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Article Information

Received: August 26, 2026

Accepted: September 27, 2026

Published: October 6, 2026

Authors' Contribution

KSM designed the study. HMM and SAAS performed experiments. HMM and KSM wrote, read, and approved the final manuscript.

Citation

Murad, H.M., Shaher, S.A.A., Mahdi, K.S., 2026. Physiopathological and Toxicological Evaluation of the Ameliorative Effects of Chicory (*Cichorium intybus* L.) against Acrylamide-Induced Hepatic Oxidative Stress and Liver Enzyme Alterations in Rats. PSM Vet. Res., 11(1): 16-27.

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Physiopathological and Toxicological Evaluation of the Ameliorative Effects of Chicory (*Cichorium intybus* L.) against Acrylamide-Induced Hepatic Oxidative Stress and Liver Enzyme Alterations in Rats

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Abstract:

Acrylamide (ACR), which is a food contaminant and is probable to cause cancer in humans, exerts its action through oxidative stress in the liver. The study began with the extraction of leaf chicory (*Cichorium intybus* L.) in order to check its efficacy against ACR-induced oxidative stress in the liver and further changes in the liver enzymes and histopathological injury. Forty-eight adult male Wistar rats were assigned into six groups (n=8). The experimental groups were: negative control (vehicle), ACR only (20 mg/kg/day), chicory only (500 mg/kg/day), ACR + chicory (100 mg/kg/day), ACR + chicory (500 mg/kg/day), and ACR + silymarin (100 mg/kg/day) as the reference group. Each treatment was administered orally for 28 days. The first two treatments were taken for 7 days before the commencement of ACR (from day 8 to day 28). One-way ANOVA and Tukey's test were the statistical methods used. ACR was found to increase MDA, ALT, AST, and ALP but reduce GSH and SOD ($p < 0.001$), and these results were also validated histologically in the form of coagulative necrosis and hemorrhage. Chicory co-administration decreased such changes in a dose-dependent way (100 against 500 mg/kg; $p < 0.001$ for each parameter). The 500 mg/kg dose had no impact on any parameter compared to silymarin ($p = 0.818-0.986$), but MDA, GSH, SOD, AST, and ALP in this group were still significantly different from control. Chicory administered by itself did not influence any parameter ($p \geq 0.76$ vs. control) and kept liver histology predominantly normal. Thus, it can be suggested that chicory extract alleviates ACR-induced liver damage in rats, presumably due to limiting oxidative stress, although the results have to be verified in long-term studies with a standardised and characterized extract.

Keywords: Acrylamide; Chicory (*Cichorium intybus* L.); Oxidative Stress; Hepatotoxicity; Histopathology; Liver Enzymes; Silymarin; Toxicology.



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INTRODUCTION

Acrylamide (ACR), a small and water-soluble vinyl monomer, is produced in carbohydrate-rich food when exposed to high temperatures through processes such as frying, baking, and roasting. It is primarily formed in the process of the Maillard reaction between asparagine and reducing sugars. Since ACR is found in a variety of foods consumed regularly, such as French fries, bread, cookies, and coffee, dietary exposure to acrylamide is virtually inevitable. The IARC considers it a Group 2A probable human carcinogen, and its neurotoxic, reproductive, and hepatotoxic effects are well known (Zhao *et al.*, 2022; Govindaraju *et al.*, 2024). When acrylamide is absorbed, it is transformed into its epoxide product glycidamide in the liver by cytochrome P450 2E1, which is the primary organ responsible for the biotransformation of ACR. ACR exerts toxic effects on the liver by generating ROS, which depletes GSH and inhibits SOD, CAT, and GPx enzyme activities, resulting in excessive lipid peroxidation, which can be measured by measuring the levels of MDA in the liver. This imbalance in redox status causes damage to hepatocyte membranes and the release of ALT, AST, and ALP into the blood, which are widely used in the study of liver injury (Wu *et al.*, 2021; Zhang *et al.*, 2023).

Given the central role of oxidative stress in ACR hepatotoxicity, antioxidant-rich plant extracts have attracted growing interest as protective agents with a favorable safety profile relative to synthetic chemoprotectants. Chicory (*Cichorium intybus* L., Asteraceae) is rich in bioactive phytochemicals — chicoric acid, chlorogenic acid, inulin, sesquiterpene lactones, and flavonoids such as quercetin and cyanidin — shown to confer antioxidant, anti-inflammatory, and hepatoprotective activity against liver injury induced by alcohol, heavy metals, and pesticides (Atef *et al.*, 2021; Birsa and Sarbu, 2023; El-Sayed *et al.*, 2024; Dang *et al.*, 2026; Duda *et al.*, 2024; Tote and Ahmed, 2025). However, despite this documented hepatoprotective profile and the well-established oxidative mechanism of ACR hepatotoxicity, chicory's ameliorative potential specifically

against ACR-induced hepatic oxidative stress and enzyme derangement remains unreported; prior protective-agent studies against ACR hepatotoxicity examined other natural compounds (wine polyphenols, ellagic acid, allicin, morin), leaving the chicory–acrylamide interaction unexplored.

