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Original article

Hepatoprotective potential of solvent-derived artichoke leaf extracts against cyclophosphamide-induced liver injury rat model

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Abstract

In this study, the protective effects of artichoke (*Cynara scolymus* L.) leaf extracts against hepatotoxicity induced by cyclophosphamide (CP), a cytotoxic agent, were investigated. Thirty female Wistar rats were randomly divided into five equal groups. The control and CP groups received 10% dimethyl sulfoxide (DMSO) via gavage for 10 days. On the 7th day, a single intraperitoneal dose of CP (200 mg/kg body weight) was administered to the rats in both the CP and the experimental groups. The rats in the experimental groups were also treated with *n*-hexane, ethyl acetate (EtOAc), and methanolic (MeOH) extracts (1g/kg body weight, via gavage) each dissolved in DMSO, for 10 days. In the LC-QTOF-MS analyses of the extracts, apigenin-7-*O*-rutinoside was present exclusively in the EtOAc extract, while this extract also had the highest concentrations of luteolin-7-O- glucoside, apigenin 7-glucoside, apigenin, oxo-octadecatrienoic acid, hydroxy-octadecatrienoic acid, and hydroxy-octadecadienoic acid. In the plasma and liver tissue samples of the CP group, levels of MDA, TNF-α, and IL-1β increased, while antioxidant markers levels and IL-10 levels decreased. Compared to the CP group, the MeOH extract group showed reduced levels of MDA, TNF-α, and IL-1β, along with increased levels of GSH (except in liver tissue), GPx, SOD, and IL-10 in both plasma and liver samples. In the EtOAc group, liver MDA levels were significantly reduced and plasma GSH levels were elevated. However, changes in plasma IL-1β, IL-10, and TNF-α levels were not statistically significant. In the *n*-hexane group, none of the examined parameters showed significant changes. Histopathological examinations of liver tissues in the CP group revealed sinusoidal dilation, hyperemia in the central veins, vacuolar degeneration, and increased Kupffer cell activation. In contrast, the EtOAc group exhibited a marked reduction in these anomalies, indicating notable histological improvement. The MeOH group showed a reduction in hyperemia and vacuolar degeneration, while the *n*-hexane group demonstrated only limited tissue recovery. Consequently, the results of this study demonstrated that the EtOAc extract, rich in diverse phenolic compounds, was the most effective in mitigating CP-induced hepatic degeneration. Meanwhile, the MeOH extract, which had the highest total phenolic content, exhibited the strongest antioxidant and anti-inflammatory effects and contributed to partial histological improvement.

Keywords: artichoke leaf, cyclophosphamide, hepatotoxicity, inflammation, oxidative stress

Introduction

Cyclophosphamide (CP) is a cytotoxic alkylating agent commonly included in treatment protocols for various malignant and autoimmune diseases, owing to its broad clinical applicability (Gupta et al. 2021). High doses of chemotherapeutic agents such as CP are often required to overcome cancer cell resistance (Chen et al. 2019). However, high-dose CP administration is associated with toxicity in multiple organs, including the heart, kidneys, and bladder, and this situation significantly limits its clinical utility (Nagi et al. 2010). The majority of these adverse effects are attributed to the hepatic metabolism of the agent, which produces the toxic metabolites acrolein and phosphoramide mustard. These metabolites, particularly acrolein and chloroacetaldehyde (CAA), induce the generation of reactive oxygen species (ROS), resulting in lipid peroxidation (LPO), protein carbonylation, and oxidative DNA damage (Ayza et al. 2022). Therefore, exploring natural and safe compounds that can enhance the antitumor efficacy of CP while mitigating its side effects is of great clinical importance.

Artichoke (*Cynara scolymus* L.), a member of the Asteraceae family, has long been utilized in traditional medicine, particularly for the treatment of hepatobiliary disorders, dyspepsia, obesity, and hyperlipidemia (Ben Salem et al. 2015). Its leaves are rich in bioactive phenolic acids, including cynarine (1,5-O-dicaffeoylquinic acid), chlorogenic acid (3-O-caffeoylquinic acid), and caffeic acid. Additionally, luteolin and apigenin glucosides are the primary flavonoids found in artichoke leaves (Biel et al. 2020). Both in vivo and in vitro studies have demonstrated that artichoke leaf extracts possess a range of pharmacological properties, including antioxidant (Biel et al. 2020), hepatoprotective (Speroni et al. 2003), and anticancer (Sokkar et al. 2020) activities. Based on these findings, several studies have reported the hepatoprotective potential of artichoke extracts against toxic various such as aflatoxin (Nasef et al. 2023), paracetamol (El Morsy and Kamel 2015), and carbon tetrachloride (CCl_4) (Mehmetcik et al. 2008).

The medicinal benefits of artichoke leaf extracts are largely attributed to their high content of polyphenolic compounds (Nasef et al. 2023). Although previous studies have demonstrated the protective effects of artichoke leaves, considered natural and safe agents, against various forms of tissue damage (Speroni et al. 2003, Ben Salem et al. 2022), their specific hepatoprotective effects in CP-induced liver toxicity remain unexplored. Given that the antioxidant activity and therapeutic efficacy of plant-based extracts can vary significantly depending on the type and concentration of phenolic constituents they contain (Speroni et al. 2003, Avci et al. 2022), it is essential to assess the biological effects of different solvent-based artichoke leaf extracts. Despite the well-documented antioxidant potential of artichoke, no study to date has systematically compared the hepatoprotective effects of its *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) extracts in the context of CP-induced liver injury. Therefore, this study aimed to investigate the potential protective roles of these extracts against CP-induced oxidative stress, focusing specifically on changes in antioxidant enzyme activity and cytokine levels in both plasma and hepatic tissue.

