

Ellagic Acid's Protective Effect Against Experimental Obstructive Hepatic Encephalopathy Associated with Jaundice

Ceyda KALENDER¹

ceyda.kalender@hotmail.com

Üsküdar University

Emel SERDAROĞLU²

Üsküdar University

Research Article

Keywords: Obstructive Jaundice, Hepatic Encephalopathy, Ellagic Acid, Oxidative Stress, Heme Oxygenase-1, Neuroinflammation

Posted Date: October 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10748926/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Ellagic Acid's Protective Effect Against Experimental Obstructive Hepatic Encephalopathy Associated with Jaundice

Abstract

Background: Obstructive jaundice can progress to hepatic encephalopathy (HE) through systemic toxin accumulation, oxidative stress, and neuroinflammation.

Aims: This study investigated the neuroprotective and hepatoprotective effects of ellagic acid (EA) in a bile duct ligation (BDL)-induced experimental HE model, focusing on oxidative stress-responsive heme oxygenase-1 (HO-1) alterations.

Methods: Forty female Wistar rats were divided into Control, Sham, EA, BDL, and BDL+EA groups. EA (25 mg/kg) was administered prophylactically via intraperitoneal (i.p.) injection for 14 days. Cognitive and locomotor performances were assessed using the Novel Object Recognition (NOR) and Open Field tests (OFT). Biochemical analyses evaluated liver enzymes, bilirubin, reduced glutathione (GSH), malondialdehyde (MDA), HO-1, and interleukin-1 β (IL-1 β) levels in serum, liver, and hippocampal tissues.

Results: BDL significantly impaired behavioral performance, increased liver enzymes, MDA, HO-1, and IL-1 β levels, and depleted GSH levels in all examined tissues ($p < 0.05$). EA administration significantly improved cognitive and locomotor outcomes, restored antioxidant status, and reduced lipid peroxidation. Furthermore, EA attenuated HO-1 and IL-1 β elevations, suggesting attenuation of oxidative stress and inflammatory responses.

Conclusions: EA exerts hepatoprotective and neuroprotective effects by restoring oxidant-antioxidant balance and suppressing neuroinflammatory responses, suggesting its potential as a therapeutic approach for liver-brain axis dysfunction.

Keywords: Obstructive Jaundice, Hepatic Encephalopathy, Ellagic Acid, Oxidative Stress, Heme Oxygenase-1, Neuroinflammation

1. Introduction

Mechanical biliary system obstruction causes obstructive jaundice, which worsens hepatobiliary dysfunction and reduces bile flow. Because bile is essential for the digestion of fats and the removal of toxins, poor flow causes bilirubin and hydrophobic bile acids to accumulate throughout the body, which damages the biliary epithelium and hepatocellular cells. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) are among the blood liver enzymes that are elevated by this mechanism, which also triggers inflammatory pathways thru Kupffer cells and pro-inflammatory cytokines (Pavlidis and Pavlidis 2018).

Hepatic encephalopathy (HE), a neuropsychiatric condition marked by cognitive, motor, and behavioral deficits, is brought on by toxic metabolites that escape liver clearance and enter the central nervous system as liver function declines. Hyperammonemia, oxidative stress, neuroinflammation, and mitochondrial dysfunction are characteristics of HE. Astrocytes in the brain detoxify ammonia by producing glutamine, which causes osmotic imbalance, swelling, and edema. In the meantime, the blood-brain barrier's enhanced permeability allows neurotoxins to enter the brain (Jayakumar et al. 2015).

Obstructive jaundice and HE are largely caused by oxidative stress and mitochondrial dysfunction. High levels of reactive oxygen species cause inflammation brought on by IL-1 β and lipid peroxidation, which harm cells. Even while the Nrf2 pathway induces HO-1 in response to long-term oxidative stress, this response is insufficient when the oxidative burden on cells increases (Hirao et al. 2020; Xu et al. 2019).

Fruits like pomegranates and strawberries include a type of polyphenols called ellagic acid (EA), which has strong anti-inflammatory and antioxidant properties. EA inhibits key inflammatory pathways like NF- κ B and MAPK, boosts endogenous antioxidant defense mechanisms, and minimizes oxidative damage by lowering lipid peroxidation. Additionally, EA lowers levels of pro-inflammatory cytokines such TNF- α and IL-1 β and improves mitochondrial integrity (Mamun et al. 2024).

The purpose of this study is to determine how well EA protects against secondary HE and obstructive jaundice caused by BDL. OFT (Open Field Test) and NORT (Novel Object Recognition Test) were used to assess motor and memory function performance, respectively. To determine how EA avoids dysregulation in the liver-brain axis, oxidative damage, neuroinflammation, and HO-1 alterations were assessed.

2. Materials and methods

2.1. Animals

The experiment was authorized by the Üsküdar University Local Ethics Committee for Animal Experiments and carried out at the Experimental Research Center in compliance with the Guide for the Care and Use of Laboratory Animals (Approval date: September 5, 2025; Meeting No: 76). The study used forty female Wistar albino rats that weighed between 200 and 250 grams and were three months old. The animals had unlimited access to food and water and were kept in a controlled environment with a 12-hour light/dark cycle and a temperature of $22 \pm 2\text{ }^{\circ}\text{C}$.

2.2. Design of Experiments

The protocol outlined by Leke et al. (2012) (Leke et al. 2012) used as a guide for the experiment's design. A computer-assisted random distribution approach was used to split the animals into five experimental groups (n = 8 in each group) following a week of acclimation. The G*Power program was used to determine the sample size ($\alpha=0.05$, power=0.80, effect size=0.40), and it was estimated that 8 animals per group would provide appropriate statistical power. The experimental protocol lasted for fourteen days. Table 1 provides details about the experimental groups.