To the best of our knowledge, the present study is therefore the first to investigate the ameliorative effect of *C. intybus* extract against ACR-induced hepatotoxicity in rats, evaluating two dose levels (100 and 500 mg/kg) to establish a dose–response relationship, benchmarking efficacy against the reference agent silymarin, and combining oxidative stress biomarkers (MDA, GSH, SOD) with a full hepatic enzyme panel (ALT, AST, ALP) and histopathological examination in a single integrated design. Accordingly, this study evaluates the ameliorative effects of chicory extract, at two dose levels, against acrylamide-induced hepatic oxidative stress, serum liver enzyme disturbances, and hepatic histopathological alterations in rats, using silymarin as the standard reference hepatoprotective agent.

MATERIALS AND METHODS

Chemicals and reagents

Acrylamide (≥99% purity) and silymarin (reference hepatoprotective standard) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Serum ALT and AST were determined using ALT/GPT and AST/GOT kits (Arena BioScien, Egypt), hepatic MDA using an MDA kit (BT Lab, China), and reduced glutathione (GSH), superoxide dismutase (SOD), and serum alkaline phosphatase (ALP) using GSH, SOD, and ALP kits (Biodiagnostic, Giza, Egypt). All other chemicals and reagents were of analytical grade.

Plant material (Chicory leaf extract)

The leaf extract of Chicory (*Cichorium intybus* L.) was sourced from a local herbal medicine seller based in Babylon Governorate, Iraq. The

extract was dried in its original packaging for the duration of the study and was freshly dissolved in distilled water immediately before each administration. The dose was calculated as mg of dry extract per kg of body weight. Since this was a commercially available extract from them, this meant that the extraction method and yield of the extract were not known.

Experimental animals and ethical approval

Forty-eight male Wistar albino rats, weighing between 180 and 220 grams, were utilized in the current study. The rats were maintained in standard laboratory conditions (temperature of $22 \pm 2^{\circ}\text{C}$ and 12 hours of light/dark cycle with humidity of 50–60%). Animals were acclimatized for a period of 7 days prior to commencement of the study. The study was approved by the Research Ethics Committee of Al-Qasim Green University (Approval No.: qgec/53/2025) and was conducted following the ARRIVE 2.0 guidelines (Percie du Sert *et al.*, 2020).

Experimental design, randomization, and treatment schedule

Following acclimatization, rats were weighed, given a unique identifying number, and assigned to six experimental groups (8 rats per group). The size of the groups was used in similar studies investigating the hepatotoxic effects of acrylamide (Karimi *et al.*, 2020). Different treatments were administered orally once a day (09:00–11:00 h) at a dose of 5 mL/kg body weight. The acrylamide was dissolved, and the chicory extract and silymarin were suspended in water. The detailed treatment pattern in the six experimental groups is as follows:

- G1 – Negative control: vehicle (distilled water) for 28 days.
- G2 – Acrylamide only: vehicle on days 1–7, then ACR (20 mg/kg/day) on days 8–28.
- G3 – Chicory only: chicory extract (500 mg/kg/day) on days 1–28, to assess its intrinsic safety.
- G4 – Acrylamide + Chicory (100 mg/kg): chicory extract (100 mg/kg/day) on days 1–28 plus ACR (20 mg/kg/day) on days 8–28.

- G5 – Acrylamide + Chicory (500 mg/kg): chicory extract (500 mg/kg/day) on days 1–28 plus ACR (20 mg/kg/day) on days 8–28.
- G6 – Acrylamide + Silymarin (100 mg/kg): silymarin (100 mg/kg/day) on days 1–28 plus ACR (20 mg/kg/day) on days 8–28 (reference hepatoprotective group).

Thus, the protective agents were given as a 7-day pre-treatment followed by 21 days of co-administration with ACR; on co-treatment days, they were administered before ACR. The ACR dose (20 mg/kg) was selected because it reliably induces oxidative hepatotoxicity in rats (Karimi *et al.*, 2020). The two chicory doses were chosen to represent a low and a high dose, and silymarin (100 mg/kg) was used as the standard reference agent, as in previous chicory hepatoprotection studies (Kim *et al.*, 2021). Body weight was recorded weekly for dose adjustment.

Blinding

A member of the laboratory, who had no role in the analyses, coded the serum samples, tissue homogenates, and histological slides before the biochemical assays and histopathological evaluation were carried out without knowledge of group allocation. Codes were only released after all of the information had been collected.

Euthanasia and sample collection

Twenty-four hours after the last dose, rats were weighed and deeply anesthetized by intraperitoneal (i.p.) injection of a ketamine–xylazine combination; the depth of anesthesia was confirmed by the loss of the pedal withdrawal reflex. Blood samples were collected by cardiac puncture (Beeton *et al.*, 2007), and euthanasia was completed by exsanguination under deep anesthesia, an acceptable method according to the AVMA Guidelines for the Euthanasia of Animals (AVMA, 2020). Blood was allowed to clot for 30 min, and serum was separated by centrifugation (3000 rpm, 15 min) and stored at -20°C until analysis. Quickly removed, washed with ice-cold saline, dried, weighed, and cut into pieces. A standardized amount of the left lateral lobe was preserved in

10% neutral buffered formalin ($\geq 10:1$ fixative-to-tissue volume) to perform histopathological tests, while the rest of the samples were stored at -80°C for further analysis.