Materials and Methods

Chemicals and plant materials

ENDOXAN (1 g i.v. infusion solution powder-containing vial, EIS Eczacıbası, Turkey) was used as a source of CP. Leaves of *Cynara scolymus* L. were bought from an organic produce market in the Ankara province, Turkey in May 2021. Leaf specimens were authenticated by Prof. Esra Kupeli Akkol from Gazi University, Department of Pharmacognosy, Faculty of Pharmacy, Ankara, Turkey, and the specimens were deposited in the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey. Artichoke leaves (500 g) powdered after drying at room temperature were macerated with 3.0 L *n*-hexane for 24 hours at room temperature. The mixture was then filtered, and the extract was obtained by distillation under vacuum with a rotary evaporator. This process was repeated 4 times (4 x 3.0 L). After drying the remaining plant material, the same process was carried out with EtOAc (4 x 3.0 L) and MeOH (4 x 3.0 L). The yields of the *n*-hexane, EtOAc, and MeOH extracts were determined as 9.60%, 0.88%, and 0.73%, respectively.

Determination of the contents of the extracts by LC-QTOF-MS

The determination of the active ingredients of the samples was carried out using LC-QTOF-MS systems. These were the Agilent 1260 series HPLC system and the Agilent 6550 iFunnel High Resolution Mass Spectrometer (Agilent Technologies, Inc., CA, USA). The MS system was used with the dual-spray Agilent Jet Stream electrospray ionization technique, and analyses were performed in negative mode. The MS operating mode was 2 GHz Extended Dynamic Range. An Agilent TC-C18(2) (4.6 mm x 150 mm x 5 μm) column was used for chromatographic separation. The Agilent MassHunter Software B06.00 was used in

the analyses, and the METLIN Metabolite database, PubChem, FooDB, and other data in the literature were compared for analyses and evaluations. The substance probabilities overlapping with the main ion values obtained in the study were also evaluated with fragmentation ions, and identifications were made.

Experimental protocol and ethics

This study was conducted using 30 female Wistar albino rats, aged 8-12 weeks and weighing between 250-300 g. The animals were obtained and bred at the Experimental Animals Application and Research Center of Afyon Kocatepe University. During the experimental period, the rats were housed in stainless steel cages under controlled environmental conditions (21 ± 2°C temperature, 55–60% relative humidity, and a 12:12 h light/dark cycle). All animals were provided ad libitum access to standard pellet feed and fresh drinking water, with no additional dietary supplements. A one-week acclimatization period was allowed before the beginning of the experiment. The experimental protocol was approved by the Local Ethics Committee for Animal Experiments of Afyon Kocatepe University (Protocol No: AKUHADYEK 11-21). All procedures were carried out in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*. The animals were randomly assigned to five groups, each consisting of six rats. CP was dissolved in isotonic saline, and the artichoke leaf extracts were prepared in 10% dimethyl sulfoxide (DMSO). The control group received 10% DMSO by oral gavage once a day for 10 consecutive days. Similarly, the CP group received DMSO for 10 days and was administered a single intraperitoneal dose of CP (200 mg/kg body weight) on day 7. This dosage was previously reported to induce hepatotoxicity in rats (Karaboga and Avci 2023). Artichoke leaf extracts (n-hexane, EtOAc, or MeOH) were administered to the experimental groups at a dose of 1 g/kg body weight by oral gavage once daily for 10 consecutive days (Mohammed et al. 2020). A single intraperitoneal injection of CP (200 mg/kg) was also given to these groups on day 7. All administrations were performed in the morning hours. On day 11, the animals were euthanized by decapitation under 4-5% isoflurane anesthesia delivered via insufflation. The consistent use of the same anesthetic protocol across all groups ensured minimal intergroup variability and maintained experimental standardization.

Preparation of plasma and tissue samples

Following anesthesia, blood samples were collected from each animal through cardiac puncture into heparinized tubes. The collected blood was centrifuged at 3000 rpm for 10 minutes at 4°C to separate the plasma, which was then carefully transferred into labeled tubes and stored at -20°C until biochemical analysis. After euthanasia, liver tissues were excised and processed immediately. A designated portion of the liver intended for biochemical evaluations was isolated and homogenized in ice-cold isotonic NaCl solution [1:10 (w/v)], first using a mechanical homogenizer (IKA-T18 ULTRA TURRAX), followed by ultrasonic homogenization (BANDELIN SONOPULS, 20 kHz). The resulting homogenate was centrifuged at 5000 rpm for 15 minutes at 4°C to obtain the supernatant, which was subsequently stored at -20°C until further use. The remaining liver tissue was preserved for histological processing.

Biochemical analysis

Plasma levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein (T. protein), and total bilirubin (T. bil.) were measured using the Abbott Architect c8000 analyzer with commercially available Abbott kits. Levels of malondialdehyde (MDA; Cat. No: E0156Ra), reduced glutathione (GSH; Cat. No: EA0113Ra), superoxide dismutase (SOD; Cat. No: E0168Ra), glutathione peroxidase (GPx; Cat. No: E1172Ra), tumor necrosis factor-alpha (TNF-α; Cat. No: E0764Ra), interleukin-1 beta (IL-1β; Cat. No: E0119Ra), and interleukin-10 (IL-10; Cat. No: E0108Ra) were determined using commercial ELISA kits (Bioassay Technology Laboratory, China). Absorbance readings were obtained using an ELISA reader (Multiskan FC, Thermo Fisher Scientific, USA) in accordance with the protocols of the manufacturers.