Table 1. Experimental Dosing and Group Allocation

Group	Nomenclature	Treatment Protocol
Group 1	Control (C)	Intraperitoneal (i.p.) injections of the vehicle solution comprising 0.1% DMSO + 0.9% saline were given to intact animals without any surgical or therapeutic intervention (Feng S et al. 2024).
Group 2	Sham (SHAM)	In order to create surgical stress, rats had a midline laparotomy, but the common bile duct was left unligated (Bolat I. 2025).
Group 3	Bile Duct Ligation (BDL)	To diagnose obstructive jaundice and subsequent hepatic encephalopathy (HE), bile duct ligation (BDL) was carried out. The vehicle (0.1% DMSO + 0.9% saline) was injected intraperitoneally; no therapy was delivered.
Group 4	Ellagic Acid (EA)	EA (Ambeed, Arlington Heights, IL, USA; Cat. No. A106247; CAS No. 476-66-4; purity $\geq 99.03\%$) was administered daily to intact rats at a dose of 25 mg/kg (i.p.) for 14 days in order to evaluate the baseline effects of ellagic acid (Ambrose SS et al. 2013). EA was given at a volume of 0.5 mL/kg body weight after being dissolved in 0.1% DMSO in regular saline.

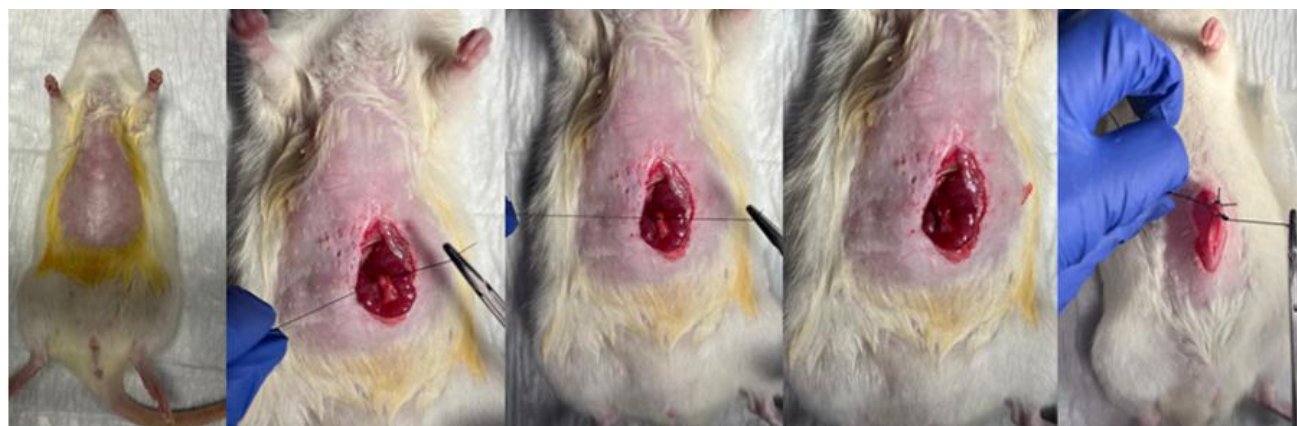
Group	Nomenclature	Treatment Protocol
Group 5	BDL + Ellagic Acid (BDL+EA)	Prophylactic EA (25 mg/kg, i.p.) was administered to rats seven days before BDL surgery and continued every day for seven days after the procedure (a total of fourteen days of treatment). EA was given at a volume of 0.5 mL/kg body weight after being dissolved in 0.1% DMSO in saline.

Notes: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile duct ligation; C, Control; DMSO, dimethyl sulfoxide; EA, ellagic acid; i.p., intraperitoneally; SHAM, sham-operated.

2.3. Surgery (Bile Duct Ligation, or BDL)

BDL produced HE-like alterations brought on by obstructive jaundice. Intramuscular injections of xylazine (5 mg/kg) and ketamine (60 mg/kg) were used to put rats to sleep. The main bile duct was accessed by a midline laparotomy after the abdominal area had been aseptically prepared. Without piercing the duct, the primary bile duct was separated and doubly ligated using 4/0 silk sutures. The abdomen was sealed in layers following this operation. With the exception of the closure of the major bile duct, the same protocol was used for animals that had sham surgery (Bolat et al. 2025) (Figure 1). Animals were administered analgesics (painkillers) for three days following surgery. Three to five milliliters of sterile isotonic saline were administered subcutaneously to prevent dehydration.

Figure 1. Induction of the obstructive jaundice model in rats: Surgical stages from exposing the common bile duct to the application of the ligature



2.4. Macroscopic and Clinical Assessment

Throughout the trial, daily observations were made of the clinical and macroscopic indicators of obstructive jaundice, including yellow skin (jaundice), alterations in coat structure, posture, spontaneous movements, and the animals' overall health (Figures 2 and 3).

Figure 2. Icteric discoloration observed in the skin, ears, and extremities of rats with induced jaundice



Figure 3. Macroscopic appearance of the abdominal region in rats with induced jaundice displaying marked yellow pigmentation in intra-abdominal tissues, bile accumulation on peritoneal surfaces, icteric discoloration of visceral organs, and deterioration of the coat structure



2.5. Test of Novel Object Recognition (NOR)

The NOR test was used to assess memory. Acclimatization, familiarization, short-term memory test, and long-term memory test were the four successive phases of the procedure. The Discrimination Index (DI) was used to measure memory in the following ways:

DI is equal to $(T_{\text{Novel}} - T_{\text{Familiar}})/(T_{\text{Novel}} + T_{\text{Familiar}})$.

In this case, "Novel" and "Familiar" stand for the time spent finding new and familiar objects, respectively. DI values range from -1, which denotes poor memory performance, to +1, which indicates a high preference for the new object (Sivakumaran et al. 2018).

2.6. Open Field Test (OFT)

The open field test (OFT) was used to measure locomotor (movement) and exploratory activities. For five minutes, each rat was put in a separate, open space measuring one by one meter. Both the average movement speed and the distance traveled were measured (Bouzbib et al. 2022).

