International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 12 No. 1, 2026
https://theaspd.com/index.php

Phytochemical Characterization and in Vivo Toxicological, Antioxidant and Hepatoprotective Assessment of Artemisia campestris L. Aerial Parts

Aymane Zouane[1]; Lynda Aïnouz[1,2]; Wahiba Zenad[1]; Katia Feddi[3]; Nassima Timsit[1]; Sofia Bouguettaya[3], Leila Bouayad[1]; Sofiane Boudjellaba[3]; Djamel Khelef[4]; Amine Berghiche[5]; Fatma Amira Hani[4]; Mohamed Zaouani[1,2]

[1] Laboratory of Food Hygiene and Quality Insurance System (HASAQ), Higher National Veterinary School/Ecole Nationale Supérieure Vétérinaire, Oued-Smar, 16000, Algiers, Algeria
[2] Laboratory of Biology and Animal Physiology, ENS-Kouba, Algiers, Algeria
[3] Laboratory for the Management of Local Animal Resources (GRAL), Higher National Veterinary School/Ecole Nationale Supérieure Vétérinaire, Oued-Smar, 16000, Algiers, Algeria,
[4] Animal Health & Production Laboratory (SPA), Higher National Veterinary School/Ecole Nationale Supérieure Vétérinaire, Oued-Smar, 16000, Algiers, Algeria
[5] Laboratory of Science and Technique of Life, Institute of Agronomic and Veterinary Sciences, Mohamed Cherif Messaadia University- Souk Ahras, Algeria.

Received: 15/08/2025 **Accepted**:20/01/2026 **Published**: 20/05/2026

ABSTRACT

*The objective of this study is to evaluate the cytoptotective effects against hepatotoxicity of the aqueous extract of the aerial parts of Artemisia campestris L.(**AEAPAC**) after qualitative phytochemical assessment. Silymarin at a dose of 50 mg/kg/day and AEAPAC at doses of 200 and 400 mg/kg/day were orally administrated to male Wistar rats during 14 days. On day 14, hepatotoxicity was induced by a single intraperitoneal injection of CCl_4 (3 ml/kg, 12% in sunflower oil). Blood samples and liver tissues were collected for biochemical, oxidative stress and histopathological examination. A comparative analysis was conducted using a positive control group with hepatotoxicity and a negative control group without hepatotoxicity. The AEAPAC phytochemical screening, revealed the presence of various bioactive compounds. In rats pre-treated with graded oral doses of the **AEAPAC**, biochemical and oxidative stress parameters results showed a significant decrease ($p < 0.01$) in serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), and malondialdehyde (MDA), along with a significant increase ($p < 0.01$) in reduced glutathione (GSH), glutathione S-transferase (GST), and glutathione peroxidase (GPx) levels. Histopathological examination revealed a significant reduction in lesions in the groups pre-treated daily with increasing oral doses of **AEAPAC**. These results are similar to those obtained in rats pre-treated with silymarin. It can be inferred that the **AEAPAC** exhibits an in vivo antioxidant activity and hepatoprotective effects against CCL_4-induced liver damage, which could be attributed to its rich composition of bioactive molecules. It would be interesting to conduct further tests in the use of **AEAPAC** as a pre-treatment for hepatotoxicity.*

Key words: *Artemisia campestris L., acute toxicity; hepatotoxicity, oxidative stress, CCL_4, phytochemical analysis*

INTRODUCTION

The liver, a metabolic hub of the animal body, is an important synthetic and metabolic organ in the body to remove toxic and harmful substances (1). While excessive administration of external chemicals or drugs, the liver is vulnerable to tissue damage, exposure to environmental toxins can may result in hepatic injury via complex mechanisms involving oxidative stress, inflammation, and necrosis (2,3). Carbon tetrachloride (CCl_4), once widely used in the chemical industry as a solvent, has been banned in most countries over the past decade due to its pronounced toxicity (3). Nevertheless, in experimental and laboratory conditions CCl_4 is one of the most commonly used in experimental models of acute and chronic liver damage. This compound is extensively used in biomedical research to assess the hepatoprotective potential of natural or synthetic substances (4). It is well accepted that CCl_4, a potent hepatotoxic chemical, is reduced by hepatic cellular cytochrome P450 2E1 (CYP2E1) in endoplasmic reticulum to produce the highly reactive free radicals including trichloromethyl (•CCl3), trichloromethyl peroxy radical (•OOCCl3) and reactive oxygen species (ROS) (5). However, excess production of these reactive free radicals within the tissues ultimately leads to oxidative stress, which plays a

crucial role in the pathogenesis of liver injury (4). ROS's capacity to interact with different types of biomolecules in the cell (4,6) results in the destruction of cell structure, contributing physiologic dysfunction, lipid peroxidation, and ultimately leading to hepatic damage (7). Conventional therapies for liver diseases often rely on synthetic drugs; however, over the past decade, there has been a marked shift toward the exploration of natural products. This evolution has stimulated extensive research aimed at identifying natural sources of antioxidants as potential and validated alternatives for the treatment of hepatic disorders (8). Herbal medicines play a significant role in managing a wide range of liver ailments, adding to extra healing activities of the liver. A great number of plants have been asserted for the treatment of liver disorders (9) . These plants are said to possess hepatoprotective properties as a result of the presence of various phytoconstituents with antioxidant potential (10). Consequently, researchers have focused considerable attention on evaluating the hepatoprotective effects of bioactive natural compounds derived from medicinal plants (11). *Artemisia campestris L.* is a perennial aromatic herb belonging to asteraceae family; it is widespread in Northern Africa and other similar Mediterranean agro-ecological zones. It is traditionally used as an herbal remedy (7). In Algeria , *A. campestris* locally named as "Dgouft", is widely used in traditional medicine as decoction for their antivenin, anti-inflammatory, antirheumatic and antimicrobial properties (7,11). The objectives of this study were to determine its qualitative composition by phytochemical screening, to conduct an acute toxicity study, and to evaluate the antioxidant and cytoprotective effects of AEAPAC in a CCL_4-induced hepatotoxicity model in Wistar rats.