Histopathological method

Liver tissues were excised and fixed in 10% neutral-buffered formalin for 48 hours, then dehydrated through a graded ethanol series (70-100%), cleared in xylene, and embedded in paraffin. Paraffin-embedded sections of 5-6 μm thickness were obtained using a rotary ultramicrotome and stained with hematoxylin and eosin (H&E). The stained slides were examined under a light microscope (Olympus BX51, Tokyo, Japan) equipped with a digital imaging system (Olympus DP20, Microscopic Digital Image Analysis System, Tokyo, Japan). The semi-quantitative scoring of liver lesions was performed according to the method described by Gibson-Corley et al. (2013). Histopathological parameters – including central vein hyperemia, sinusoidal dilatation, vacuolar degeneration, and Kupffer cell activation – were evaluated and scored on

Table 1. Identified compounds for the phytochemical profile of artichoke leaf extracts.

ID	Compound name	Ion formula	Theoretical ion (*m/z*)	Measured ion (*m/z*)	Margin of error (ppm)	Retention time (min)	References
1	Caffeoyl hexoside	$C_{15}H_{17}O_9$	341.0878	341.0885	-1.81	2.99	Abu-Reidah et al. 2013
2	Quinic acid	$C_7H_{11}O_6$	191.0561	191.0561	0.00	3.32	Yang et al. 2020
3	Syringic acid O-hexoside	$C_{15}H_{19}O_{10}$	359.0984	359.0968	4.46	10.55	Abu-Reidah et al. 2013
4	Caffeoyl quinic acid isomer	$C_{16}H_{17}O_9$	353.0878	353.0876	0.57	12.09	Abu-Reidah et al. 2013
5	Pinoresinol-4-O-β-D-glycoside	$C_{26}H_{31}O_{11}$	519.1872	519.1864	1.87	14.57	Abu-Reidah et al. 2013
6	Dicaffeoyl quinic acid isomer 1	$C_{25}H_{23}O_{12}$	515.1195	515.1172	4.46	14.97	Luca et al. 2022
7	Luteolin-7-O-glycoside	$C_{21}H_{19}O_{11}$	447.0933	447.0922	2.86	15.39	Yang et al. 2020
8	Dicaffeoyl quinic acid isomer 2	$C_{25}H_{23}O_{12}$	515.1195	515.1167	5.62	15.66	Abu-Reidah et al. 2013
9	Apigenin-7-O-rutinoside	$C_{27}H_{29}O_{14}$	577.1563	577.1553	1.99	15.95	Yang et al. 2020
10	Apigenin 7-glycoside	$C_{21}H_{19}O_{10}$	431.0984	431.098	0.93	16.15	Abu-Reidah et al. 2013
11	Apigenin	$C_{15}H_9O_5$	269.0455	269.0454	0.78	18.94	Yang et al. 2020
12	Oxo-octadecatrienoic acid	$C_{18}H_{27}O_3$	291.1966	291.1966	0.00	21.4	Luca et al. 2022
13	Hydroxy octadecatrienoic acid	$C_{18}H_{29}O_3$	293.2122	293.2119	1.02	21.8	Luca et al. 2022
14	Hydroxy octadecadienoic acid	$C_{18}H_{31}O_3$	295.2279	295.2277	0.52	21.95	Luca et al. 2022

a scale of 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe).

Statistical analysis

The normality of the biochemical data distribution was assessed using the Shapiro–Wilk test. Homogeneity of variances across groups was initially evaluated using one-way analysis of variance (ANOVA). Since the data did not meet the assumptions of normal distribution, the non-parametric Kruskal-Wallis test was employed for group comparisons. For the biochemical parameters, where statistically significant differences were detected, Dunn's test was applied as a post hoc non-parametric multiple comparison method to identify the sources of variation among the groups. For the analysis of histopathological data, Duncan's multiple range test was used to determine statistically significant differences between groups. A p-value of <0.05 was considered statistically significant in all tests. All statistical analyses were performed using SPSS version 22.0.

Results

Active ingredients of the extracts determined by LC-QTOF-MS

The list of compounds identified by the LC-QTOF-MS system, along with the contents of the *n*-hexane, EtOAc, and MeOH extracts derived from artichoke leaves, is presented in Tables 1 and 2. In total, 14 compounds were identified through comparison to the literature, and the signal intensities of these compounds in the respective extracts are illustrated in Fig. 1.

Dicaffeoyl quinic acid isomer 1, luteolin-7-*O*-glycoside, apigenin, oxo-octadecatrienoic acid, hydroxy octadecatrienoic acid, and hydroxy octadecadienoic acid were detected in the *n*-hexane extract. All 14 compounds listed in the Table 2 were detected in the EtOAc extract. Based on its signal intensity values, apigenin-7-*O*-rutinoside was found only in the EtOAc extract. The signal intensities of luteolin-7-*O*-glycoside, apigenin 7-glycoside, apigenin, oxo-octadecatrienoic acid, hydroxy octadecatrienoic acid, and hydroxy octadecadienoic acid were higher in the EtOAc extract than in the MeOH extract. The presence of caffeoyl hexoside, quinic acid, syringic acid *O*-hexoside, caffeoyl quinic acid isomer, pinoresinol 4-*O*-*β*-D-glycoside, and dicaffeoyl quinic acid isomers was detected at higher rates in the MeOH extract compared to the other extracts.