2.7. Gathering Tissue and Blood Samples

Rats were given profound anesthesia with xylazine (8 mg/kg) and 100 mg/kg after behavioral analysis. To get serum samples, a heart puncture was used to draw blood. Commercial ELISA kits were used to measure the levels of HO-1 and IL-1 β after the hippocampus and liver tissues were extracted and homogenized to estimate GSH and MDA levels by spectroscopy.

2.8. Calculating Levels of Reduced Glutathione (GSH)

The Ellman method, in which sulfhydryl groups interact with 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) to generate a yellow chromophore, was used to quantify GSH concentrations. A spectrophotometer was used to measure the solution's absorbance at 412 nm, and GSH contents were expressed in tissue $\mu\text{mol/mg}$ (Christodoulou et al. 2022).

2.9. Calculating the Levels of Malondialdehyde (MDA)

MDA concentrations were measured using the TBARS technique to determine the extent of lipid peroxidation. MDA concentrations were expressed in tissue nmol/mg , and the colored product's absorbance was measured at 532 nm (Christodoulou et al. 2022).

2.10. HO-1 and IL-1 β Level Determination

According to manufacturer instructions, commercially available sandwich ELISA kits were used to measure the quantities of HO-1 and IL-1 β in serum, liver, and hippocampus tissue homogenates. HO-1 concentrations were measured using the Rat Heme Oxygenase-1 ELISA Kit (Bioassay Technology Laboratory; Cat. No. E0676Ra). Conversely, the Rat Interleukin-1 β ELISA Kit (Cat. No. E0119Ra) was used to quantify IL-1 β quantities. Both assays have analytical sensitivities of 0.05 ng/mL and detection ranges of 0.120 ng/mL. A microplate reader was used to measure optical densities at 450 nm.

2.11. Statistical Analysis

Researchers who were blinded to the treatment groups conducted all behavioral and metabolic assessments. GraphPad Prism 10 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analyzes. Results were presented as mean $\pm$ SEM standard error (SEM) with a significance threshold of $p < 0.05$ after a one-way analysis of variance (ANOVA) with Tukey post hoc test was carried out.

3. Results

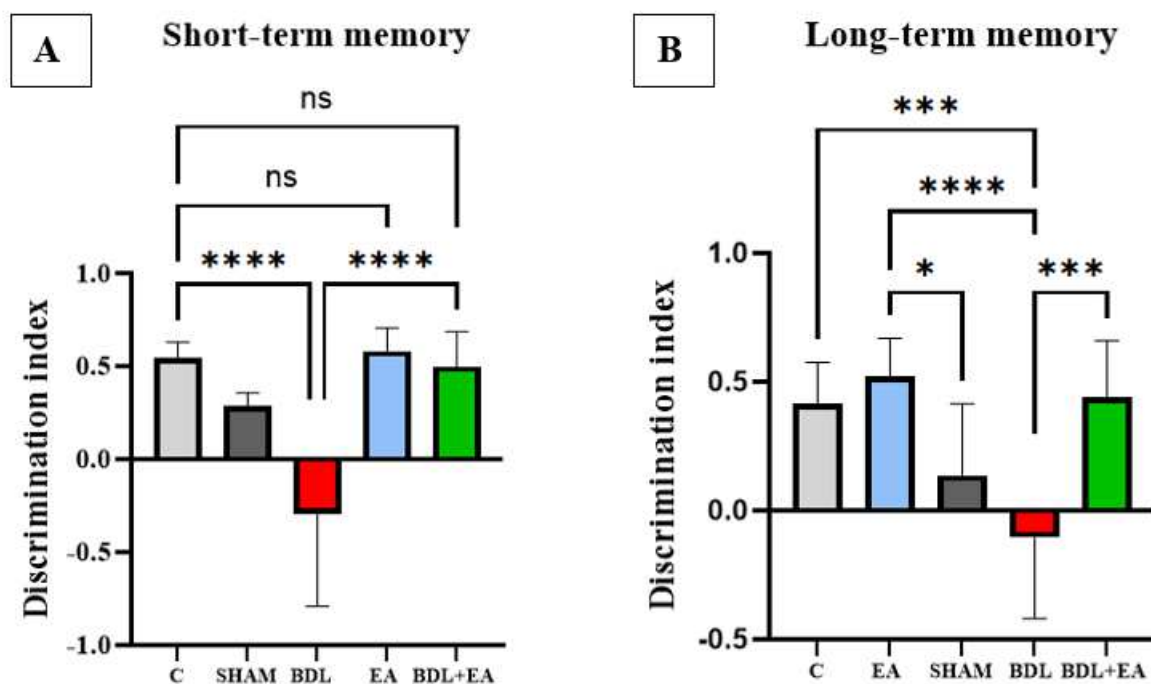
3.1. EA Reduces Cognitive Impairment Caused by BDL

The NORT exam was used to assess how BDL and EA therapy affected memory performance. The Discrimination Index (DI) in both short-term and long-term memory tests was used to evaluate memory performance.

One-way analysis of variance (ANOVA) revealed a statistically significant difference between the experimental groups in short-term memory ability ($p < 0.05$). Rats in the BDL group had significantly lower DI than rats in the Control, SHAM, and EA groups, according to post hoc analysis using the Tukey test ($p < 0.05$). DI was observed to be considerably higher in the BDL+EA group than in the BDL group (Figure 4A).

The DI values associated with the experimental groups' long-term memory ability also showed a statistically significant variance ($p < 0.05$). When compared to other groups, the BDL group's long-term memory performance DI values decreased statistically considerably ($p < 0.05$), but the EA group's DI values grew significantly (Figure 4B). All of these findings show that BDL causes significant cognitive deficits in both short-term and long-term memory function, which can be lessened with EA therapy.