Preparation of liver homogenate and biochemical analyses

A weighed portion of liver was homogenized in ice-cold phosphate buffer (pH 7.4) to obtain a 10% (w/v) homogenate (1 g tissue per 10 mL buffer) and centrifuged under cooling (4°C); the supernatant was used for the assays. Because every homogenate was prepared at the same tissue-to-buffer ratio, hepatic MDA, GSH, and SOD are expressed per volume of 10% liver homogenate (nmol/mL, $\mu\text{mol/L}$, and U/mL, respectively) and are therefore normalized to wet tissue weight; results were not normalized to tissue protein content. Hepatic MDA (lipid peroxidation marker) was measured using the MDA kit (BT Lab, China) according to the manufacturer's instructions; GSH content was determined using the GSH kit (Biodiagnostic, Egypt), which is based on Ellman's method, i.e., the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) by free sulfhydryl groups (Ellman, 1959); and SOD activity was assayed spectrophotometrically using the SOD kit (Biodiagnostic, Egypt). Serum ALT and AST activities were determined using the ALT/GPT and AST/GOT kits (Arena BioScien, Egypt), and ALP activity using the ALP kit (Biodiagnostic, Egypt), all according to the manufacturers' instructions.

Histological tissue processing

The samples collected from the liver were preserved in 10% neutral buffered formalin and subsequently analyzed through histopathological means. The liver biopsy samples underwent typical histological processing. Paraffin blocks were sectioned at $4\text{--}5\ \mu\text{m}$ thickness using a rotary microtome, mounted on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E). Stained sections were examined and photographed under a light microscope at $100\text{--}400\times$ magnification (Slaoui and Fiette, 2011). One section per animal ($n = 8$ per group) was evaluated.

Semi-quantitative histopathological grading

Hepatic lesions were evaluated semi-quantitatively on coded H&E-stained sections from all animals ($n = 8$ per group), following general principles for histo-pathological scoring in research (Gibson-Corley *et al.*, 2013). Six lesions were assessed: coagulative necrosis, hemorrhage/ congestion, mononuclear inflammatory cell infiltration, microvesicular fatty change, Kupffer-cell proliferation, and sinusoidal/central vein dilatation. For each section, ten non-overlapping fields were examined at $400\times$, and each lesion was graded according to its extent as absent ($-$, 0), mild ($+$, 1; $<25\%$ of fields affected), moderate ($++$, 2; $25\text{--}50\%$), or severe ($+++$, 3; $>50\%$). The grade assigned to each group corresponds to the predominant (modal) grade among the eight animals of that group. For comparison among groups, the numerical grades of the six lesions were summed to give a cumulative lesion score for each group (range 0–18). Because this grading summarizes group-level morphological findings, it was not subjected to inferential statistical testing.

Statistical analysis

Data are expressed as mean $\pm$ SD ($n = 8/\text{group}$). Comparisons among groups were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) *post hoc* test for all pairwise comparisons, using SPSS statistical software (IBM Corp., Armonk, NY, USA) (McDonald, 2014). F statistics and exact p values are reported; p values smaller than 0.001 are given as $p < 0.001$. A p-value < 0.05 was considered statistically significant.

RESULTS

Effect of Chicory on hepatic oxidative stress and antioxidant parameters

One-way ANOVA showed that there were significant variations in MDA, GSH, and SOD levels among all groups (F (5, 42), 187.4; F (5, 42), 94.6; F (5, 42), 95.0 ($p < 0.001$; Table 1, Figure 1). According to the study, the MDA level

in the liver in the Acrylamide-treated group (G2) increased by 154%, while the GSH content and SOD activity decreased by 50% and 44%, respectively, when compared to the control group (G1). Adding chicory to the Acrylamide treatment decreased the above effects dose-dependently. Compared to group G2, both G4 and G5 groups showed lower MDA and higher GSH and SOD levels compared to those in group G2 ($p < 0.001$), where the effect of the high dose (G5) was indeed much better than that of the low one in terms of G4 vs. G5 ($p <$

0.001; Table 3). (G1 vs. G5: MDA $p < 0.001$, GSH $p = 0.001$, SOD $p = 0.003$) but did not differ significantly from the silymarin reference group (G5 vs. G6: MDA $p = 0.818$, GSH $p = 0.892$, SOD $p = 0.889$). Silymarin itself also produced incomplete normalization of MDA ($p = 0.001$) and GSH ($p = 0.022$) relative to control. Chicory administered alone (G3) did not differ from control for any oxidative stress parameter (MDA $p = 0.946$, GSH $p = 0.761$, SOD $p = 0.855$), supporting its safety at the tested dose.

Table 1. Hepatic oxidative stress and antioxidant parameters (mean $\pm$ SD, $n = 8$ /group).