Biochemical and oxidative stress parameters

Plasma AST, ALT, ALP, and T. protein levels were elevated in the CP group compared to the control group. Treatment with all extracts significantly reduced AST, ALT, and ALP levels relative to the CP group ($p<0.05$), while a significant decrease in T. protein levels was observed only in the EtOAc group. Changes in T. bil. levels were not statistically significant in any of the experimental groups (Table 3). As shown in Tables 4 and 5, plasma and liver MDA levels were significantly increased, while GSH, GPx, and SOD levels were significantly decreased ($p<0.01$) in the CP group compared to the control. Liver MDA levels were reduced in both the MeOH and EtOAc extract groups, whereas a signi-

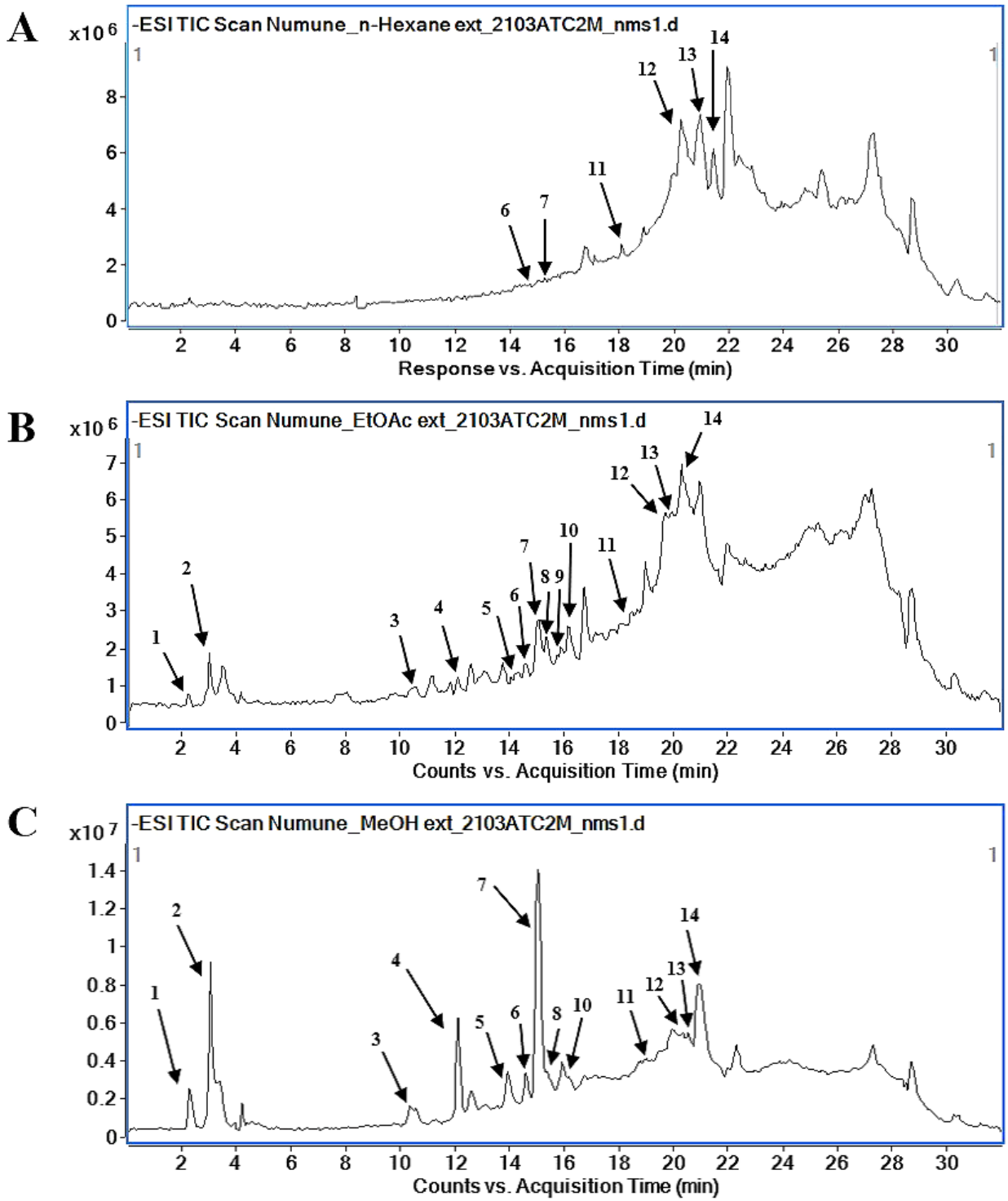

Fig. 1. Total ion chromatogram of the extracts prepared from *Cynara scolymus* leaves. The numbers assigned to the molecules identified in the chromatograms correspond to those listed in Tables 1 and 2. Abbreviations: A: *n*-Hexane extract. B: Ethyl acetate extract. C: Methanolic extract.

ficant reduction in plasma MDA levels ($p<0.01$) was observed only in the MeOH group. Although liver GSH levels showed no statistically significant increase in any extract group, plasma GSH levels were significantly elevated in the EtOAc and MeOH groups ($p<0.01$). Both liver and plasma GPx and SOD levels increased significantly ($p<0.01$) only in the MeOH group compared to the CP group. In the analyses of cytokines, liver and plasma TNF-α and IL-1β levels were significantly elevated ($p<0.01$), and IL-10 levels were significantly reduced in the CP group relative to the controls. Treatment with the MeOH extract led to significant decreases in TNF-α and IL-1β and a significant increase in IL-10 levels in both liver and plasma ($p<0.01$).

Table 2. Mass detector (MS) signal intensities of the compounds identified in the extracts.

Compound name	Retention time (min)	MS signal intensities (1/1000)		
		n-Hexane	EtOAc	MeOH
Caffeoyl hexoside	3.12	Not found	4374	33150
Quinic acid	3.39	Not found	302	8334
Syringic acid O-hexoside	10.48	Not found	2773	8990
Caffeoyl quinic acid isomer	12.09	Not found	1473	16813
Pinoresinol 4-O-β-D-glycoside	14.63	Not found	1380	5344
Dicaffeoyl quinic acid isomer 1	15.04	96	6656	65170
Luteolin-7-O-glycoside	15.30	21	7602	3299
Dicaffeoyl quinic acid isomer 2	15.66	Not found	755	75388
Apigenin-7-O-rutinoside	15.95	Not found	2267	Not found
Apigenin 7-glycoside	16.15	Not found	8318	272
Apigenin	19.02	47	9452	21
Oxo octadecatrienoic acid	21.40	15701	2451	1238
Hydroxy octadecatrienoic acid	21.80	22687	10005	3227
Hydroxy octadecadienoic acid	21.95	12266	7440	5471

Abbreviations: CP: Cyclophosphamide. EtOAc: Ethyl acetate extract. MeOH: Methanolic extract.