Experimental section

Plant material

The *A. campestris L.* plant, were collected in May 2023 in the Aïn Deheb region ***(Fig. 1)***, which is located in the steppe highlands, at 52 km south-west of the wilaya of Tiaret in Algeria. The specimen was identified by the botany laboratory of the Faculty of Pharmacy at the University of Algiers and authenticated by comparison with a specimen from the herbarium of the National School of Agronomy in Algiers. The aerial parts of *A. campestris L.* were washed to remove impurities, then dried at room temperature in the open air and away from light in order to preserve their phytochemical integrity. After drying, they were ground into a fine powder.

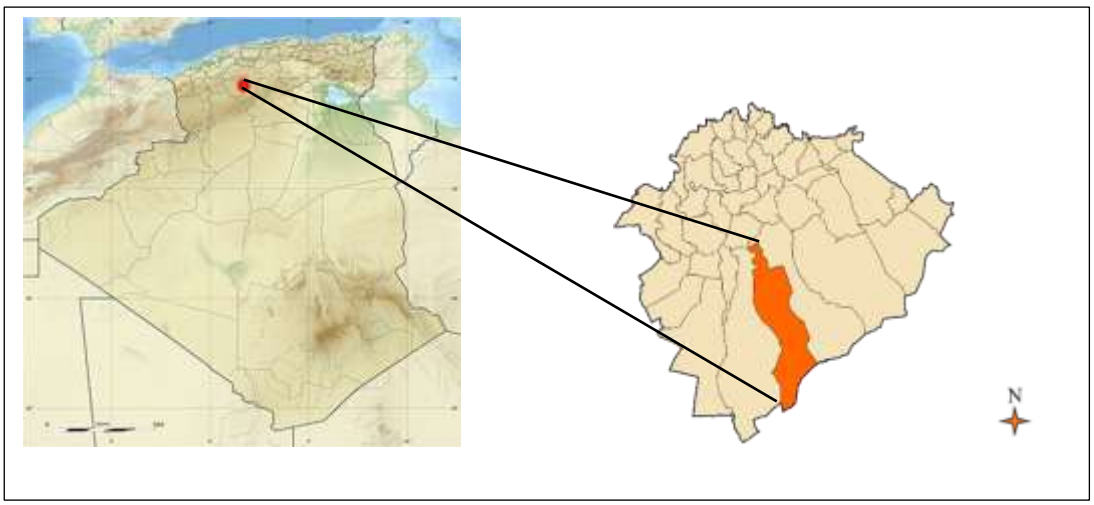


Figure 1 : Ain Deheb region, wilaya of Tiaret in Algeria

Preparation of the samples

The aqueous extract of the aerial parts of *A. campestris L.* was prepared according to a method described by **Guede-Guina *et al.* (année 20)**. 50 grammes of aerial parts of *A. campestris L.* powder was dissolved in 500 milliliters of distilled water and homogenized with a blender. The homogenate was left to macerate for 72 hours and then filtered through Whatman paper. The plant residue was rewashed three times with small volumes of distilled water to ensure complete extraction of water-soluble constituents. The combined filtrates were concentrated under reduced pressure at 40 °C and subsequently lyophilized to obtain a dry powder. The resulting aqueous extract of aerial parts of *A. campestris L* (AEAPAC) was stored in airtight containers at 4 °C until further analysis.

Chemical reagents

The chemicals and reagents used in this study were of high analytical quality and were from Sigma (St. Louis, MO), Merk (Mannheim, Germany), Biochemical (Germany) and (Troikaa Pharmaceuticals, India). Biochemical parameters were measured by using standard and colorimetric commercial kits (SPINREACT, Sant Esteve De Bas (GI). SPAIN), following the manufacturer's protocols.

Phytochemical analysis

Preliminary phytochemical screening of the plant extract was carried out using standard qualitative methods in order to identify the presence of major secondary metabolites. The phyto-components examined were: Flavonoids, saponins, tannins, coumarin, carbohydrate and phenols. The analyses were based on characteristic color changes and precipitation reactions specific to each class of bioactive compounds.

Animals and housing

Male and female Wistar rats were obtained from the animal breeding division of Pasteur Institute (IPA) of Kouba, (Algiers, Algeria). The rats were housed in an animal facility (ENSV Oued Smar, Algiers) in separate cages under controlled conditions of humidity, lighting, and temperature (50 ± 10% RH, 12-h light/dark, 23 ± 2 °C). They were given water and standard diet *ad libitum* throughout the experiment. All in vivo experiments commenced after a 7-day adaptation period and were carried out in compliance with the institutional guidelines for animal care as well as the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) following approval by the local Ethical Committee of the Houari Boumediene University of Sciences and Technology (USTHB), Algeria (approval number 45/DGLPAG/DVA.SDA.14).

Acute toxicity study

The acute oral toxicity was evaluated in healthy 200 ± 20 g female rate, following Organization for Economic Cooperation and Develepment (OECD) Guideline No. 423. The rats (n=5) were administered a single oral AEAPAC dose of 2000 mg/kg body weight, while the vehicle-treated control group (n=5) received the same volume of water. Animals were individually and closely observed during the first four hours for toxic signs and symptoms of seizures hyperactivity, changes in behavior (alertness, mood and motor activity, isolement), sedation, fur erection, urination, mucous membranes, respiration, autonomous parameters (eyes, salivation and diarrhea), neurological changes and mortality. After the acute phase, animals were kept under observation once daily for 14 extra days.

Hepatoprotective activity

Healthy 230 ± 30 g male rats were randomly divided into 5 groups (n = 6). The rats were labelled, and body weight was recorded. Pellets and water were provided *ad libitum*. Different groups ***(table 1)*** of rats were treated as follows: **Group I – Negatif group (Neg G)**: received 2 mL distilled water orally per day. At the 14 day, they received an intraperitoneal (i.p.) injection of fresh sunflower oil (3 mL kg^{-1}). **Group II – Positif group (Pos G)**: received 2 mL distilled water orally per day. At the 14 day, they received an i.p. injection (3 mL kg^{-1}) of fresh mixture of 30 % of CCl_4 and sunflower oil; **Group III - Silymarin-treated group** (Syl G) received as reference drug 2 mL of silymarin 50 mg kg^{-1} orally per day then challenged acutely by an i.p injection (3 mL kg^{-1}) of fresh mixture of 30 % of CCl_4 and sunflower oil at the 14 day. **Group IV -AEAPAC 200 mg/kg treated group: AEAPAC 200 G,** received 2 mL of **AEAPAC 200** mg kg^{-1} orally per day then challenged acutely by an i.p. injection (3 mL kg^{-1}) of fresh mixture of 30 % of CCl_4 and sunflower oil at the 14 day. **Group V -AEAPAC 400 mg/kg treated group: AEAPAC 400 G,** received 2 mL of **AEAPAC 400** mg kg^{-1} orally per day then challenged acutely by an i.p. injection (3 mL. kg^{-1}) of fresh mixture of 30 % of CCl_4 and sunflower oil at the 14 day. 24 hours after hepatotoxicity induction, rats were weighted and blood samples were performed, after a 12 hour fasting period, on animals through heart puncture under anaesthesia with intraperitoneal injection of 150 mg kg^{-1} body weight. Blood samples were collected in heparin tubes. The plasma was collected by centrifuging at 3000×g for 5 min a t 4 ◦C and kept at –20 ± 1 ◦C for biochemical parameters assays. After; rats were dissected to collect livers, which were twice washed with saline solution to determine the oxidative stress status.