Figure 4. Visual memory performance quantified via the Discrimination Index (DI) during the Novel Object Recognition Test (NORT) across experimental groups: (A) short-term memory outcomes and (B) long-term memory outcomes



3.2. EA Enhances Exploratory and Locomotor Behaviors Following BDL

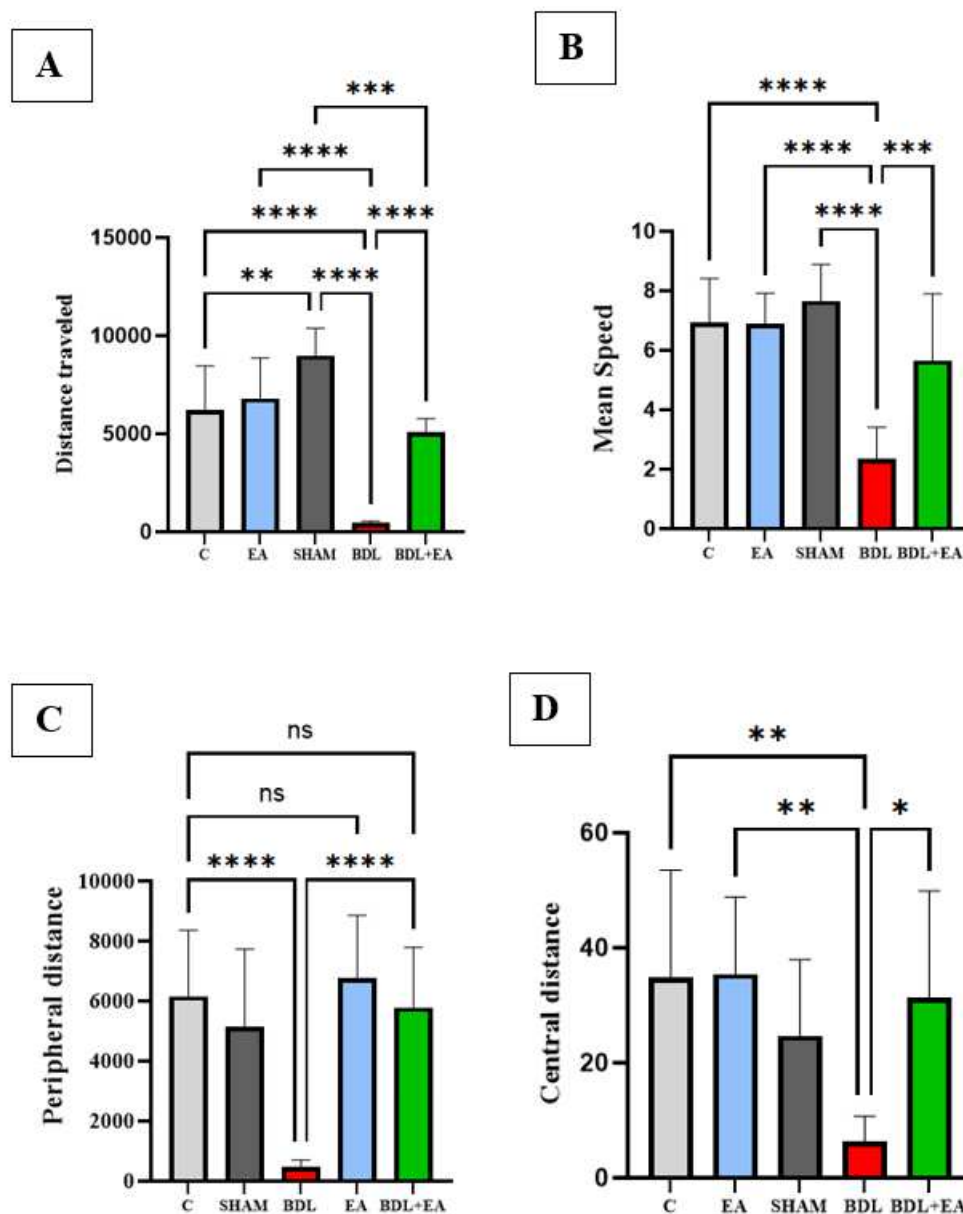
The OFT (Open Field Test) was used to evaluate locomotor and exploratory behaviors.

There was a significant difference in the overall distance walked between the experimental groups ($p < 0.05$). Rats in the BDL group exhibited significantly less locomotor behavior than those in the Control, SHAM, and EA groups ($p < 0.05$). The overall distance walked by BDL rats increased significantly after EA administration (Figure 5A). Following BDL surgery,

locomotor speed likewise considerably decreased ($p < 0.05$), but EA dramatically enhanced locomotor speed in comparison to BDL rats that were not treated (Figure 5B).

There were significant group differences in peripheral area activity ($p < 0.05$). The peripheral area's typical exploratory activity was effectively restored by EA therapy (Figure 5C). In a similar vein, there were significant group differences in activity in the center area ($p < 0.05$). Central exploration was significantly reduced in rats in the BDL group ($p < 0.05$). On the other hand, the EA group showed an increase in central activity (Figure 5D).

Figure 5. Open field test parameters measured in rats: (A) total distance traveled (total path length in cm), (B) mean speed (mean velocity in cm/s), (C) distance traveled in the peripheral zone (outer zone along the walls), and (D) distance traveled in the central zone (inner open area)



3.3. EA Reduces Systemic Biochemical Alterations Caused by BDL

However, following BDL surgery, results from blood biochemical tests showed a marked deterioration in liver and kidney function (Table 2). In this case, BDL resulted in elevated serum levels of AST, ALT, and total bilirubin ($p < 0.05$), indicating that the liver had successfully sustained cholestatic injury. Furthermore, the test revealed low serum creatinine levels and high uric acid levels ($p < 0.05$). The previously reported biochemical alterations were physiologically improved by EA treatment.