Group	MDA (nmol/mL homogenate)	GSH (μ mol/L homogenate)	SOD (U/mL homogenate)
G1 – Negative control	2.31 $\pm$ 0.22 ^d	8.42 $\pm$ 0.61 ^a	9.18 $\pm$ 0.54 ^{ab}
G2 – Acrylamide only (20 mg/kg)	5.87 $\pm$ 0.41 ^a	4.21 $\pm$ 0.37 ^d	5.12 $\pm$ 0.43 ^e
G3 – Chicory only (500 mg/kg)	2.18 $\pm$ 0.19 ^d	8.76 $\pm$ 0.58 ^a	9.46 $\pm$ 0.49 ^a
G4 – Acrylamide + Chicory (100 mg/kg)	4.36 $\pm$ 0.35 ^b	5.63 $\pm$ 0.42 ^c	6.58 $\pm$ 0.47 ^d
G5 – Acrylamide + Chicory (500 mg/kg)	3.12 $\pm$ 0.27 ^c	7.31 $\pm$ 0.53 ^b	8.21 $\pm$ 0.51 ^c
G6 – Acrylamide + Silymarin (100 mg/kg)	2.94 $\pm$ 0.25 ^c	7.58 $\pm$ 0.49 ^b	8.47 $\pm$ 0.46 ^{bc}

Hepatic values are expressed per volume of 10% (w/v) liver homogenate (i.e., normalized to wet tissue weight). Within a column, means sharing a superscript letter do not differ significantly (one-way ANOVA followed by Tukey's HSD test, $p \geq 0.05$). ANOVA: MDA $F(5,42) = 187.4$; GSH $F(5,42) = 94.6$; SOD $F(5,42) = 95.0$; all $p < 0.001$.

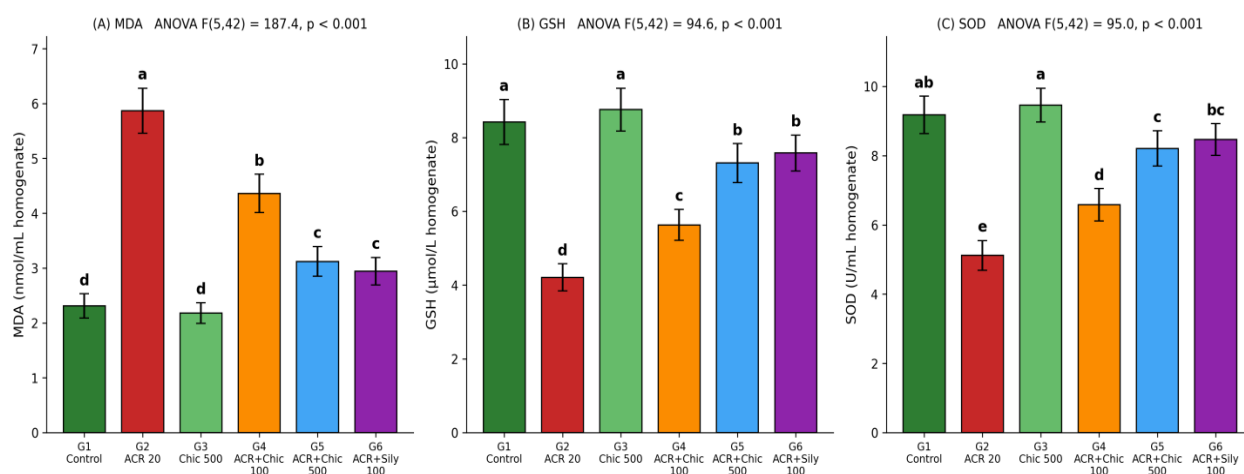


Fig. 1. Effect of chicory on hepatic (A) MDA, (B) GSH, and (C) SOD in acrylamide-intoxicated rats. Bars represent mean $\pm$ SD ($n = 8$ /group). Bars sharing a letter do not differ significantly (Tukey's HSD, $p \geq 0.05$). G1, negative control; G2, ACR 20 mg/kg; G3, chicory 500 mg/kg; G4, ACR + chicory 100 mg/kg; G5, ACR + chicory 500 mg/kg; G6, ACR + silymarin 100 mg/kg.

Effect of Chicory on serum liver enzyme activities

The analysis results presented in table (2) and figure (2) showed that there were major differences among different groups regarding ALT, with $F(5,42) = 93.2$. In addition, for AST and ALP, the indicators were $F(5,42) = 98.6$ and $F(5,42) = 75.5$, respectively, where all results were under 0.001 (Table 3). All differences among groups were significant in the case of acrylamide intoxication (G2), which showed an increase of 85% in ALT, 72% in AST, and 56% in ALP. Co-administration of chicory varied depending on the amount of chicory used: a

positive effect was noticed with both doses compared to G2 results. Moreover, the dose of 500 mg/kg provided greater results than the amount of 100 mg/kg. One negative point of the study is that AST and ALT results in G5 were significantly different from control, while ALP level was without significant differences. Moreover, there were no major differences between G5 and the control group (G6) results either. Thus, it is possible to draw the conclusion that chicory did not change the activity. In addition to this, the results obtained correspond to changes in oxidative stress, indicating that the mechanism used is connected to the redox process.