Table 1: Study design of hepatoprotective activity

Experimental group	Code	Animals number	Administration doses	Hepatotoxicity model induction
Group I - Negatif control group	**Neg CG**	6	Na Cl 0.9% (2ml/rat/day)	i.p. injection of fresh sunflower oil (12 % v/v)
Group II - Positif control group	**Pos CG**	6	Na Cl 0.9% (2ml/rat/day)	

Group III Silymarin-treated group	**Syl TG**	6	Silymarin (50 mg /rat/day)	i.p. injection of fresh mixture of 30% CCl 4 in sunflower oil (12 % v/v)
Group IV - AEAPAC 200 mg/kg treated group	**AEAPAC 200 TG**	6	**AEAPAC** (200mg/kg/d)	
Group IV - AEAPAC 400 mg/kg treated group	**AEAPAC 400 TG**	6	**AEAPAC** (400mg/kg/d)	

Biochemical analysis

Biochemical parameters and oxidative stress analysis were measured by spectrophotometry (Jenway spectrophotometer, UK) and with (AE-600 Biochemical analyser, Erma inc. - Japan). Common plasma biochimical parameters, i.e. AST, ALT, PAL and Total Bilirubine (TB) levels were measured using standard and colorimetric commercial kits (SPINREACT, SantEsteve De Bas (GI). SPAIN), following the manufacturer's protocols.

Liver supernatant MDA, GSH, GST and GPx) assay

MDA level was estimated according to the method of (Ohkawa et al., 1979) (13). An aliquot of 100 µL was added to a reaction mixture containing 50 µL of 8.1% sodium dodecyl sulfate, 375 µL of 20.0% acetic acid (pH 3.5), 375 µl of 0.8% thiobarbituric acid (TBA). Samples were then boiled for 1h at 95 °C and centrifuged at 3000 g for 10 min. The concentration is calculated using the molar extinction coefficient of the MDA-TBA complex at 532 nm of 1.56 x 105 mmol. L-1.cm-1. GSH was evaluated by measuring the absorbance of free thiol (SH) groups at 412 nm (15). 150 mL of was mixed with 300 mL of 0.25% sulfosalicylic acid and incubated at +4°C for 1h. Afterward, all was centrifuged at 3000 g for 10 min at room temperature. 200 mL of the resulting supernatant containing free SH groups was mixed with 100 mL of 0.004% 5,5′ dithiobis2-nitrobenzoic acid (DNTB) and 800 mL of 200 mM phosphate buffer, pH=8. The activity of GST was measured according to the method of Habig et al. (1974). Add to 830 µL of sodium phosphate buffer (0.1 M; pH 7.4), 100 µL reduced glutathione (0.1 M), 20 µl of and 50 µl of 1-chloro 2,4-dinitro benzene (0.02 M). Molar extinction coefficient of 9.6 × 103 M−1 cm−1 with 340 nm was used to calculate the enzyme activity. GPx activity was determined according to the method of Flohe and Gunzler (1984). The rate of oxidation of GSH by H_2O_2 was used as measure of GPx activity. Reaction mixture of 1 ml was prepared which contained 0.3 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of hydrogen peroxide (1 mM) and 0.3 ml of liver supernatant. After incubation at 37°C for 15 min, reaction was terminated by the addition of 0.5 ml of 5% TCA. Tubes were centrifuged at 1500g for 5 min and the supernatant was collected. Totally, 0.2 ml of phosphate buffer (0.1 M, pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm (17).

Histological analysis

Livers fixed in 4% formol were washed, then lightened by dipping into xylene, and finally embedded in paraffin. Sections of 5 µm were stained with hematoxylin and eosin and examined under a light microscope. Microscopic observation was performed to verify changes in tissues. Digital images were obtained with a Leica optical microscope connected to an AmScop 3.7 camera.

Statistical analysis

Statistical analysis was performed using SPSS 17 (SPSS Inc., Chicago, IL, USA). Data was presented as mean ± standard error of the mean (SEM). For post hoc analysis, multiple comparisons were analyzed using one-way ANOVA and Tukey test. A P value less than 0.05 was considered as statistically significant.

Results

Phytochemical screening

A phytochemical analysis of the aqueous extract of *A. campestris* was carried out to detect the presence of various phytochemical constituents (**Tab. 2**). The following bioactives substances were identified : flavonoids, saponins, coumarins, carbohydrates, phenols and true tannins. However, the results show the absence of anthocyanins, leucoanthocyanins, condensed tannins, gallic tannins, starches and alkaloids.

Table 2: Phytochemicals in the aqueous extract of *A. Campestris*

Phytochemicals	*Test results*
Anthocyanins	-
Leucoanthocyanins	-

Flavonoids		+
Saponins		+++
Tannins	*True tannins*	+++
	Condensed tannins	-
	Gallic tannins	-
Alkaloids		-
Coumarins		+++
Carbohydrates		+++
Starch		-
Phenols		+++

Acute Toxicity

The acute toxicity of **AEAPAC** evaluated orally in Wistar female rats at a dose of 2000 mg/kg body weight, showed that the observations made during the 24 hours wich follow administration product, revealed no behavioral alterations, clinical signs of toxicity, or mortality. Furthermore, monitoring of the animals over a 14-day period showed no notable changes in autonomic responses or body weight. These results indicate that the **AEAPAC**, can be considered non-toxic up to a dose of 2000 mg/kg body weight.