Table 3. Plasma biochemical parameters in experimental rat groups.

	Control	CP	*n*-Hexane	EtOAc	MeOH	P
AST (U/I)	38.8 ± 4.21^{a}	82.8 ± 8.81^{c}	56.6 ± 4.16^{b}	62 ± 14.16^{b}	64.6 ± 9.94^{b}	0.000
ALT (U/I)	13.4 ± 3.21^{a}	28.2 ± 4.09^{b}	16 ± 3.81^{a}	17.8 ± 5.36^{a}	17.8 ± 3.03^{a}	0.000
ALP (U/I)	67 ± 20.33^{a}	267.2 ± 52.05^{b}	67.2 ± 6.14^{a}	80.2 ± 10.43^{a}	71.2 ± 20.3^{a}	0.000
T. protein (g/dl)	5.52 ± 0.16^{a}	6.06 ± 0.64^{b}	$5.64\pm0.21^{a,b}$	5.28 ± 0.15^{a}	5.54 ± 0.35^{a}	0.033
T. bil.(mg/dl)	0.1±0.017	0.002±0.004	0.008±0.013	0.004±0.009	0.004±0.009	0.796

Different letters in the same row show statistically significant differences ($p<0.05$). Abbreviations: AST: Aspartate transaminase. ALT: Alanine transaminase. ALP: Alkaline phosphatase. CP: Cyclophosphamide. EtOAc: Ethyl acetate extract. MeOH: Methanolic extract. NS: Insignificant. T. protein: Total protein. T. bil.: Total bilirubin.

Table 4. Plasma oxidant-antioxidant and cytokine parameters in experimental rat groups.

	Control	CP	*n*-Hexane	EtOAc	MeOH	P
MDA (nmol/ml)	5.21 ± 0.48^{c}	10.47 ± 0.25^{a}	8.01 ± 0.56^{ab}	6.83 ± 0.53^{abc}	6.50 ± 0.44^{bc}	0.001
GSH (nmol/ml)	48.54 ± 9.20^{a}	28.68 ± 2.03^{c}	36.41 ± 2.26^{bc}	41.50 ± 3.07^{ab}	42.48 ± 2.70^{ab}	0.001
GPx (U/ml)	415.88 ± 10.91^{a}	272.70 ± 12.41^{c}	320.04 ± 9.94^{bc}	362.29 ± 12.57^{abc}	384.62 ± 7.78^{ab}	0.001
SOD (U/ml)	16.51 ± 0.89^{a}	7.92 ± 0.56^{c}	10.58 ± 0.95^{bc}	13.53 ± 0.88^{abc}	14.22 ± 1.24^{ab}	0.001
IL-1β (pg/ml)	125.48 ± 6.70^{a}	243.92 ± 18.02^{c}	193.42 ± 6.62^{bc}	157.21 ± 6.39^{abc}	141.99 ± 7.02^{ab}	0.001
IL-10 (pg/ml)	307.18 ± 7.14^{a}	135.18 ± 11.62^{c}	218.83 ± 19.53^{bc}	251.99 ± 17.15^{abc}	259.91 ± 18.88^{ab}	0.001
TNF-α (pg/ml)	76.67 ± 5.07^{c}	234.17 ± 32.61^{a}	172.39 ± 10.01^{ab}	118.86 ± 5.97^{abc}	97.15 ± 6.19^{bc}	0.001

Different letters in the same row show statistically significant differences ($p<0.01$).
Abbreviations: CP: Cyclophosphamide. EtOAc: Ethyl acetate extract. GSH: Glutathione. GPx: Glutathione peroxidase. IL-1β: Interleukin-1beta. IL-10: Interleukin 10. MeOH: Methanolic extract. MDA: Malondialdehyde. SOD: Superoxide dismutase. TNF-α: Tumor necrosis factor alpha.

Table 5. Liver oxidant-antioxidant and cytokine parameters in experimental rat groups.

	Control	CP	*n*-Hexane	EtOAc	MeOH	P
MDA (nmol/mg)	1.39±0.22^{b}	2.87±0.23^{a}	1.85±0.10^{ac}	1.65±0.18^{bc}	1.66±0.08^{bc}	0.001
GSH (μmol/g)	38.87±3.72^{a}	25.10±3.21^{b}	29.03±6.04^{ab}	35.62±2.91^{ab}	34.20±2.69^{ab}	0.001
GPx (U/mg)	145.22±6.24^{a}	74.74±4.12^{c}	90.43±3.44^{bc}	108.71±3.48^{abc}	116.85±4.07^{ab}	0.001
SOD (U/mg)	129.17±1.87^{a}	84.64±2.26^{c}	104.49±1.48^{abc}	113.80±1.68^{abc}	118.61±1.31^{ab}	0.001
IL-1β (pg/g)	62.28± 2.43^{c}	127.87± 7.63^{a}	92.80±2.01^{ab}	79.64±3.04^{abc}	76.14± 3.38^{bc}	0.001
IL-10 (pg/g)	111.93±4.14^{a}	42.76±1.60^{c}	63.88±2.91^{bc}	83.23±3.73^{abc}	89.80±4.97^{ab}	0.001
TNF-α (ng/g)	41.40±2.60^{c}	79.25±7.07^{a}	60.06±5.57^{ab}	49.70±3.90^{abc}	43.41±1.69^{bc}	0.001

Different letters in the same row show statistically significant differences ($p<0.01$).
Abbreviations: CP: Cyclophosphamide. EtOAc: Ethyl acetate extract. GSH: Glutathione. GPx: Glutathione peroxidase. IL-1β: Interleukin-1beta. IL-10: Interleukin 10. MeOH: Methanolic extract. MDA: Malondialdehyde. SOD: Superoxide dismutase. TNF-α: Tumor necrosis factor alpha.