Table 2. Serum liver enzyme activities (mean $\pm$ SD, n = 8/group).

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
G1 – Negative control	42.6 $\pm$ 3.8 ^{cd}	91.4 $\pm$ 6.2 ^{de}	126.8 $\pm$ 8.5 ^{de}
G2 – Acrylamide only (20 mg/kg)	78.9 $\pm$ 5.7 ^a	157.6 $\pm$ 10.4 ^a	198.4 $\pm$ 12.6 ^a
G3 – Chicory only (500 mg/kg)	40.8 $\pm$ 3.5 ^d	88.7 $\pm$ 5.9 ^e	121.5 $\pm$ 7.9 ^e
G4 – Acrylamide + Chicory (100 mg/kg)	65.2 $\pm$ 4.9 ^b	132.8 $\pm$ 8.7 ^b	174.3 $\pm$ 10.8 ^b
G5 – Acrylamide + Chicory (500 mg/kg)	49.1 $\pm$ 4.1 ^c	104.6 $\pm$ 7.1 ^c	141.7 $\pm$ 9.2 ^c
G6 – Acrylamide + Silymarin (100 mg/kg)	47.3 $\pm$ 3.9 ^{cd}	101.8 $\pm$ 6.8 ^{cd}	138.5 $\pm$ 8.7 ^{cd}

Within a column, means sharing a superscript letter do not differ significantly (one-way ANOVA followed by Tukey's HSD test, $p \geq 0.05$). ANOVA: ALT $F(5,42) = 93.2$; AST $F(5,42) = 98.6$; ALP $F(5,42) = 75.5$; all $p < 0.001$.

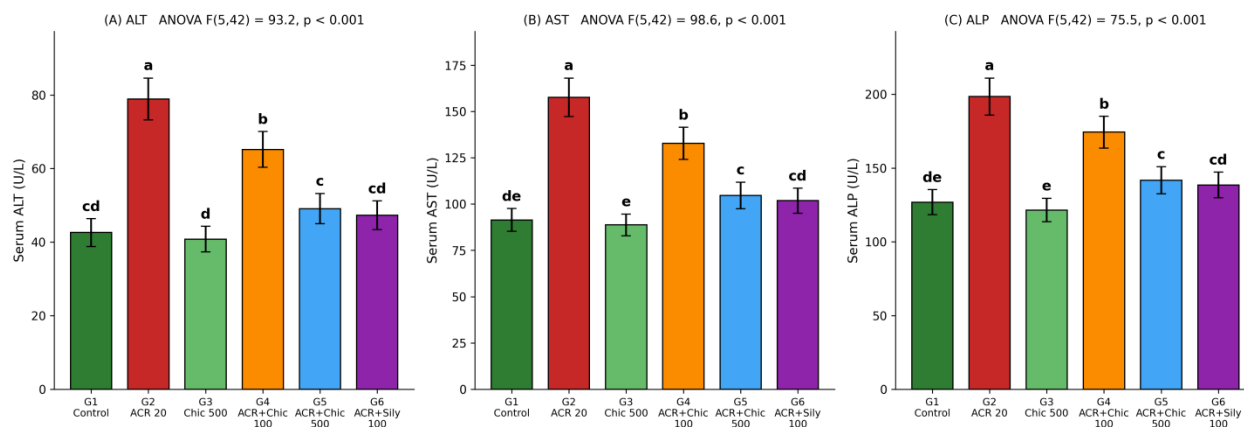


Fig. 2. Effect of chicory on serum (A) ALT, (B) AST, and (C) ALP activities in acrylamide-intoxicated rats. Bars represent mean $\pm$ SD (n = 8/group). Bars sharing a letter do not differ significantly (Tukey's HSD, $p \geq 0.05$). G1, negative control; G2, ACR 20 mg/kg; G3, chicory 500 mg/kg; G4, ACR + chicory 100 mg/kg; G5, ACR + chicory 500 mg/kg; G6, ACR + silymarin 100 mg/kg.

Table 3. Exact p values (Tukey's HSD) for the principal pairwise comparisons.

Comparison	Purpose	MDA	GSH	SOD	ALT	AST	ALP
G1 vs G2	ACR effect	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G1 vs G3	Safety of chicory alone	0.946	0.761	0.855	0.962	0.981	0.884
G2 vs G4	Low-dose protection	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G2 vs G5	High-dose protection	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G2 vs G6	Silymarin protection	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G4 vs G5	Dose dependence	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
G5 vs G6	High-dose chicory vs silymarin	0.818	0.892	0.889	0.962	0.977	0.986
G1 vs G5	Residual injury, high dose	< 0.001	0.001	0.003	0.052	0.016	0.042
G1 vs G6	Residual injury, silymarin	0.001	0.022	0.057	0.285	0.095	0.180

p < 0.05 indicates a statistically significant difference. G1, negative control; G2, ACR; G3, chicory 500 mg/kg; G4, ACR + chicory 100 mg/kg; G5, ACR + chicory 500 mg/kg; G6, ACR + silymarin 100 mg/kg.