Serum Biochimical analysis

Biochemical results are reported in **Table 3.** In the Pos CG, treated with CCl_4, we observed a significant increase ($p < 0.001$) in serum AST, ALT, ALP, and TB compared to the Neg CG AST, ALT, ALP , and BT. Treatment with silymarin resulted in a highly significant reduction ($p < 0.01$) in AST, ALT and ALP activities and in BT level compared with the Pos CG. Administration of the **AEAPAC** at a dose of 200 mg/kg resulted in a significant decrease ($p < 0.05$) in enzyme activities of AST, ALT and ALP and TB level compared with the Pos CG. At the higher dose of 400 mg/kg, a more pronounced and highly significant improvement ($p < 0.01$) was observed (AST: 102.0 ± 1.17 U/L; ALT: 92.95 ± 3.18 U/L; ALP: 195.4 ± 2.603 U/L; TB: 1.063 ± 0.078 mg/dL), approaching the values recorded in the **Syl TG.** When comparing results obtained in Syl TG and AEAPAC 400 TG with the Neg CG, no significant differences were noted for liver enzymes (AST, ALT, ALP). Furthermore, we observed that AST and ALT levels were slightly lower in tthe AEAPAC 400 TG, when compared with the Syl TG. The almost complete restoration of liver biochemical parameters and the absence of significant differences compared to the Neg CG showed the ability of the tested products to preserve liver integrity. The TB level in the AEAPAC 400 TG was slightly higher than that of the Syl TG, although it remained lower than that of the Pos CG. The obtanied values were closer to those obtained in Neg CG, which suggests an equivalent, or possibly slightly greater, hepatoprotective potential of the AEAPAC 400 TG.

Liver supernatent Oxidative stress parameters

Exposure to carbon tetrachloride (CCl_4) caused a significant alteration in hepatic antioxidant defense systems (Tab. 3), as evidenced by a marked increase in MDA activity and a reduction in GST activity, GSH levels, and GPx activity compared to the Neg CG ($p < 0.001$). More specifically, GST levels decreased from (62.2 ± 2.57 µmol/ml) in the Neg CG to (22.3 ± 0.98 µmol/ml) in the Pos CG. In contrast, the MDA increased from (3.44 ± 0.11 µmol/ml) in the control group to (6.01 ± 0.14 µmol/ml) in the Pos CG . Similarly, GSH and GPx concentrations decreased from (29.8 ± 1.62 µmol/ml) to (9.86 ± 0.57 µmol/ml) and from (2.22 ± 0.01 µmol/ml) to (1.56 ± 0.09 µmol/ml), respectively. Administration of silymarin resulted in a significant improvement ($p < 0.01$) in those antioxidant parameters, with MDA levels reaching (4.39 ± 0.1 µmol/ml), GST (37.7 ± 0.78 µmol/ml), GSH (17.9 ± 1.92 µmol/ml) and GPx (1.89 ± 0.14 µmol/ml), compared to the Pos CG. The AEAPAC, in rats group treated at the dose of 200 mg/kg produced a moderate improvement, with values of MDA (4.55 ± 0.10 µmol/ml), GST (26.7 ± 1.150 µmol/ml), GSH (14.3 ± 1.219 µmol/ml), and GPx (1.79 ± 0.078 µmol/ml). At the dose of 400 mg/kg of AEAPAC, a greater improvement was observed, with values of MDA (4.30 ± 0.22 µmol/ml), GST (33.5 ± 1.27 µmol/ml), GSH (15.7 ± 1.15 µmol/ml), and GPx (1.99 ± 0.03 µmol/ml), indicating a dose-dependent response. No significant differences were observed when comparing the AEAPAC 200 mg/kg treated group and the AEAPAC 400 mg/kg treated group, regarding the levels of antioxidant enzymes (GPx, GST), GSH and MDA. However, a statistically significant difference ($p < 0.05$) was observed for the GST parameter, suggesting a dose-dependent modulation of this enzyme by the extract. These results indicate that the AEAPAC treated group exerted comparable effects to those of Sylimarin, the reference drug.

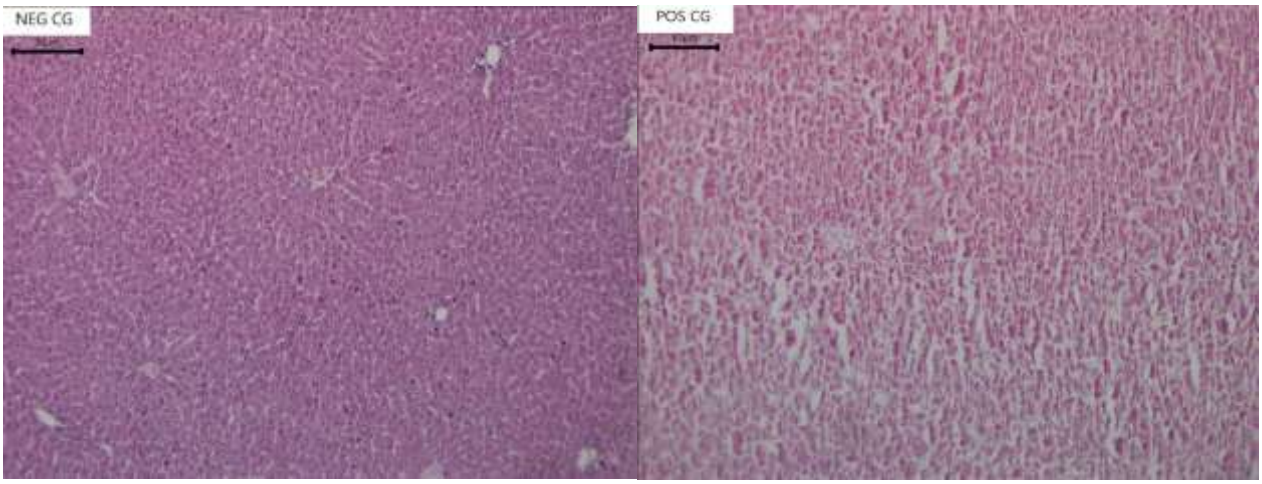
Table 3 : Biochemical markers in rats plasma and liver supernatent.