Table 6. Histopathological scoring of liver tissue in experimental groups.

Liver tissue	Control	CP	*n*-Hexane	EtOAc	MeOH	p<0.05
Hyperemia in vena centralis	0.0±0.0^{d}	2.27±0.17^{a}	1.77±0.21^{ab}	0.73±0.23^{c}	1.41±0.35^{b}	0.001
Sinusoidal dilation and hyperemia	0.0±0.0^{c}	2,10±0.26^{a}	1.60±0.22^{a}	0.73±0.23^{b}	1.60±0.22^{a}	0.001
Vacuolar degeneration	0.0±0.0^{d}	2.27±0.17^{a}	1.77±0.21^{b}	1.10±0.00^{c}	1.77±0.21^{b}	0.001
Kupffer cells	0.0±0.0^{c}	2.10±0.37^{a}	1.77±0.21^{a}	0.73±0.23^{b}	1.77±0.21^{a}	0.001

Values bearing different letters in the same row indicate statistically significant differences in histopathological scores ($p<0.05$).
Abbreviations: CP: Cyclophosphamide. EtOAc: Ethyl acetate extract. MeOH: Methanolic extract.

No statistically significant changes in these cytokine levels were observed in the other extract groups compared to the CP group.

Histopathological findings

The mean histopathological scores of the groups are presented in Table 6. No significant histopathological alterations were observed in the control group [Fig. 2(A1)]. In contrast, the CP group exhibited marked hyperemia in the central vein, sinusoidal dilation and hyperemia, vacuolar degeneration, and increased Kupffer cell activation compared to the control group [Fig. 2(A2)]. Compared to the CP group, the MeOH extract group showed a significant reduction in hyperemia and vacuolar degeneration in the central vein [Fig. 2(A5)], while the *n*-hexane group exhibited a decrease only in vacuolar degeneration [Fig. 2(A3)]. In the EtOAc group, all examined pathological changes, including central vein hyperemia, sinusoidal dilation and hyperemia, vacuolar degeneration, and Kupffer cell activation, were significantly reduced compared to the CP group [Fig. 2(A4)].

Discussion

In this study, we examined the potential protective effects of artichoke leaf extracts, prepared using different solvents, against CP-induced acute liver toxicity. While the cytotoxicity of CP limits its therapeutic applications, oxidative stress resulting from redox imbalance following drug exposure has also been implicated in the development of various biochemical and physiological abnormalities (Nagi et al. 2010). Previous research has indicated that the antioxidant capacity and therapeutic efficacy of medicinal plant extracts may vary significantly depending on the type and concentration of phenolic constituents present in them (Speroni et al. 2003, Avci et al. 2022). In our earlier study, the total phenolic contents of *n*-hexane, EtOAc, and MeOH extracts were quantified as 0.167 mg, 0.917 mg, and 5.735 mg GAE/g extract, respectively, based on gallic acid equivalence (Albayrak et al. 2022). According to our phytochemical analysis in this study, luteolin-7-*O*-glycoside, apigenin 7-glycoside, apigenin, oxo-octadecatrienoic acid, hydroxy-octadecatrienoic acid, and hydroxy-octadecadienoic acid were present at higher concentrations in the EtOAc extract compared to the MeOH extract, and apigenin-7-*O*-rutinoside was uniquely detected in the EtOAc extract. Notably, only 8 out of the 14 identi-