Table 2. Serum Routine Biochemical Profiles Across Experimental Groups

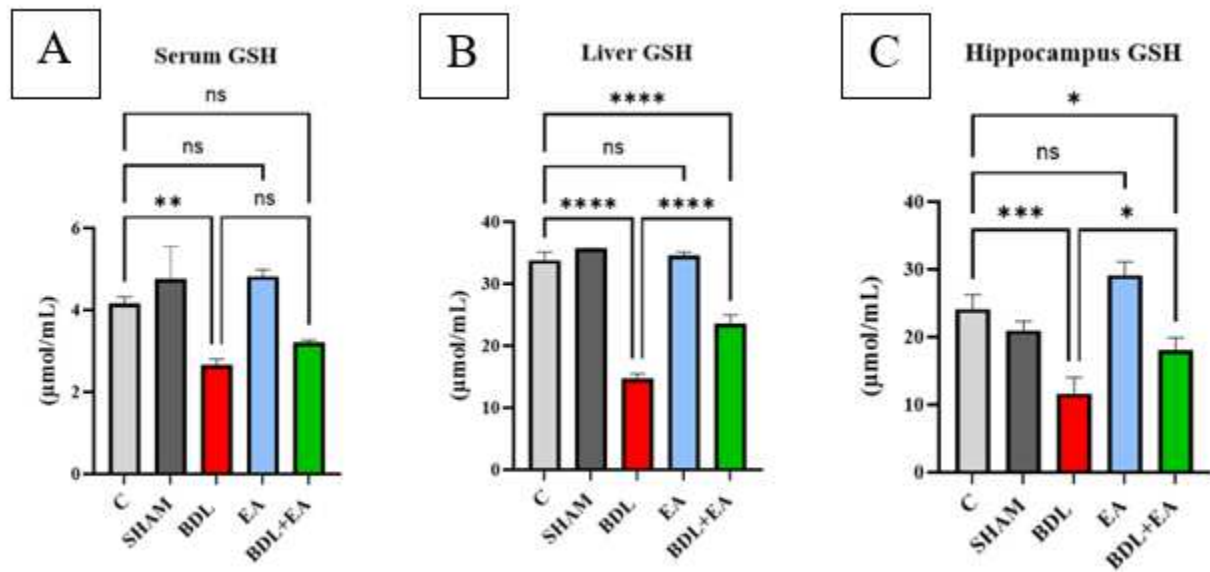
	AST (U/L) (Mean $\pm$ SEM)	ALT (U/L) (Mean $\pm$ SEM)	Bilirubin (mg/dL) (Mean $\pm$ SEM)	Creatinine (mg/dL) (Mean $\pm$ SEM)	Uric Acid (mg/dL) (Mean $\pm$ SEM)
C	151.2 $\pm$ 23.08	88.59 $\pm$ 1.56	0.195 $\pm$ 0.075	0.995 $\pm$ 0.118	0.865 $\pm$ 0.205
SHAM	151.1 $\pm$ 23.76	87.57 $\pm$ 1.54	0.192 $\pm$ 0.076	0.991 $\pm$ 0.116	0.862 $\pm$ 0.201
BDL	587.3 $\pm$ 68.46	117 $\pm$ 2.08	7.423 $\pm$ 1.154	1.888 $\pm$ 0.012	1.643 $\pm$ 0.129
EA	128.0 $\pm$ 0.58	83.33 $\pm$ 1.76	0.100 $\pm$ 0.000	0.885 $\pm$ 0.052	0.717 $\pm$ 0.044
BDL+EA	175.3 $\pm$ 33.83	57.67 $\pm$ 7.22	3.067 $\pm$ 0.949	1.000 $\pm$ 0.058	0.867 $\pm$ 0.088

Notes: Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical assessments were performed using One-Way Analysis of Variance (ANOVA) followed by Tukey's HSD post-hoc test, with Levene's test utilized to verify the homogeneity of variances. ALT, alanine aminotransferase; AST, aspartate aminotransferase; C, Control; SHAM, sham-operated; BDL, bile duct ligation; EA, ellagic acid

3.4. EA Increases GSH Levels to Restore Antioxidant Capacity

GSH levels were tested in the hippocampus, liver, and serum. When compared to the Control, SAM, and EA groups, the bile duct ligation technique caused a statistically significant drop in GSH levels in the organs ($p < 0.05$). GSH levels were higher in EA-treated mice than in BDL mice ($p < 0.05$). GSH levels, however, continued to be statistically substantially lower than those of the control group ($p < 0.05$) (Figure 6).

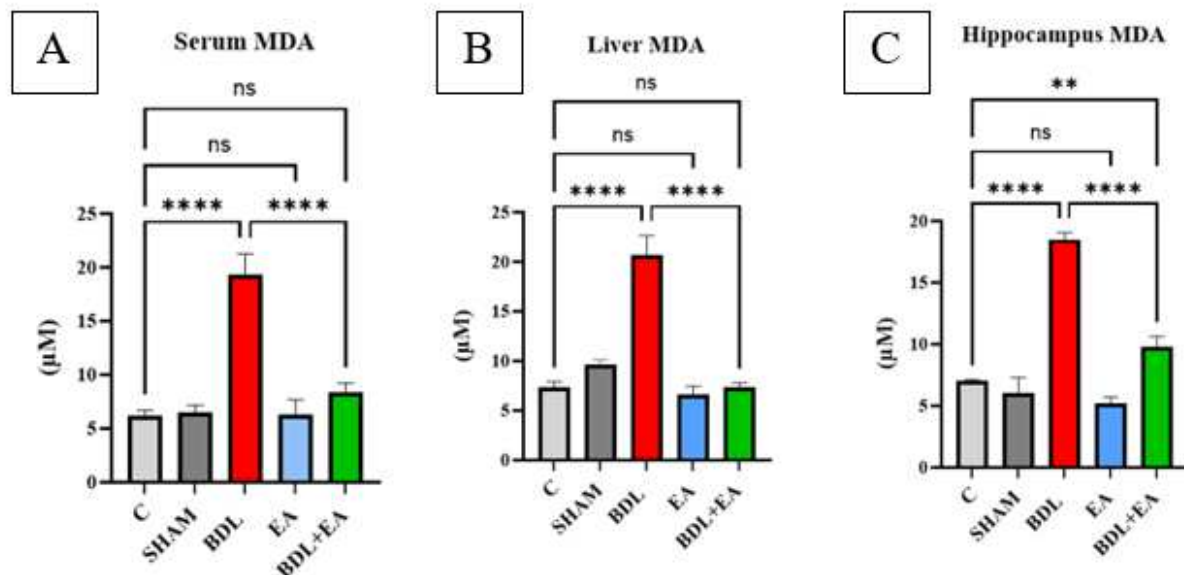
Figure 6. Effects of ellagic acid on GSH levels in (A) serum, (B) liver, and (C) hippocampus.