Histopathological findings

The analysis of liver slides stained with H&E corroborated the biochemical outcomes mentioned earlier. For instance, in the group that was designated as the negative control (G1, Figure 3A), one could observe the normal structure of the liver, with normal liver cells arranged to generate a structure that contains a centrally positioned vein. The liver cells showed clearly eosinophilic cytoplasm and rounded cell nuclei. The group receiving only acrylamide (G2, Figure 3B) showed the most severe alterations, characterized by necrosis and bleeding, which is an indicator of the damage caused to liver cells. As for the group receiving chicory (G3, Figure 3C), the examination showed that normal details of structure remained intact, with only sporadic findings of multiple nuclei in the liver cells, with no signs of necrosis, inflammation, or appearance of fat in them.

Using the low dose of chicory (G4, 100 mg/kg; Figure 3D) resulted in some improvement, with mild microvesicular fatty change in hepatocytes and slight aggregation of mononuclear cells and Kupffer cells, showing that residual damage cannot be avoided at that dose. The results seen in the high dose of chicory (G5, 500 mg/kg; Figure 3E) were much better since there was

only focal mononuclear infiltration beside the central vein, mild proliferation of Kupffer cells, and normal architecture of hepatic cords. The effect of the control preparation silymarin (G6; Figure 3F) was quite similar, with extremely subtle changes, like slight dilation of sinusoids and moderate proliferation of Kupffer cells without any remarkable disorganization of hepatic cords. All parameters indicating histopathological damage of the studied groups are shown in figure (4). In the group using acrylamide alone (G2), the cumulative damage score was the highest (8/18), as a result of severe (+++) coagulative necrosis and hemorrhagic/congestive states. Low-dose chicory (G4) was effective in lowering the overall score to 4/18 due to the absence of necrosis or hemorrhage, although mild (+) inflammation and moderate (++) fatty change occurred. On the other hand, the use of a higher dose of chicory (G5) reduced the score to 2/18, showing mild (+) infiltration and proliferation of Kupffer cells, which were almost the same as the features observed in the silymarin group (G6; 3/18), showing moderate (++) proliferation of Kupffer cells and mild (+) sinusoidal dilatation as the only effects. The control (G1) and chicory-only (G3) groups showed no lesions (0/18). Overall, the severity gradient (G2 > G4 > G6 ≈ G5 > G3 = G1).

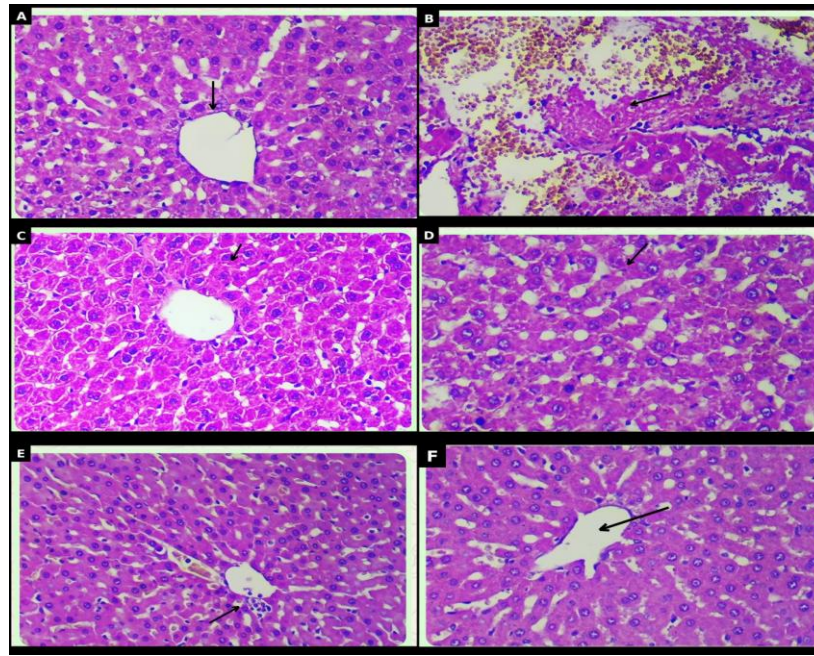


Fig. 3. Representative photomicrographs of H&E-stained rat liver sections from the six experimental groups. **(A)** G1, negative control: normal hepatic cords radiating from the central vein (arrow). **(B)** G2, ACR only: coagulative necrosis with extensive hemorrhage and red blood cell extravasation (arrow). **(C)** G3, chicory only: essentially normal parenchyma with occasional multinucleated hepatocytes (arrow). **(D)** G4, ACR + chicory 100 mg/kg: microvesicular fatty change of hepatocytes (arrow) with mild mononuclear and Kupffer-cell aggregation. **(E)** G5, ACR + chicory 500 mg/kg: focal mononuclear cell infiltration beside the central vein (arrow). **(F)** G6, ACR + silymarin 100 mg/kg: mild dilatation of the central vein and sinusoids (arrow) with Kupffer-cell proliferation. H&E, original magnification 400 $\times$.