	Males Rats groups	Neg CG	Pos CG	Syl TG	AEAPAC 200 TG	AEAPAC 400 TG
Plasma parameters	AST U/L	91,93±3,21^{a}	163,2±7,2^{e}	102,9±1,43^{ac}	137,0±3,76^{d}	102,0±1,17^{ac}
	ALT U/L	86,25±6,32^{a}	164,2±7,204^{d}	94,11±3,06^{ac}	114,3±3,35^{bc}	92,95±3,18^{a}
	ALP U/L	184,9±4,28^{a}	379,7±10,50^{c}	211,9±7,21^{a}	251,0±4,16^{b}	195,4±2,60^{a}
	TB (mg/dL)	0,778±0,05^{a}	2,433±0,19^{c}	0,94±0,03^{a}	1,75±0,09^{b}	1,063±0,08^{a}
Liver supernatent Oxydatitf stres	MDA (µmol/ 100g per liver tissue)	3,44±0,11^{a}	6,01±0,14^{b}	4,39±0,10^{ad}	4,55±0,10^{cd}	4,30±0,22^{ac}
	GST (µmol/100g per liver tissue)	61,2±2,57^{c}	21,3±0,98^{a}	36,7±0,78^{b}	25,7±1,15^{a}	32,5±1,27^{b}
	GSH (µmol/100g per liver tissue)	28,8±1,62^{e}	9,86±0,57^{a}	16,9±1,92^{bcd}	13,3±1,22^{ac}	14,7±1,15^{ad}
	GPx (µmol/100g per liver tissue)	2,12±0,01^{c}	1,46±0,09^{a}	1,79±0,14^{abc}	1,69±0,08^{ab}	1,89±0,03^{bc}

Data were subjected to oneway ANOVA followed by Tukey's multiple comparisons tests. The change of letter within the same line indicate a significant difference.

Histological Analysis

In Neg CG **(fig. 2, A),** no significant histopathological alterations were observed. It's serves as a reference for normal tissue integrity. In contrast, we observed, in the Pos. CG **(fig. 2, B)**, the presence of hepatocellular degeneration, necrosis, and alteration of the lobular architecture of the liver, particularly with a modification of the hepatocytes architectonic structures, which is typical of CCL_4-induced lesions. In the Syl TG **(fig. 2, C)**, the micrographic highlighted apparition of small localized inflammatory areas and subtle changes in liver architecture in the form of hepatocyte trabeculae, suggesting a form of protective efficacy, but insufficient to fully restore liver structure. In rats treated with the AEAPAC at the dose of 200 mg/kg treated group **(fig. 2, D)**, there is evidence of early mitosis, a mild inflammatory reaction, and moderate degeneration accompanied by enlargement of the central vein, which could suggest an early hepatic response to stimulation by the extract, but without complete reparative effects. At the dose of 400 mg/kg of AEAPAC **(fig. 2, E)**, the lobular architecture of the liver is generally preserved, with mild inflammatory infiltration and localized necrotic foci in certain tubular areas. The maintenance of the hexagonal organization of hepatic lobules indicates better structural protection, likely due to the hepatoprotective effect of the extract at the higher dose.



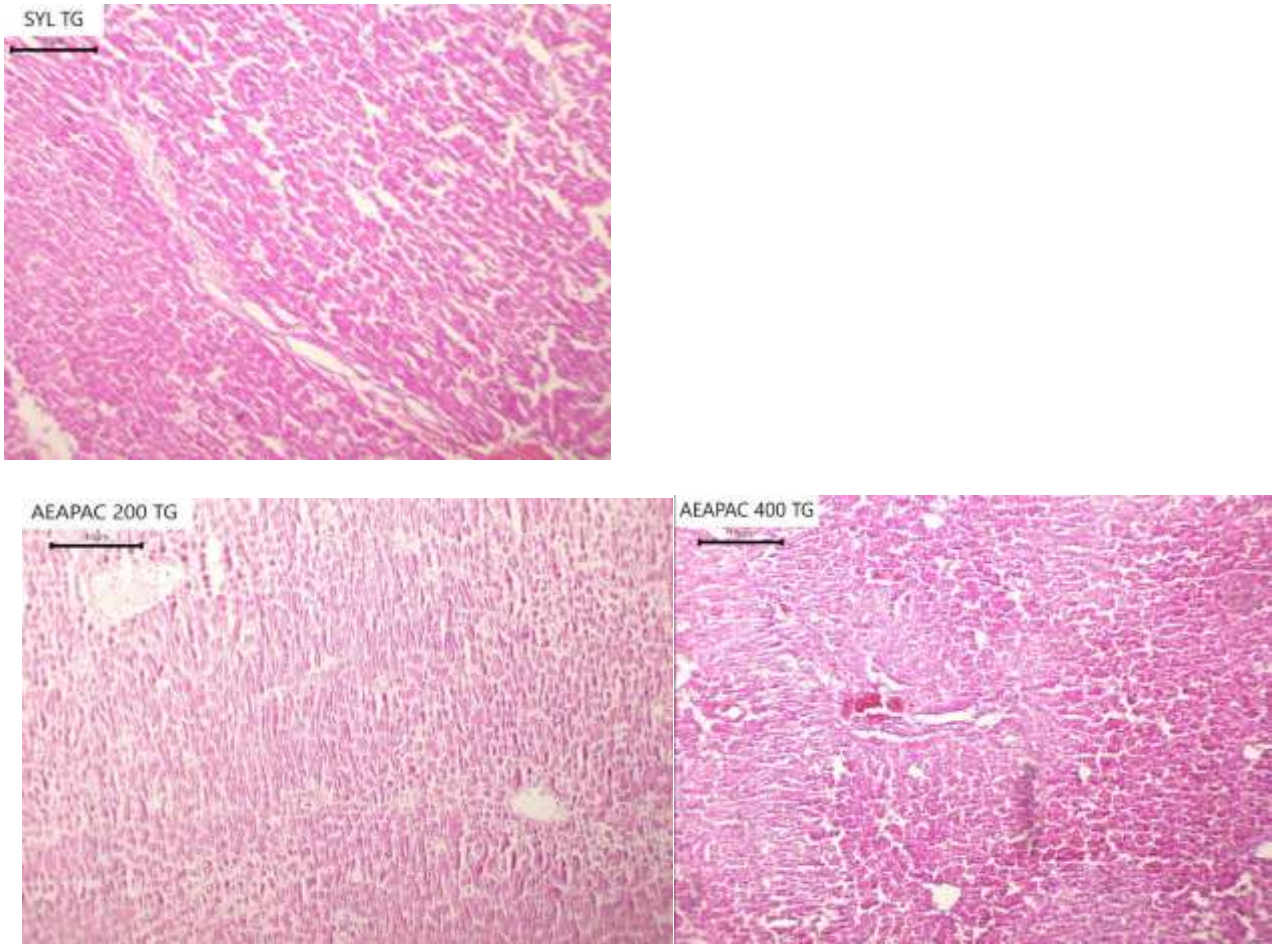


Figure 2 : Histopathological changes in rat's liver. HE staining. **Scale Bar = 10 µm.**

