ($p \leq 0.05$). Additionally, GR5 showed statistically significant increases in hepatic GSH and SOD concentrations compared to GR4 ($p \leq 0.05$).

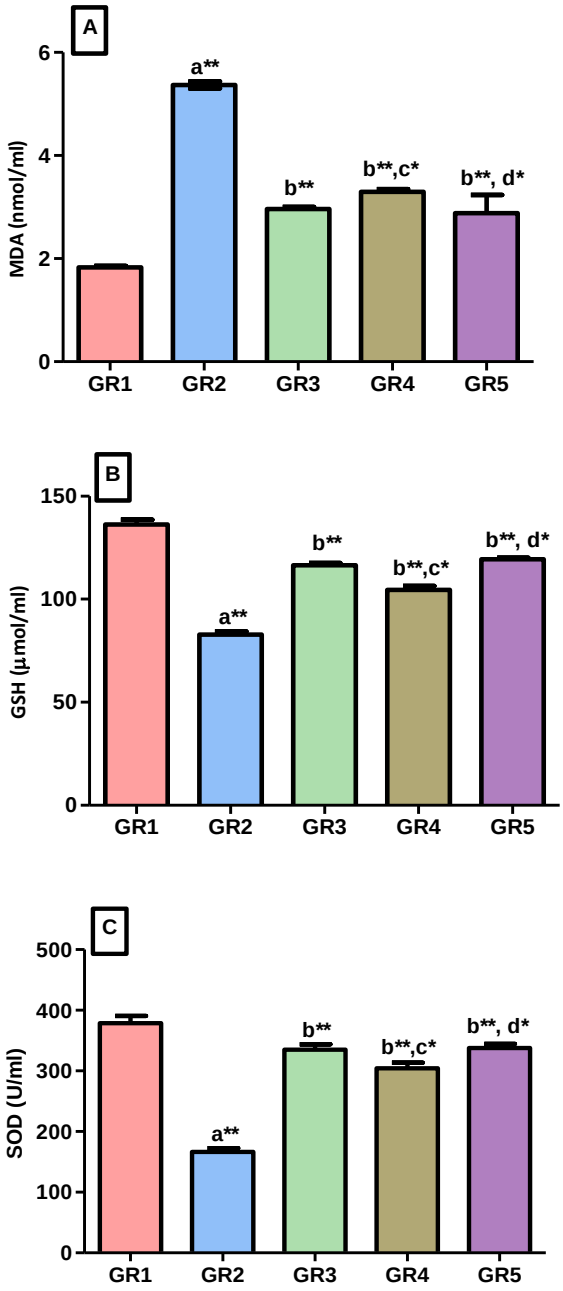

Figure 1: Effects of *A. annua* leaves ethanolic and aqueous extracts on plasma oxidative stress/ antioxidants indices quantified in CCl_4-induced hepatorenal toxicity in rats.

Values were presented as mean ± SE (n=8). [a] Significantly differ compared to GR1, [b] significantly differ compared to GR2. [c] significantly differ from GR3, [d] significantly differ from GR4 ($^{*}p \leq 0.05$ and $^{**}p \leq 0.01$).

MDA: Malondialdehyde; GSH: Reduced glutathione; SOD: Superoxide dismutase.

GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

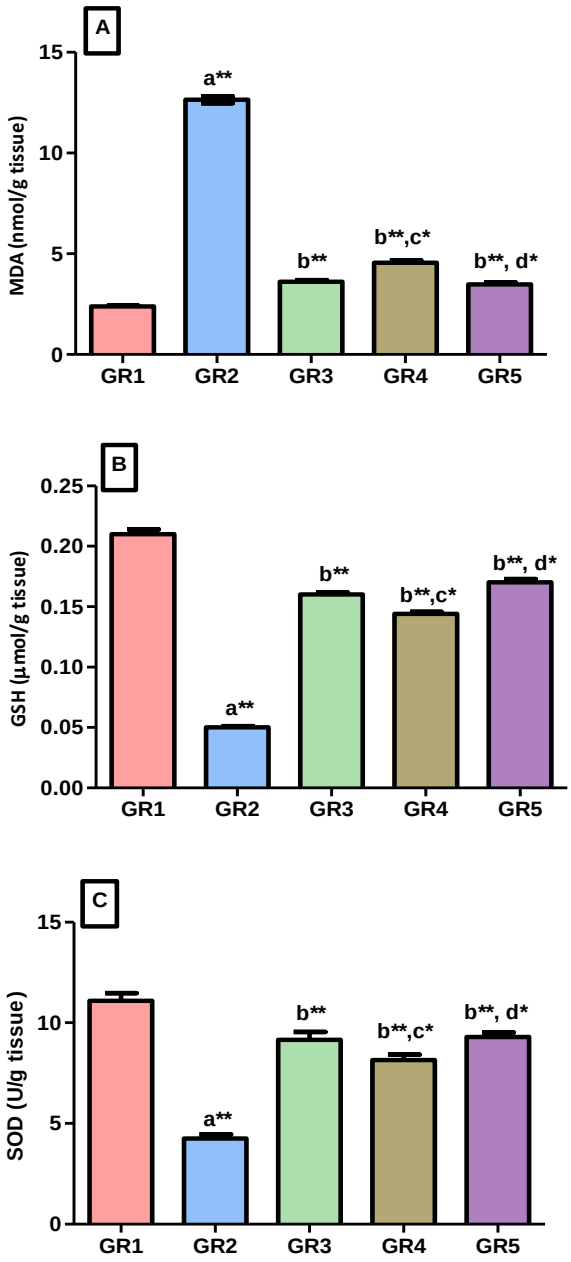

Figure 2: Effects of *A. annua* leaves ethanolic and aqueous extracts on hepatic oxidative stress/ antioxidants indices quantified in CCl_4-induced hepatorenal toxicity in rats.

Values were presented as mean ± SE (n=8). [a] Significantly differ compared to GR1, [b] significantly differ compared to GR2. [c] significantly differ from GR3, [d] significantly differ from GR4 ($^{*}p \leq 0.05$ and $^{**}p \leq 0.01$).

MDA: Malondialdehyde; GSH: Reduced glutathione; SOD: Superoxide dismutase.

GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

Effects of *A. annua* leaves ethanolic and aqueous extracts liver histopathology

Sections of GR1 rats' livers showed normal hepatocytes (Figure 3 A). Liver sections of GR2 rats showed severe vacuolar degeneration of the hepatocytes with inflammatory cell infiltrations around blood vessels (Figure 3 B). High power photo showed fibrous connective tissue proliferation in the portal area, dilated central vein, and newly formed bile ductulus (Figure 3 C). Liver sections of GR3 rats showed an apparently normal structure except for slight vacuolar degeneration of hepatocytes with pericentral inflammatory cell infiltrations (Figure 3 D). Liver sections of GR4 rats showed vacuolar degeneration of hepatocytes with inflammatory cell infiltrations around the blood vessels (Figure 3 E). The liver sections of GR5 rats showed normal structure except for the slight degeneration of hepatocytes (Figure 3 F).

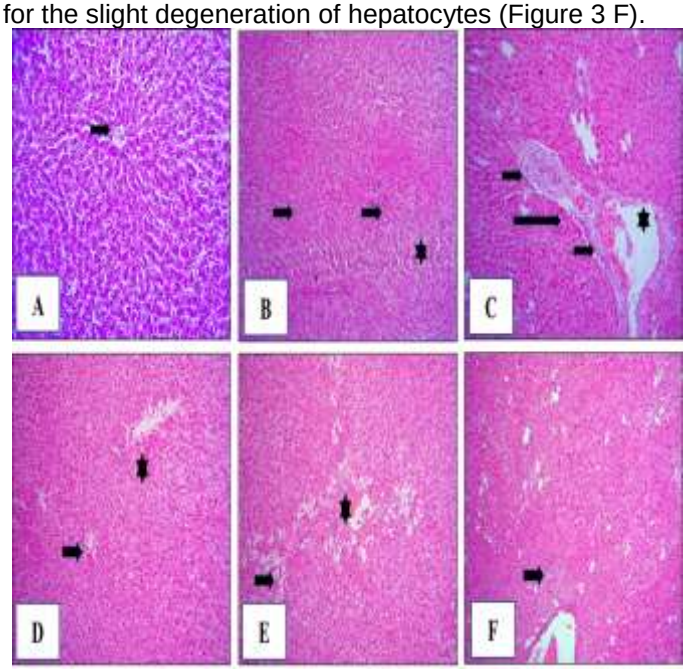

Figure 3: Effects of *A. annua* leaves ethanolic and aqueous extracts on liver histopathology (H&E x200 for all photos except photo c H&E x400).