3.5. EA Reduces Lipid Peroxidation After BDL

The BDL group had significantly higher levels of MDA, a marker of lipid peroxidation, in their serum, liver, and hippocampus ($p < 0.05$). MDA concentration was substantially lower following EA therapy than in the control BDL group ($p < 0.05$). MDA levels, however, continued to be much greater than in the control group ($p < 0.05$) (Figure 7).

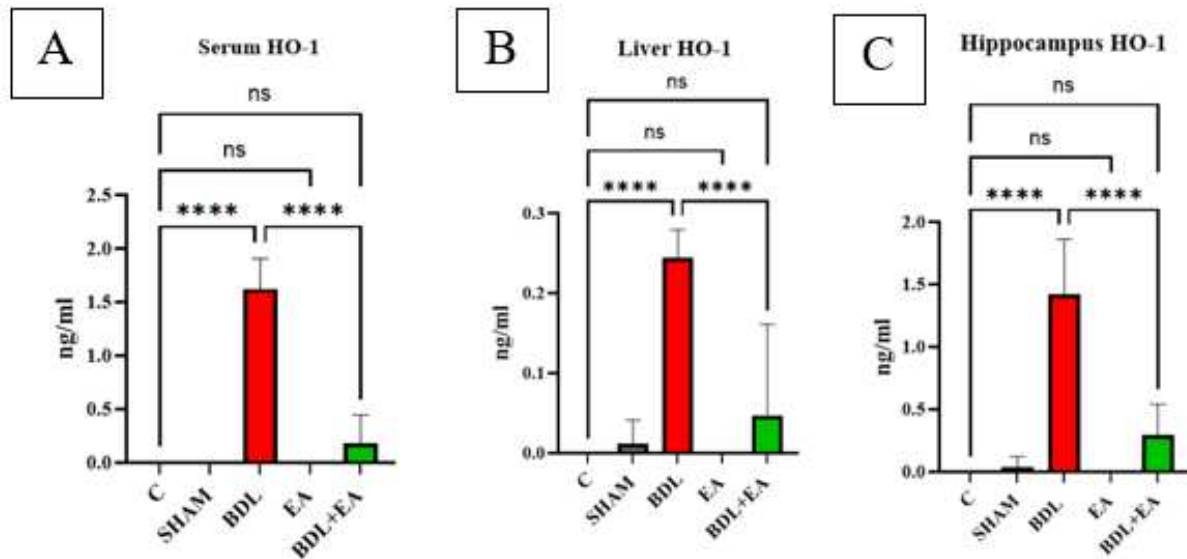
Figure 7. Effects of ellagic acid on MDA levels in (A) serum, (B) liver, and (C) hippocampus.



3.6. EA Lowers HO-1 Expression Derived from BDL

The BDL group's serum, liver, and hippocampal HO-1 levels were found to be considerably higher ($p < 0.05$); this suggests enhanced cellular resilience to oxidative stress. In comparison to the untreated BDL group, EA dramatically lowered HO-1 levels ($p < 0.05$), suggesting less oxidative stress (Figure 8). The results should not be regarded as direct modification of the Nrf2 pathway because Nrf2 levels were not assessed in this investigation.

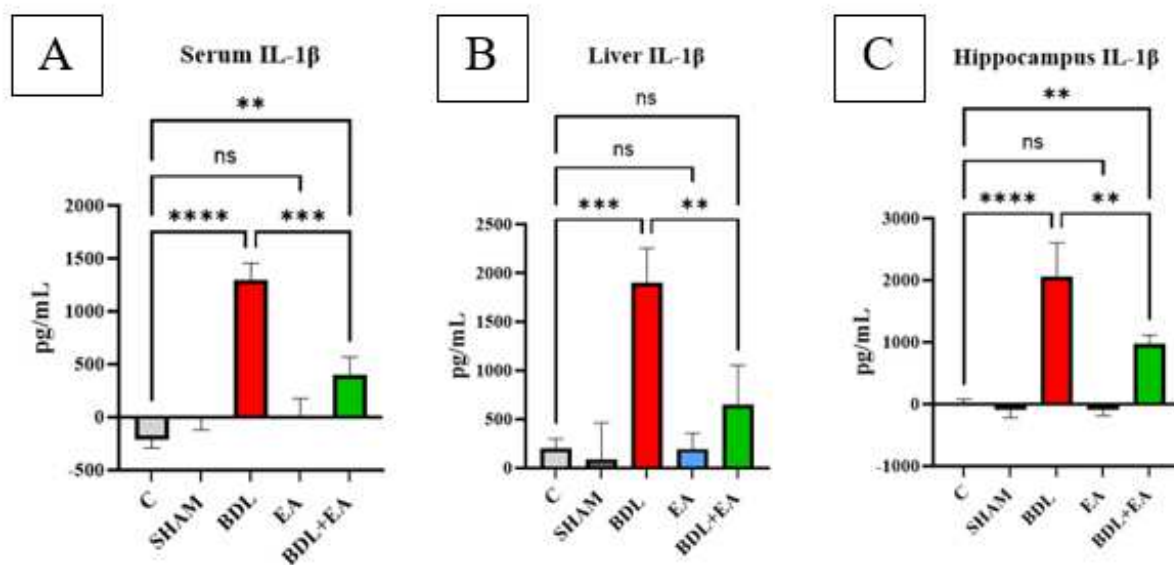
Figure 8. Effects of ellagic acid on HO-1 concentrations in (A) serum, (B) liver, and (C) hippocampus.