Neg CG, no significant histopathological alterations were observed. **Pos. CG**, presence of hepatocellular degeneration, necrosis, and alteration of the lobular architecture. **Syl TG**, small localized inflammatory areas and subtle changes in liver architecture in the form of hepatocyte trabeculae. **AEAPAC 200 TG**, a mild inflammatory reaction, and moderate degeneration accompanied by enlargement of the central vein. **AEAPAC 400 TG**, the lobular architecture is preserved, with mild inflammatory infiltration and localized necrotic foci in certain tubular areas.

DISCUSSION

The liver is a major organ that is normally the target for toxicity due to its role in the clearing and metabolizing of chemicals via a process called detoxification. These plants are also said to possess hepatoprotective properties as a result of the presence of various phytoconstituents with antioxidant potential. The antioxidant activity and the presence of some phytoconstituents have been reported for parts of *A. compestris* (10). These phytochemicals have been described to have antioxidant and hepatoprotective potential (21). Based on the traditional use of the aerial parts of *A. campestris* L. and supported by promising literature reports, the present study was undertaken to evaluate the hepatoprotective potential of its aqueous extract against carbon tetrachloride (CCl_4)-induced hepatotoxicity in male Wistar rats.

The acute toxicity test of the aqueous extract of A. campestris L. demonstrated that this extract is safe up to a dose of 2,000 mg/kg body weight in albino Wistar rats, with no abnormalities or mortality observed, thus confirming its safety. This is consistent with studies conducted by **Dib** and **El Alaoui-Faris (2019) and Mammeri et al. (2022) (11,24),** which agree with the results indicating that the aqueous extract and essential oil of A. campestris are non-toxic according to OECD Protocol 423.

The administration of CCl_4 at a dose of 3 mL/kg bw of 30% CCl 4 and sunflower oil in male Wistar rats led to elevated serum enzyme levels. However, pretreatment with **AEAPAC** at doses of 200 and 400 mg/kg significantly reduced serum concentrations of AST, ALP, ALT and TB. These results are similar to those obtained in **Silymarin-treated group** witch is the reference drug. Our results are consistent with those obtained by **Dib**

and El Alaoui-Faris, 2019 and Saadaoui et al., 2023 (4,11)**.** Indeed**,** CCl_4 intoxication has been shown to generate free radicals such as nitric oxide, triggering a cascade of events that ultimately result in hepatic injury in rats (25). This was reflected by the significant elevation of serum ALP, AST, ALT, and TB levels. According to previous studies (11,26), these alterations are indicative of hepatocyte dysfunction, cellular leakage, and loss of functional membrane integrity (27). In the liver, CCl_4 is metabolized by the enzyme CYP2E1 into reactive trichloromethyl and trichloromethylperoxy radicals(2,28) .These radicals bind to unsaturated fatty acids in the membranes of hepatocytes, mitochondria, and the endoplasmic reticulum, thereby initiating a chain lipid peroxidation process that progresses from structural damage to hepatocyte and intracellular organelle death (2,29). In CCl_4-intoxicated rats, the marked elevation of ALP, AST, ALT, and TB indicated altered hepatic parenchymal integrity and cellular injury(25) **.** In contrast, silymarin, considered the reference hepatoprotective agent, nearly normalized these parameters, confirming its strong protective role against liver damage(11)**.** Pretreatment with the **AEAPAC** also resulted in a significant reduction in ALT and AST levels, particularly at doses of 400 mg/kg/d, with values approaching those of the Neg CG and Syl TG. This suggests an effective restoration of hepatic parenchymal integrity. Hence, the **AEAPAC** demonstrates a comparable efficacy, likely attributable to the presence of bioactive compounds capable of stabilizing cellular membranes and limiting hepatic enzyme leakage. These findings are consistent with previous studies reporting that Artemisia extracts and silymarin reduce lipid peroxidation and stabilize cell membranes, thereby limiting transaminase leakage (11).

Regarding ALP, all three pretreatments significantly reduced enzyme levels, the two doses of the **AEAPAC** were more effective than the reference drug, indicating improved biliary function and protection against the cholestatic effects of CCl_4. Additionally, the elevation of TB observed in the Pos CG was markedly attenuated in the **Syl TG, AEAPAC 200 TG and AEAPAC 400 TG**, with maximal efficacy for silymarin. An increase in TB generally suggests an icteric condition(20). CCl_4-induced hyperbilirubinemia may result from excessive heme breakdown or obstruction of bile ducts, which impairs conjugation and leads to the release of unconjugated bilirubin by damaged hepatocytes (20). Thus, normalization of total bilirubin following administration of the aqueous extract highlights their hepatoprotective effect by mitigating the harmful impact of the hepatotoxic compound. These results align with earlier studies showing that silymarin normalizes ALP and bilirubin levels by limiting cholestasis and promoting hepatocyte regeneration(4). Similarly, Dib et al. (2021) reported that *A. campestris* extract significantly attenuates ALP and bilirubin elevation in a similar hepatotoxicity model (30).