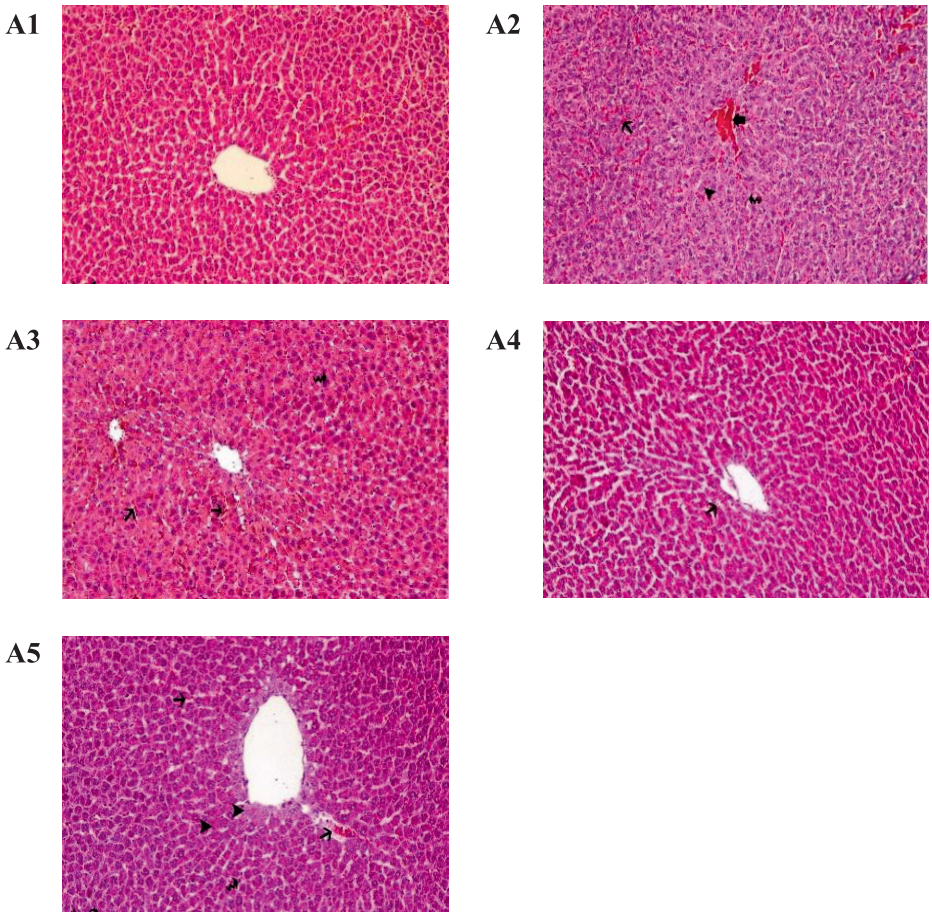

Fig. 2. Histopathological appearance of the liver tissue in experimental rat groups. Abbreviations: A1: Control group, A2: CP group, A3: *n*-Hexane extract, A4: Ethyl acetate extract, A5: Methanolic extract. All sections were stained with hematoxylin and eosin (H&E). The original magnification: 20X objective; scale bar=100 µm. Thick arrows indicate hyperaemia of the vena centralis in the liver tissue (Fig. 2-A2). Thin arrows indicate sinusoidal dilation and hyperaemia in the liver tissue (Fig. 2-A2, Fig. 2-A3, Fig. 2-A4, Fig. 2-A5), respectively. Arrowheads indicate areas of vacuolar degeneration in hepatocytes (Fig. 2-A2, Fig. 2-A3, Fig. 2-A5). The curved arrow shows an increase in Kupffer cells in the liver tissue (Fig. 2-A2, Fig. 2-A3, Fig. 2-A5), respectively.

fied compounds were found in the *n*-hexane extract. These findings highlight that solvent selection is a critical factor influencing extraction efficiency (Sokkar et al. 2020). Furthermore, since polar solvents tend to dissolve polar compounds and non-polar solvents dissolve non-polar compounds, the chemical composition of each extract varies accordingly. As a result, the biological activity of an extract may also differ depending on the solvent used. In terms of polarity, the solvents employed in this study can be ranked from the most non-polar to the most polar as follows: *n*-hexane > ethyl acetate > methanol. Supporting this, Albayrak et al. (2022) reported that MeOH and EtOAc extracts exhibited higher antioxidant activity and contained greater amounts of total phenolic compounds compared to *n*-hexane extracts, which aligns with the findings of this study.

In general, elevated serum levels of cytoplasmic enzymes such as AST, ALT, and ALP are indicative of hepatobiliary dysfunction, impaired bile secretion, and in some cases, skeletal disorders, thus reflecting a toxicological profile (Abdelfattah-Hassan et al. 2019, Florek et al. 2023). Indeed, numerous studies have shown that hepatotoxic agents like CCl_4 and CP induce liver injury, typically marked by increased transaminase levels (Aktay et al. 2000, Mehmetcik et al. 2008). In our study, the significant rise in AST, ALT, and ALP levels observed in the CP group compared to the control group was attributed to the generation of ROS triggered by toxic metabolites such as acrolein and CAA, which are produced during the hepatic metabolism of CP. The resulting oxidative stress and lipid peroxidation lead to hepatocyte membrane damage, facilitating the release of transaminases into the bloodstream (El Morsy et al. 2015). Consistently, Abdelfattah-Hassan et al. (2019) also reported elevated AST, ALT, and ALP levels following CP administration (20 mg/kg, i.p., for two weeks), which aligns with our findings, although they additionally noted a decrease in total protein levels – an outcome not observed in our study. In our study, CP administration resulted in a significant increase in T. protein levels, whereas the changes in T. bil. levels were

not statistically significant. Similar observations have been reported in previous studies. For example, Hu et al. (2014) demonstrated that eight liver-specific protein markers increased in a dose-dependent manner within the first 24 hours following the administration of both low (150 mg/kg) and high (300 mg/kg) doses of acetaminophen. Similarly, naphthalene exposure was shown to elevate T. protein levels alongside increased serum transaminases, which was attributed to hepatic inflammation (Chukwunonyelum et al. 2016). These findings suggest that the increase in T. protein levels may stem from hepatocellular injury and systemic inflammatory processes. Additionally, Hu et al. (2014) proposed that elevated T. protein levels may result from the release of cytoplasmic and nuclear proteins into the bloodstream due to membrane leakage or proteolysis following cellular damage. Accordingly, it is plausible that the administration of CP at a dose of 200 mg/kg induces similar pathological mechanisms, leading to the observed elevation in serum T. protein levels. The assessment of tissue damage resulting from increased ROS production or the inactivation of cellular proteins is crucial for detecting pathological alterations (Senthilkumar et al. 2006). It has been demonstrated that CP administration at various doses elevates the levels of enzymes such as AST, ALT, ALP, CK, and LDH and induces histopathological alterations in liver tissue (Senthilkumar et al. 2006, Avci et al. 2016), which supports the findings of the present study.

According to the histopathological findings of this study, the adverse alterations observed in the liver tissues of the CP-treated rats are consistent with reports indicating that acrolein, a toxic metabolite of CP, promotes excessive ROS production by overwhelming the antioxidant defense system (McCarroll et al. 2008). Furthermore, the report stating that high-dose CP administration (200 mg/kg) led to sinusoidal dilation, hyperemia, degeneration, necrosis, and steatosis in the liver (Karaboga and Avci 2023) also supported our results.