Coagulative necrosis	—	+++	—	—	—	—
Hemorrhage / congestion	—	+++	—	—	—	—
Mononuclear cell infiltration	—	+	—	+	+	—
Microvesicular fatty change	—	—	—	++	—	—
Kupffer-cell proliferation	—	+	—	+	+	++
Sinusoidal / central vein dilatation	—	—	—	—	—	+
Cumulative lesion score (0-18)	0	8	0	4	2	3
	G1 Control	G2 ACR	G3 Chic 500	G4 ACR+Chic 100	G5 ACR+Chic 500	G6 ACR+Sily 100
— absent (0) + mild (1) ++ moderate (2) +++ severe (3)						

Fig. 4. Semiquantitative histopathological grading of hepatic lesions in the six experimental groups (n = 8 sections per group, ten fields per section at 400 $\times$). Each cell shows the predominant grade of the lesion in the group: — absent (0), + mild (1; <25% of fields), ++ moderate (2; 25–50%), +++ severe (3; >50%). The bottom row gives the cumulative lesion score (sum of the six lesion grades; range 0–18). G1, negative control; G2, ACR 20 mg/kg; G3, chicory 500 mg/kg; G4, ACR + chicory 100 mg/kg; G5, ACR + chicory 500 mg/kg; G6, ACR + silymarin 100 mg/kg.

DISCUSSION

The present findings demonstrate that acrylamide administration induced a state of marked hepatic oxidative stress, evidenced by elevated MDA and reduced GSH and SOD, accompanied by a parallel rise in serum ALT, AST, and ALP — a biochemical pattern that is highly consistent with previous reports on ACR-induced hepatotoxicity. Zhang et al. (2023) comprehensively reviewed the mechanisms of ACR hepatotoxicity and confirmed that ACR exposure disrupts the hepatic antioxidant defense system, depleting SOD, CAT, GPx, and GSH while promoting lipid peroxidation and liver enzyme leakage, mechanisms that mirror the pattern observed in the acrylamide-only group (G2) of the present study (Zhang et al., 2023).

Similarly, Karimi et al. (2020) reported that oral ACR administration (20 mg/kg) in Wistar rats as used in the present study significantly decreased hepatic GSH, SOD, GPx, and CAT activities while increasing MDA, ALT, AST, and ALP, changes that were reversed by the antioxidant ellagic acid in a dose-dependent manner (Karimi et al., 2020). Banc et al. (2022) similarly showed that ACR-intoxicated Wistar rats exhibited elevated MDA and depleted GSH together with increased AST/ALT, and that co-administration of a polyphenol-rich red wine extract restored antioxidant enzyme activity and reduced hepatic enzyme leakage, underscoring the protective potential of polyphenolic antioxidants against ACR hepatotoxicity (Banc et al., 2022).

The ameliorative effect of chicory observed in the present study, particularly at the 500 mg/kg dose (G5), is consistent with a growing body of evidence supporting the antioxidant and hepatoprotective properties of *Cichorium intybus* L. across diverse hepatotoxicity models. Krepkova et al. (2023) demonstrated that a chicory extract from the aerial parts of the plant normalized serum liver enzymes and reduced oxidative stress in rats with mercury chloride-induced acute liver injury, in addition to exerting hypolipidemic and hypoglycemic effects (Krepkova et al., 2023). Kim et al. (2021) reported that *C. intybus* root extract reduced alcohol-induced elevations in liver enzymes and

enhanced antioxidant and alcohol-metabolizing enzyme activity in rats, with efficacy comparable to silymarin used as the positive control — an experimental design parallel to that adopted in the present study (Kim et al., 2021).

More recently, Pathak et al. (2024) showed that a hydroethanolic leaf extract of *C. intybus* significantly improved catalase, SOD, GST, and GSH levels and reduced lipid peroxidation in the liver of rats exposed to the heavy metals lead and nickel, and identified cyanidin and rutin — flavonoid constituents of chicory — as showing strong molecular docking affinity for hepatoprotective protein targets, providing a plausible molecular basis for the antioxidant effects observed at the biochemical level (Pathak et al., 2024). Likewise, Maletha et al. (2022) showed that chicory leaf powder exerted hepatoprotective and nephroprotective effects against imidacloprid-induced subchronic toxicity in White Leghorn cockerels, with efficacy comparable to a silymarin-treated reference group, in agreement with the comparable outcomes of the chicory- and silymarin-treated groups in this study (Maletha et al., 2022).

Duda et al. (2024) further consolidate chicory's pharmacological profile, attributing its hepatoprotective, antioxidant, and anti-inflammatory activities to its rich content of polyphenols, sesquiterpene lactones, and inulin, which act through free-radical scavenging, upregulation of endogenous antioxidant enzyme transcription, and suppression of inflammatory mediators such as NF-κB (Duda et al., 2024). This mechanistic profile is corroborated by other reviews and experimental studies confirming that chicory's chicoric acid, chlorogenic acid, and flavonoid content underlie its multi-target antioxidant and hepatoprotective activity (Birsa and Sarbu, 2023; El-Sayed et al., 2024; Pouille et al., 2022). The interaction of these mechanisms may potentially account for the dose-dependent suppression of the alterations in MDA, GSH, and SOD, as well as the decrease in ALT, AST, and ALP due to co-treatment with chicory in the current study, and is in agreement with the hypothesis that chicory exerts its ameliorative effect against acrylamide largely by reversing oxidative stress and maintaining

hepatocellular membrane integrity. Unfortunately, the extract was not phytochemically analyzed in this study to be able to discern its principal protective constituents.