Photo A represented the liver section of a rat from the GR1, showed normal hepatocytes and portal vein (arrow). Photo B represented the liver section of a rat from the GR2 showed severe vacuolar degeneration of hepatocytes (arrows) and inflammatory cell infiltrations around blood vessels (star). Photo C represented a high-power magnification of the liver section of a rat from the GR2, showed fibrous connective tissue proliferation in the portal area (arrows), dilated central vein (stars), and newly formed bile ductulus (large arrows). Photo D represented the liver section of a rat from the GR3 showed an apparently normal structure except for slight vacuolar degeneration of hepatocytes (arrow) and mild pericentral inflammatory cell infiltrations (star). Photo E represented the liver section of a rat from the GR4 showed vacuolar degeneration of hepatocytes (arrow) and inflammatory cell infiltrations around blood vessels (star). Photo F represented the liver section of a rat from the GR 5 showed an apparently normal structure except for the slight degeneration of hepatocytes (arrow).

GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

Effects of *A. annua* leaves ethanolic and aqueous extracts on kidney histopathology

Sections of GR1 rats' kidneys showed normal renal tubules and glomerular tuft (Figure 4 A). Kidney sections of GR2 rats showed necrosis and degeneration of epithelial lining renal tubules together with glomerular tufts and inflammatory cells infiltrations (Figure 4 B). High power photo showed inflammatory cells infiltrations, interstitial hemorrhages, widening space of Bowman's capsule severe necrosis, and degeneration of epithelial lining renal tubules and glomerular tufts (Figure 4 C). Kidney sections of GR3 rats showed an apparently normal structure except mild degeneration of the epithelial lining renal tubules with the presence of hyaline casts within few renal tubules (Figure 4 D). Kidney sections of GR4 rats showed vacuolation of the blood vessel wall with perivascular connective tissue proliferation (Figure 4 E). The kidney sections of GR5 rats showed an apparently normal structure except for slight vacuolation of the blood vessel wall with perivascular connective tissue proliferation and narrow space of Bowman's capsule (Figure 4 F).

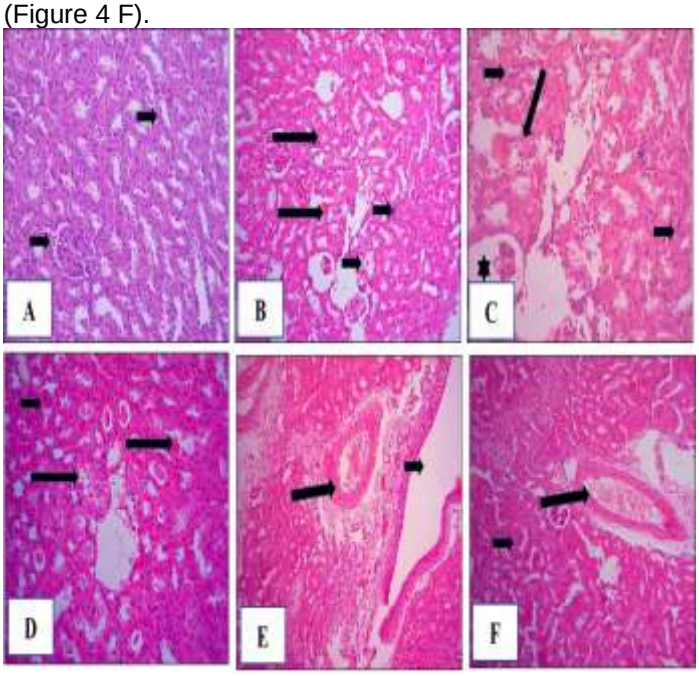

Figure 4: Effects of *A. annua* leaves ethanolic and aqueous extracts on kidney histopathology (H&E x200 for all photos except photo c H&E x400).