Cynarine is commonly regarded as the primary compound responsible for the biological activity of artichoke plant extracts. However, flavonoid glycosides derived from apigenin and luteolin, found at high concentrations in EtOAc extracts, also exhibit potent antioxidant and hepatoprotective properties (He et al. 2019). Zheng et al. (2005) reported that apigenin-7-glucoside inhibited both the elevation of serum transaminase activities and the formation of MDA in cases of CCl_4-induced liver injury. In our study, T. bil. levels did not change significantly in any of the extract groups. Pre-treatment with the extracts (1.0 g/kg) attenuated the CP-induced elevations in serum AST, ALT, ALP, and T. protein levels, indicating the protective effects of flavonoids and phenolic compounds on hepatocellular membrane integrity. In a similar study, Mehmetcik et al. (2008) reported that artichoke leaf extract reduced ALT, AST, and MDA levels and improved histopathological damage in a CCl_4-induced liver injury model. Additionally, other studies have shown that artichoke extracts reduced transaminase enzyme activities in liver injuries caused by paracetamol and CCl_4 (Aktay et al. 2000, Florek et al. 2023). These findings are in agreement with the results of our study. Among the treatment groups, the EtOAc extract demonstrated the most pronounced improvement against CP-induced liver damage. In the MeOH group, hyperemia and vacuolar degeneration in the central vein were reduced, while the *n*-hexane group showed no marked histological improvements. Overall, histopathological evaluations revealed that especially the EtOAc and MeOH extracts mitigated CP-induced degenerative alterations in hepatic tissue, which was further supported by reductions in serum enzyme activities.

Acrolein, a toxic metabolite of CP, increases ROS production through xanthine oxidase activation, thereby depleting antioxidant molecules (Chen et al. 2019). This elevated ROS generation disrupts macromolecular functions, induces oxidative stress, and enhances lipid peroxidation levels (Fouad et al. 2016). MDA is a key marker of ROS-induced lipid peroxidation, while components of the antioxidant defense system, such as GSH, GPx, and SOD, help protect cell membranes from oxidative damage (Bacak Gullu and Avci 2013, Denk et al. 2022, Florek et al. 2023). In this study, CP administration led to elevated MDA levels in both plasma and liver tissue, alongside decreased levels of GSH, GPx, and SOD. These changes indicate that the cellular antioxidant defense system was overwhelmed by oxidative stress resulting from CP metabolites, leading to the depletion of antioxidant enzymes. These findings were consistent with previous reports (Fouad et al. 2016, Abdelfattah-Hassan et al. 2019). Additionally, the increase in plasma transaminase levels and the histopathological alterations observed further supported the occurrence of lipid peroxidation and hepatic injury induced by CP. Similarly, it has been reported that acrolein forms adducts with GSH, induces ROS formation, causes cellular damage, and exerts mutagenic effects (Senthilkumar et al. 2006). Artichoke extracts are known to be rich in caffeoylquinic acids and flavonoids with potent antioxidant properties (El Morsy et al. 2015). Our results demonstrated that the MeOH extract reduced MDA levels and enhanced the activities of SOD and GPx, suggesting a mitigating effect on oxidative hepatocellular damage. Both in vitro and in vivo studies have shown that compounds such as cynarine, chlorogenic acid, and flavonoids present in artichoke

leaf extracts possess ROS-scavenging and metal-chelating abilities, thereby protecting biological molecules from oxidative injury (Ben Salem et al. 2015, Salekzamani et al. 2019). These compounds exert antioxidant effects by scavenging ROS, inhibiting lipid peroxidation, and preserving cell membrane integrity. Furthermore, the concentration and composition of phenolic compounds in an extract influence its pharmacological properties (Speroni et al. 2003). In agreement with our findings, a previous in vitro study revealed that the MeOH extract of artichoke leaves contained higher levels of total phenolic compounds compared to other extracts (Albayrak et al. 2022). In this study, dicaffeoylquinic acid isomers were found at higher concentrations in the MeOH extract than in the other extracts. Meanwhile, the EtOAc extract exhibited a broader diversity of phenolic compounds, whereas the *n*-hexane extract displayed both the lowest diversity and the lowest total phenolic content (Albayrak et al. 2022). These findings suggested that both the quantity and diversity of phenolic constituents are closely linked to the antioxidant potential of extracts. Nevertheless, further comprehensive studies are needed to identify the specific phenolic compounds or flavonoids in artichoke leaf extracts that are primarily responsible for the observed biological effects.

Proinflammatory cytokines such as TNF-α and IL-1β are key mediators in chemokine production during acute inflammatory responses. IL-10, an anti-inflammatory cytokine, exerts immunosuppressive effects by inhibiting the activity of proinflammatory cytokines (Yazar et al. 2010). Our findings were consistent with previous reports indicating that both low (20 mg/kg) (Germoush and Mahmoud 2014) and high (200 mg/kg) (Abdelfattah-Hassan et al. 2019) doses of CP administration reduce antioxidant levels and elevate proinflammatory cytokines such as TNF-α and IL-1β. In this study, when the extract groups were compared to the CP group, only the MeOH extract group showed a significant reduction in TNF-α and IL-1β levels, along with an increase in IL-10 levels in both liver and plasma. These results suggest that phenolic compounds in the MeOH extract may inhibit cellular apoptosis and inflammation by exerting antioxidant effects against the oxidative stress and inflammation induced by CP administration.

The findings of this study demonstrated that the EtOAc extract, which contained the greatest diversity of phenolic compounds, was the most effective in mitigating CP-induced hepatic cell degeneration. Moreover, the MeOH extract, which exhibited the highest total phenolic content, provided partial histopathological improvement in the liver and showed the most pronounced antioxidant and anti-inflammatory effects in both plasma and tissue. Therefore, considering its bioactive compound profile, the MeOH extract of artichoke leaves appears to be a promising, natural, and safe supportive agent for alleviating the adverse hepatic effects of chemotherapeutic agents when used in combination therapy. Nonetheless, further investigations are warranted to identify the specific phenolic or flavonoid constituents responsible for these biological activities.

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