3.7. EA Lowers IL-1 β Levels to Reduce Inflammatory Reactions

The hippocampus, liver, and serum of rats receiving BDL surgery showed a statistically significant rise in IL-1 β levels ($p < 0.05$). In every tissue examined, mice treated with EA had statistically lower levels of IL-1 β than BDL animals not treated with EA ($p < 0.05$). In contrast to the control group, IL-1 β levels in the hippocampus continued to be considerably greater ($p < 0.05$). This indicates that the neuroinflammatory process has been somewhat suppressed (Figure 9).

Figure 9. Effects of ellagic acid on IL-1 β concentrations in (A) serum, (B) liver, and (C) hippocampus.



4. Discussion

Hepatic encephalopathy (HE) is a multifactorial neurological condition that is brought on by liver injury and dysfunction and presents with symptoms such neuropsychiatric abnormalities, motor impairments, and cognitive deficiencies. Although hyperammonemia is thought to be one of the main causes of HE, recent research has revealed that oxidative stress, neuroinflammation, mitochondrial dysfunction, and the body's lack of antioxidants are the main causes of the illness. Effective therapeutic approaches for HE are still missing, despite tremendous advancements in our knowledge of the molecular causes of HE. In order to assess the potential of EA, a model of HE was produced in rats with BDL in this investigation. The findings demonstrated that rats given BDL experienced increased oxidative stress, inflammatory reactions, liver dysfunction, decreased locomotor activity, and cognitive deficits. However, it was discovered that EA improved cognitive and locomotor abilities, decreased inflammation, raised antioxidant levels, and lessened the symptoms of rats given BDL.

One of the main clinical manifestations of HE is neurological dysfunction, which is linked to altered motor behavior, diminished attention, and poor memory formation. As seen by lower DI values in NORT and lower exploration and locomotor activity in OFT, BDL-treated rats in this study showed impaired recognition memory. These results are in line with earlier research demonstrating that cholestasis and HE are linked to behavioral impairments mediated by hyperammonemia, oxidative stress, and neuroinflammatory activation, as well as hippocampal dysfunction and decreased synaptic plasticity (Felipo 2013; Cabrera-Pastor et al. 2019; Farbood et al. 2015).

Crucially, high systemic ammonia buildup that eludes normal hepatic clearance is a result of chronic liver failure. This ongoing ammonia excess is the main cause of neuroinflammatory acceleration in the central nervous system; it directly activates microglia and causes a strong rise in pro-inflammatory cytokines, particularly IL-1 β (Drago et al. 2026). Astrocyte dysfunction, mitochondrial damage, and excessive ROS production can all be exacerbated by excessive ammonia accumulation brought on by poor liver detoxification, which can ultimately impede neural communication and cognitive functions. The hippocampus is especially vulnerable to these pathophysiological alterations because of its significant role in learning and memory. In the current investigation, BDL-induced rats' recognition memory and locomotion were considerably enhanced by EA therapy. These positive outcomes might be linked to EA's capacity to lessen inflammatory signaling and oxidative damage, protecting hippocampus function and neuronal integrity (Farbood et al. 2015; Islam et al. 2025; Jalouli et al. 2025)

BDL-induced cholestasis causes significant liver dysfunction thru bile acid buildup, decreased bile secretion, and progressive hepatocellular destruction in addition to neurological impairment. Our findings, which demonstrated notable increases in serum AST, ALT, and bilirubin levels after BDL induction, supported the establishment of liver injury and were consistent with earlier experimental investigations. Hepatocyte membrane rupture and mitochondrial injury are reflected in increased aminotransferase activities, whereas poor liver clearance and cholestatic development are indicated by elevated bilirubin levels (Widyawati et al. 2023; Zia et al. 2026). These biochemical alterations were considerably decreased with EA treatment, suggesting enhanced liver function. By lowering systemic inflammation and preventing the buildup of harmful metabolites linked to the course of HE, liver function restoration may also enhance neurological outcomes (Wang et al. 2022).

One of the primary causes of brain and liver damage during the onset of HE is oxidative stress. In our research, we found that the blood, liver, and hippocampus had significantly lower GSH levels and higher MDA concentrations, indicating

lower antioxidant reserves and more lipid peroxidation. The findings are consistent with previous research demonstrating that oxidative stress arises from mitochondrial damage in cholestasis, with elevated ROS generation and oxidative damage in both peripheral and central organs (Christodoulou et al. 2022; Simicic et al. 2022; Najibi et al. 2022)

MDA is a biomarker of oxidative stress-induced membrane damage, whereas GSH is involved in controlling intracellular redox equilibrium (Rudrapal M et al. 2022). Thus, a significant imbalance in the redox system is indicated by a drop in GSH levels and a rise in MDA accumulation (Wojtunik-Kulesza et al. 2025; Wójcik-Borowska et al. 2025)

Cellular response to oxidative stress involves the inducible antioxidant enzyme HO-1, which is often seen to be active when antioxidant pathways are engaged. Thus, depending on the existence of oxidative stress, the results of the current experiment demonstrated that protective mechanisms were triggered by the rise in HO-1 concentration in the serum, liver, and hippocampus of BDL group rats. However, rather than providing protection against oxidative stress, chronic activation of HO-1 may indicate persistent oxidative stress, mitochondrial malfunction, and metabolic abnormalities (Froh et al. 2007; Ryter et al. 2006). Following EA therapy, HO-1 levels were considerably lowered (Zhang et al. 2024; Loboda et al. 2016).