Photo A represented the kidney section of a rat from the GR1 showed normal renal tubules, glomerular tuft, and Bowman's capsule (arrow). Photo B represented the

kidney section of a rat from the GR2 showed necrosis and degeneration of epithelial lining renal tubules (arrows) and some glomerular tufts and inflammatory cell infiltrations (large arrow). Photo C represented a high-power magnification of the kidney section of a rat from the GR2, showed inflammatory cell infiltrations (arrow), interstitial hemorrhages (large arrow), widening space of Bowman's capsule (star), severe necrosis, and degeneration of epithelial lining renal tubules and glomerular tufts. Photo D represented the kidney section of a rat from the GR3 showed an apparently normal structure except for mild degeneration of the epithelial lining renal tubules (arrow) and hyaline casts within renal tubules (large arrow). Photo E represented the kidney section of a rat from the GR4 showed vacuolation of the blood vessels wall (arrow) and perivascular connective tissue proliferation (large arrow). Photo F represented the kidney section of a rat from the GR 5, showed an apparently normal structure and Bowman's capsule except for slight vacuolation of the blood vessels wall (arrow) with perivascular connective tissue proliferation (large arrow).

GR1: control; GR2: CCl_4; GR3: SIL (100 mg/ kg) + CCl_4; GR4: *A. annua* aqueous extract (150 mg/kg) + CCl_4; GR5: *A. annua* ethanolic extract (150 mg/kg) + CCl_4.

DISCUSSION

This study elucidated the protective effect of *A. annua* leaves aqueous and alcoholic extracts against CCl_4-induced hepatorenal toxicity in rats. Hepatorenal damage is a known toxicity of CCl_4 (Elsawy et al. 2019). Overproduction of ROS and oxidative damage determine the degree of CCl_4 toxicity (Sun et al. 2022). BW is a crucial factor in determining the harmful effects of chemicals (Gangar and Koul, 2008). In this study, the BWG percentage in the CCl_4 group decreased significantly relative to the control group. These results agree with Lee et al. (2019) and Hijazy (2021), who also found that CCl_4 reduced the BWG percentage of rats. It has been hypothesized that the reduction in BW by CCl_4 injection is the consequence of direct toxicity of CCl_4 and/or indirect toxicity through liver damage. These findings are consistent with Pradeep, et al (2005), who indicated that BW changes following the CCl_4 dose had been utilized as a useful predictor of CCl_4-related organ damage (El–Bana et al. 2015).

The results of this study showed significant increases in plasma ALT, AST, and ALP levels, although significant decreases in TP, A, G, and A/G ratio occurred in the CCl_4-administered group. According to earlier research, these findings point to hepatocyte malfunction, cellular leakage, and a loss of the liver's functional integrity in the cell membrane (Khan et al. 2012). In this research, *A. annua* leaves aqueous, and alcoholic extracts treatment apparently prevented the liver damage induced by CCl_4. Due to its naturally occurring polyphenolic properties, *A. annua* may function as a membrane-stabilizing agent that prevents enzyme leakage and preserves liver enzyme homeostasis. The primary ingredient of *A. annua* leaves extract, flavonoids, has a potent antioxidant action to reduce oxidative stress generated by ROS, which may explain why plasma hepatic transaminases are inhibited (Al-Musawi et al. 2022). The observed decrease in hepatic enzyme leakage in this research is consistent with (Al-Musawi et al. 2022 and Salah et al. 2022), which showed that following *A. annua* therapy, AST, and AL levels were decreased.

The present research also showed that rat liver sections treated with *A. annua* extract before exposure to CCl_4 reduced histopathological changes. Our findings are corroborated by Salah et al. (2022), which claimed that *A annua* extract improves liver sections featuring normal-appearing hepatocytes with almost normal nuclei, normal portal vein, blood sinusoids, and Kupfer cells with minimum vacuolization cytoplasm. Moreover, the ROS scavenging activity, antioxidant and anti-inflammatory properties of *A. annua* were essential for keeping liver health and hepatic preservation (Young et al. 2020). Artemisinin, a family of sesquiterpene trioxane lactone antimalarials, was found to boost the hepatocyte cell membrane's stability and guard against its damage. This preservation may arise from a reduction in the expression of inflammatory cytokines and inducible nitric oxide synthase as well as nuclear factor kappa beta (NF-κB) stimulation (Xiaoyan et al. 2017).