Within the limits of the present design, the high-dose chicory group (G5) did not differ significantly from the silymarin reference group (G6) for any biochemical parameter ($p = 0.818$ – 0.986), and both groups showed a similarly mild histological picture. However, the absence of a statistically significant difference does not demonstrate therapeutic equivalence, because the study was neither designed nor powered as an equivalence comparison, and several parameters in G5 remained significantly different from control values. These results therefore support a protective effect of chicory in this experimental model, but they are insufficient to propose chicory as an alternative or adjunct to standard hepatoprotective therapy; such a claim would require longer-term studies, a phytochemically standardized extract, dose–response, pharmacokinetic and safety data, and ultimately clinical evidence.

The histopathological findings of the present study reinforce and visually confirm the biochemical pattern of ACR-induced hepatotoxicity and its amelioration by chicory. The severe coagulative necrosis and hemorrhage observed in the acrylamide-only group (G2) are consistent with the histopathological damage — necrosis, hemorrhage, vascular congestion, and inflammatory exudates — reported by Erfan et al. (2021) in ACR-intoxicated rat liver, and with the hepatic alterations described by Tomaszewska et al. (2022) in weaned Wistar rats prenatally exposed to ACR, both of which attributed these changes to ACR-driven oxidative and inflammatory hepatocellular injury (Erfan et al., 2021; Tomaszewska et al., 2022). Similarly, Karimi et al. (2020) documented comparable histopathological alterations — including necrosis, congestion, and leukocyte infiltration — in ACR-treated Wistar rats at the same 20 mg/kg dose used here, which were significantly attenuated by co-treatment with the antioxidant ellagic acid, a pattern that parallels the marked histological improvement observed

with chicory co-treatment in the present study (Karimi et al., 2020).

The dose-dependent reduction in Kupffer cell proliferation, mononuclear infiltration, and fatty change observed with increasing chicory dose (G4 → G5) mirrors the histopathological amelioration reported for *C. intybus* extract in other hepatotoxicity models: Krepkova et al. (2023) documented restoration of near-normal hepatic histoarchitecture with chicory extract in mercury chloride-induced liver injury, while Pathak et al. (2024) similarly reported reduced hepatocellular degeneration and inflammatory infiltration with *C. intybus* leaf extract against lead- and nickel-induced hepatotoxicity, attributing these histological improvements to the free-radical scavenging activity of chicory's flavonoid and polyphenol constituents (Krepkova et al., 2023; Pathak et al., 2024). The histological picture in the high-dose chicory and silymarin groups is rather mild, as it consists solely of either focal inflammatory infiltration or simple sinusoidal dilation and lacks necrosis, which agrees with the biochemical result indicating that chicory 500 mg/kg does not show much difference from silymarin in this study.

LIMITATIONS OF THE STUDY

Some limitations need to be pointed out. Firstly, the study was short-term (28 days), consisting only of male rats, thus lacking consideration of sex differences and chronic low-dose dietary impact. Additionally, the source of chicory leaf extract was a private vendor, which does not allow knowing its extraction method or its yield, while a lack of independent verification and chemical standardization limits the reproducibility of the results and makes it impossible to relate the results to particular compounds. Thirdly, only two levels of chicory extract were tested, and the study was preventive in nature, utilizing pre- and co-treatment methodologies instead of applying therapy. Fourthly, the oxidative stress studies included only MDA, GSH, and SOD, while such enzymes as catalase and glutathione peroxidase, as well as indicators of Nrf2/Keap1 pathways, were not investigated. Fifthly,

histopathological assessment was carried out in a semi-quantitative way; it was just descriptive among the groups rather than being tested statistically. Lastly, the comparison with silymarin was not powerful enough for equivalence testing in the former study.

CONCLUSION

In conclusion, the administration of acrylamide caused pronounced oxidative stress in the liver, as well as leakage of liver cell enzymes and severe morphological damage, such as coagulative necrosis and hemorrhage in rats, providing support for the hypothesis regarding redox-based mechanisms of acrylamide toxicity. The concomitant use of chicory (*Cichorium intybus* L.) extract helped to diminish the above-mentioned alterations in a dose-dependent manner. The use of this plant extract at the dose of 500 mg/kg showed similar results to those of the reference drug, silymarin, with respect to several parameters that did not reach their initial levels in control animals. The administration of chicory showed no sign of toxicity at the studied doses. All in all, the current study shows that chicory can be beneficial in preventing the effects of acrylamide-induced liver damage in rats. However, before making any practical conclusions, further experiments with a standardized extract should be undertaken, involving prolonged exposure and assessment of mechanisms.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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