Hepatoprotective activity is recognized as being linked to antioxidant activity, since it involves damage mediated by free radicals. Within the cellular antioxidant arsenal, GST is considered one of the most potent antioxidant defense enzymes, and glutathione (GSH) acts as an essential buffer (1), maintaining cellular redox homeostasis (31,32). This protein also metabolizes hydrogen peroxide and lipid peroxides by acting as a cofactor of glutathione peroxidase (GPx)(33). Malondialdehyde (MDA), a toxic aldehyde metabolite , is produced during the peroxidation of polyunsaturated fatty acids (34). It generally indicates exacerbated lipid peroxidation leading to tissue damage; the increased concentrations of GST and GSH together with the reduction of MDA in the groups treated with the AEAPAC in our study indicate its antioxidant potential. A high level of MDA reflects intense lipid peroxidation and the collapse of antioxidant defenses(7). An elevation of MDA in hepatic tissues is a key indicator of CCl_4-induced toxicity (35). In this experiment, MDA levels were significantly increased in the Pos CG, but reduced to values close to normal in the groups treated AEAPAC 400 TG. These findings are consistent with the results reported by (4,30); as well as for silymarin . Therefore, the hepatoprotective effect of the AEAPAC can be attributed to their antioxidant properties. The enzyme GST participates in the conjugation of GSH with xenobiotic substrates, thereby contributing to their detoxification and maintaining cellular homeostasis. This indicates a stimulation of conjugation mechanisms and a restoration of the cellular defense potential (20). CCl_4-induced toxicity caused a decrease in both GST and GSH activity; however, a significant and marked increase was observed in the groups pretreated with AEAPAC for both GST and GSH, showing values very close to the silymarin treatement. This effect, comparable to that of silymarin, suggests that the bioactive metabolites of the extract act as potent antioxidants (8). Our results are consistent with those of (27), who demonstrated an improvement in the activity of antioxidant enzymes (GST, GPx) in CCl_4-intoxicated rats treated with plant extracts . Similarly,(27) highlighted the protective role of flavonoids derived from *Artemisia* species against oxidative stress. Furthermore,(26) confirmed that *A. campestris* extract exerts a significant hepatoprotective effect through its ability to restore GSH levels and reduce lipid peroxidation.

GPx protects cells from free radicals by reducing hydroperoxides using GSH as a substrate(26) (26). In this study, reduced GPx activity was observed in the Neg CG, compared to the Pos CG. Moreover, an increase in GPx activity was noted in the groups pretreated with AEAPAC, with values comparable to the Pos CG and even

higher than those of the reference treatment. The elevation of GSH and GPx levels in the hepatic tissues of rats treated with AEAPAC and silymarin may result either from GSH regeneration or from an enhanced synthesis of glutathione

In the histopathological analysis of the liver in rats exposed to carbon tetrachloride (CCl_4), severe alteration of the hepatic architecture was observed, marked by centrilobular necrosis, vacuolar degeneration of hepatocytes, and inflammatory infiltration. These alterations result from the metabolism of CCl_4 by CYP2E1 into trichloromethyl radicals and trichloromethyl peroxide, which initiate chain lipid peroxidation and lead to the destruction of cell membranes (2,29)

In rats pretreated with the AEAPAC, the liver alterations induced by CCl_4 were significantly attenuated. Histological examination showed a reduction in foci of necrosis, partial restoration of hepatocyte cords, and decreased inflammatory infiltration, indicating active cell regeneration. These protective effects can be attributed to the extract's richness in secondary metabolites with antioxidant and cytoprotective activities, confirming the role of the natural constituents of AEAPAC in stabilizing cell membranes and reducing oxidative stress. Our findings are consistent with those reported by (27), who demonstrated the protective role of *Artemisia* species extracts against experimentally induced oxidative liver damage in rats.

CONCLUSION

The cytoprotective effect of AEAPAC, highlighted by histopathological results corroborate the biochemical findings, in particular the decrease in the hepatic enzymes (ALAT, ASAT, PAL), and the BT level, and the restoration of antioxidant defenses (GSH, GPx, GST). These observations demonstrate the ability of AEAPAC to attenuate enzymatique and oxidative damage, reduce hepatic lesions and promote liver regeneration.

Conflicts of interest: None to declare

REFERENCES

1. Wang Y, Deng X, Liu Y, Wang Y, Luo X, Zhao T, et al. Protective effect of Anneslea fragrans ethanolic extract against CCl_4-induced liver injury by inhibiting inflammatory response, oxidative stress and apoptosis. Food and Chemical Toxicology. 2023 May;175:113752.
2. Kostic T, Popović D, Perisic Z, Stanojevic D, Dakic S, Saric S, et al. The hepatoprotective effect of aminoguanidine in acute liver injury caused by CCl_4 in rats. Biomedicine & Pharmacotherapy. 2022 Dec;156:113918.
3. Teschke R. Liver Injury by Carbon Tetrachloride Intoxication in 16 Patients Treated with Forced Ventilation to Accelerate Toxin Removal via the Lungs: A Clinical Report. Toxics. 2018 Apr 27;6(2):25.
4. Saadaoui N, Mathlouthi A, Zaiter A, El-Bok S, Mokni M, Harbi M, et al. Phytochemical profiling, antioxidant potential and protective effect of leaves extract of tunisian Vitis vinifera autochthonous accessions against acute CCl_4-injured hepatotoxicity in mice. Heliyon. 2023 May;9(5):e16377.
5. Khan RA, Khan MR, Sahreen S. CCl_4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement Altern Med. 2012 Dec;12(1):178.
6. Zheng Z, Xue Y, Jia J, Wei L, Shang W, Lin S. Tiopronin-induced membranous nephropathy: a case report. Renal Failure. 2014 Oct;36(9):1455–60.
7. Ghlissi Z, Sayari N, Kallel R, Bougatef A, Sahnoun Z. Antioxidant, antibacterial, anti-inflammatory and wound healing effects of Artemisia campestris aqueous extract in rat. Biomedicine & Pharmacotherapy. 2016 Dec;84:115–22.
8. Sefi M, Fetoui H, Soudani N, Chtourou Y, Makni M, Zeghal N. Artemisia campestris leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. Pathology - Research and Practice. 2012 Mar;208(3):157–62.
9. Anti-inflammatory and antioxidant potential of Artemisia dracunculus L. aqueousextract against acetic acid induced ulcerative colitis in male Wistar rats. IJEB [Internet]. 2022 Dec 26 [cited 2025 Nov 1];61(1). Available from: http://op.niscpr.res.in/index.php/IJEB/article/view/41130
10. Cimanga RK, Nsaka SL, Tshodi ME, Mbamu BM, Kikweta CM, Makila FBM, et al. In vitro and in vivo antiplasmodial activity of extracts and isolated constituents of Alstonia congensis root bark. Journal of Ethnopharmacology. 2019 Oct;242:111736.
11. Dib I, El Alaoui-Faris FE. Artemisia campestris L.: review on taxonomical aspects, cytogeography, biological activities and bioactive compounds. Biomedicine & Pharmacotherapy. 2019 Jan;109:1884–906.
12. Hani FA, Ainouz L, Rabia SH, Zahnit W, Zenad O, Boudjellaba S, et al. In Vivo Effects of Punica Granatum against Ulcerative Colitis, Nephroprotection, Inflammation and Acute Oral Toxicity. Medycyna Weterynaryjna. 2026;82(1):40–8.
13. Aïnouz L, Zaouani M, Remichi H, Boudjellaba S, Chabane K, Benaziza D. Coronary Artery and Myocardium Injuries Induced by a High Cholesterol and Fructose Diet in Growing Male and Female Wistar Rats. BUASVMCN-VM [Internet]. 2022 Oct 24 [cited 2026 Jan 5];78(2). Available from: https://journals.usamvcluj.ro/index.php/veterinary/article/view/buasvmcn-vm%3A2021.0012
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 1979 June;95(2):351–8.
15. Boudjellaba S, Saadi H, Zaouani M, Ainouz L, Hani AF, Remichi H, et al. Assessment of Plasma Oxidative Stress Biomarkers and Metabolic Parameters of Dairy Heifers: Relationship with Success at First Service. BUASVMCN-VM [Internet]. 2022 Oct 24 [cited 2025 Dec 25];78(2). Available from: https://journals.usamvcluj.ro/index.php/veterinary/article/view/buasvmcn-vm%3A2021.0007
16. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-Transferases. Journal of Biological Chemistry. 1974 Nov;249(22):7130–9.