Efficient antioxidant responses are indicated when ROS are produced in excess relative to the cell's capacity. Lipid peroxidation may contribute to ROS-mediated liver injury. One of the by-products of lipid peroxidation is MDA, both *A annua* extracts could significantly reduce MDA levels in the plasma and liver. Superoxide dismutase (SOD) and reduced glutathione (GSH) are two antioxidants designed to protect cells from ROS. SOD, an enzyme in the mitochondria that contain manganese, SOD anions into hydrogen peroxide (H_2O_2) (Reiter et al. 2000). GSH is a crucial regulator for antioxidant enzymes and a significant water-phase scavenger that defends the mitochondria from endogenous reactive oxygen (Ho et al. 2006). In our study, both *A annua* extracts increased liver and plasma SOD and GSH. The emergence of artemisinin and its derivatives in *A. annua* is well-documented (Eugene et al. 2014). While artemisinin was initially used to treat malaria, numerous studies have revealed that artemisinin and its related compounds also positively impact cancer, viruses, fungi, parasites, inflammation, and oxidative stress (Tutun et al. 2019). *A annua* is also known for its essential oils with antioxidant properties (Fabien et al. 2002 and Showkat et al. 2013).

The kidney likewise benefited from *A annua's* protective properties observed in the liver. Urine, uric acid, and creatinine, which are the three leading renal indicators, exhibit significant decreases following administration of *A annua*, which may be caused by an antioxidative stress mechanism. Similar to the findings of this investigation, administering an ethanolic extract of *A*

annua L. dramatically lowers urea levels in rats exposed to lead acetate, while having no impact on the blood creatinine levels raised by lead acetate (Al-Musawi et al. 2022). *A annua L.* has reportedly been shown to reduce proteinuria and stop the progression of renal dysfunction (Jiawei et al. 2020). Numerous studies have also claimed that artemisinin can alleviate kidney impairment in rats by reducing the expression of metalloproteinase, protein kinase C (PKC), and platelet-derived growth factor B (PDGF-B) (Gleeson et al. 2019 and Xiang et al. 2019). One study found that taking 2 to 4g of cordyceps powder and 0.6g of the artemisinin from the herb *A annua* for three years restored renal function as determined by creatinine clearance in 61 patients with lupus nephritis (Ahmad et al. 2014). Moreover, the findings of a prior study revealed that artemisinin could protect the kidney against doxorubicin-induced renal damage, most likely due to its antioxidant properties and the reduction of NF-κB, inducible nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNFα), and caspase-3 upregulation (Tutun et al. 2019). *A annua L.* further improved malaria nephropathy (Xia et al. 2020). The nephroprotective effect of *A annua* leaf extract against CCl_4-induced kidney injury was first reported in the current investigation.

According to previous reports, the phenolic chemicals luteolin, luteolin-7-glucoside, kaempferol, quercetin, rutin, and others were present in high amounts in *A. annua* (Yizhong et al. 2004 and Kim et al. 2014). The current investigation also showed that phenolic chemicals were present in both the ethanolic and aqueous extracts of the *A annua*, but that the ethanolic extract had a higher concentration. This could account for our findings, which indicated that the ethanolic extract provided more pronounced hepatorenal protection.

CONCLUSION

In conclusion, both *A annua* extracts (aqueous and ethanolic) prevented CCl_4-induced hepatorenal damage through reduced oxidative stress and improved antioxidant status. However, the effectiveness of treatment with the alcoholic extract was superior to the efficacy of the water extract. These results support the idea that high levels of bioactive components may be present in the alcoholic extract.

CONFLICT OF INTEREST

The authors declared that the present study was performed without any conflict of interest.

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AUTHOR CONTRIBUTIONS

IA and NA designed the study. HE and EF prepared the extracts, performed animal treatments, samples and tissue collection. IA and MM data analysis and wrote the manuscript. All authors reviewed the manuscript, read and approved the final version.

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