17. Djeffal A, Messarah M, Boumendjel A, Kadeche L, Feki AE. Protective effects of vitamin C and selenium supplementation on methomyl-induced tissue oxidative stress in adult rats. Toxicol Ind Health. 2015 Jan;31(1):31–43.
18. Cai T, Cai B. Pharmacological activities of esculin and esculetin: A review. Medicine. 2023 Oct 6;102(40):e35306.
19. Wang Z, Ma L, Zhang X, Xu L, Cao J, Jiang W. The effect of exogenous salicylic acid on antioxidant activity, bioactive compounds and antioxidant system in apricot fruit. Scientia Horticulturae. 2015 Jan;181:113–20.
20. Okoro IO, Okoro EO, Isoje FE, Oyubu G. Protective effects of Alstonia congensis Methanolic extract against CCl_4 induced liver damage in Wistar rats. Scientific African. 2022 Sept;17:e01315.
21. Akrout A, Gonzalez LA, El Jani H, Madrid PC. Antioxidant and antitumor activities of Artemisia campestris and Thymelaea hirsuta from southern Tunisia. Food and Chemical Toxicology. 2011 Feb;49(2):342–7.
22. Cikman O, Soylemez O, Ozkan OF, Kiraz HA, Sayar I, Ademoglu S, et al. Antioxidant Activity of Syringic Acid Prevents Oxidative Stress in l-arginine–Induced Acute Pancreatitis: An Experimental Study on Rats. International Surgery. 2015 May;100(5):891–6.
23. Kumar S, Prahalathan P, Raja B. Antihypertensive and antioxidant potential of vanillic acid, a phenolic compound in L-NAME-induced hypertensive rats: A dose-dependence study. Redox Report. 2011 Sept;16(5):208–15.
24. Mammeri B, Bahri F, Kouidri M, Boudani B, Arioui F. Evaluation of chemical composition, anti-inflammatory, antibacterial activity and synergistic effect between antibiotics and the essential oil of *Artemisia Campestris L.* J Appl Biol Sci. 2022 May 29;16(2):230–47.
25. Algefare AI, Alfwuaires M, Famurewa AC, Elsawy H, Sedky A. Geraniol prevents CCl_4-induced hepatotoxicity via suppression of hepatic oxidative stress, pro-inflammation and apoptosis in rats. Toxicology Reports. 2024 June;12:128–34.
26. Saoudi M. Antioxidant and Protective Effects of Artemisia campestris Essential Oil Against Chlorpyrifos-Induced Kidney and Liver Injuries in Rats.
27. Dib I, Angenot L, Mihamou A, Ziyyat A, Tits M. Artemisia campestris L.: Ethnomedicinal, phytochemical and pharmacological review. Journal of Herbal Medicine. 2017 Mar;7:1–10.
28. Li L, Li W, Kim Y ho, Lee YW. Chlorella vulgaris extract ameliorates carbon tetrachloride-induced acute hepatic injury in mice. Experimental and Toxicologic Pathology. 2013 Jan;65(1–2):73–80.
29. Popović D, Kocić G, Katić V, Zarubica A, Janković Veličković L, Ničković VP, et al. Anthocyanins Protect Hepatocytes against CCl_4-Induced Acute Liver Injury in Rats by Inhibiting Pro-inflammatory mediators, Polyamine Catabolism, Lipocalin-2, and Excessive Proliferation of Kupffer Cells. Antioxidants. 2019 Oct 4;8(10):451.
30. Sihem T, Hafsia B, Intissar E, Fatma R, Nasr HB. The Preventive Effect of Artemisia Campestris Infusion Against Hepatotoxicity.
31. Dalton TP, Chen Y, Schneider SN, Nebert DW, Shertzer HG. Genetically altered mice to evaluate glutathione homeostasis in health and disease. Free Radical Biology and Medicine. 2004 Nov;37(10):1511–26.
32. Zhai K, Duan H, Wang W, Zhao S, Khan GJ, Wang M, et al. Ginsenoside Rg1 ameliorates blood–brain barrier disruption and traumatic brain injury via attenuating macrophages derived exosomes miR-21 release. Acta Pharmaceutica Sinica B. 2021 Nov;11(11):3493–507.
33. Roy R, Roy J, Liya IJ, Ahmed J, Akter A, Basher MA. Antioxidant and hepatoprotective activities of methanolic extract of Fimbristylis miliacea (L.) Vahl. Phytomedicine Plus. 2023 May;3(2):100449.
34. Aliahmat NS, Noor MRM, Yusof WJW, Makpol S, Ngah WZW, Yusof YAM. Antioxidant enzyme activity and malondialdehyde levels can be modulated by Piper betle, tocotrienol rich fraction and Chlorella vulgaris in aging C57BL/6 mice. Clinics. 2012;67(12):1447–54.
35. Mondal M, Sarkar C, Saha S, Hossain MN, Norouzi R, Mubarak MS, et al. Hepatoprotective activity of andrographolide possibly through antioxidative defense mechanism in Sprague-Dawley rats. Toxicology Reports. 2022;9:1013–22.