Another significant factor in the development of HE is inflammatory activation. In this investigation, BDL markedly raised IL-1 β levels in serum, liver, and hippocampus tissues, indicating systemic and central inflammatory reactions. High IL-1 β signaling: Neuronal dysfunction and cognitive impairment have been linked to microglial activation in HE experimental models (Felipo 2013). IL-1 β levels were considerably lowered by EA therapy, suggesting that inflammatory signaling pathways were suppressed. However, compared to peripheral inflammatory alterations, neuroinflammatory processes within the central nervous system may take longer periods of resolution, as evidenced by the incomplete normalization of hippocampus IL-1 β levels (Mazrooei et al. 2023).

By lowering oxidative stress and inflammation and enhancing liver function, the protective effects linked to EA in this study seem to result from the management of many pathogenic processes. EA may interfere with the pathogenic interplay between liver damage, systemic inflammation, and brain dysfunction by increasing antioxidant capacity, lowering lipid peroxidation, and regulating inflammatory responses (Weiskirchen et al. 2021). By showing the positive benefits of EA in a BDL-induced HE rat model, the current findings build on these discoveries and highlight EA's potential as a multifunctional pharmacological treatment that targets both cerebral and hepatic problems.

There are certain limitations that should be noted, even tho this study offers some significant insights into the protective function of EA against HE in the BDL animal model. Initially, the study only used one animal model for HE caused by obstructive jaundice. The model can accurately depict several pathological alterations that might lead to cholestasis and HE in the body, but it is unable to replicate the intricate, multi-step processes that underlie the actual illness. It would be advantageous to take into account additional animal models for HE, such as hyperammonemia or other liver failure models, in order to further validate the protective role of EA. The fact that the molecular mechanisms of EA's activity remain unknown despite the evaluation of oxidative stress and inflammation is another drawback of the study.

5. Conclusion

In summary, our study shows that HE production after BDL causes oxidative stress, inflammation, liver malfunction, and cognitive impairment in female rats. Utilizing EA has been shown to enhance cognitive and motor abilities, prevent liver damage by blocking AST, ALT, and bilirubin, restore redox balance by raising GSH and reducing MDA production, and lessen inflammation by downregulating (suppressing) the expression of the IL-1 β gene. According to the aforementioned results, EA regulates oxidative stress and inflammation in an animal model of BDL-induced HE, acting as a hepatoprotective (liver-protective) and neuroprotective (nervous system-protective) agent. More molecular research should be done, especially on the Nrf2/HO-1 pathway's function in HE development and how it relates to neuroprotection.

Reference

- Ambrose SS, Solairaj P, Subramoniam A (2013) Effectiveness of ellagic acid on isoniazid-rifampicin induced liver damage in rats. *J Pharmacol Pharmacother* 4:60-62. <https://doi.org/10.4103/0976-500X.107685>
- Bolat İ, Bolat M, Kiliçlioğlu M, Çelik A, Yılmaz H et al. (2025) A Study of the Effects of Boric Acid on Postoperative Testicular Adhesions Resulting from Experimental Laparotomy in Rats. *Biol Trace Elem Res* 203:3181–3194. <https://doi.org/10.1007/s12011-024-04400-4>
- Bouzbib U, Leger M, Quignon A (2022) Locomotor activity assessment protocols in rodent models. *Neurosci Biobehav Rev* 135:104592. <https://doi.org/10.1016/j.clinre.2021.101784>
- Cabrera-Pastor A, Llansola M, Montoliu C, Malaguarnera M, Balzano T et al. (2019) Peripheral inflammation induces neuroinflammation that alters neurotransmission and cognitive and motor function in hepatic encephalopathy: underlying mechanisms and therapeutic implications. *Acta Physiol* 226:e13270. <https://doi.org/10.1111/apha.13270>
- Christodoulou MC, Orellana Palacios JC, Hesami G, Jafarzadeh S, Lorenzo JM et al. (2022) Spectrophotometric Methods for Measurement of Antioxidant Activity in Food and Pharmaceuticals. *Antioxidants* 11:2213. <https://doi.org/10.3390/antiox11112213>
- Drago AA, Yanofsky S, Rohner R, Baral K, Spade L et al. (2026) Acute-on-chronic liver failure and immune dysregulation: systemic inflammation and immunoparesis paradox. *Hepatol Commun* 10:e00917. <https://doi.org/10.1097/HC9.0000000000000917>
- Farbood Y, Sarkaki A, Dianat M, Khodadadi A, Haddad MK et al. (2015) Ellagic acid prevents cognitive and hippocampal long-term potentiation deficits and brain inflammation in rat with traumatic brain injury. *Life Sci* 124:120–127. <https://doi.org/10.1016/j.lfs.2015.01.013>
- Felipo V (2013) Hepatic encephalopathy: effects of liver failure on brain function. *Nat Rev Neurosci* 14:851–858. <https://doi.org/10.1038/nrn3587>
- Feng S, Liu L, Cheng Y, Zhou M, Zhu H et al. (2024) Icaritin promotes functional recovery in rats after spinal cord injury by inhibiting YAP and regulating PPM1B ubiquitination to inhibiting the activation of reactive astrocytes. *Front Pharmacol* 15:1434652. <https://doi.org/10.3389/fphar.2024.1434652>
- Froh M, Conzelmann L, Walbrun P, Netter S, Wiest R et al. (2007) Heme oxygenase-1 overexpression increases liver injury after bile duct ligation in rats. *World J Gastroenterol* 13:3478–3486. <https://doi.org/10.3748/wjg.v13.i25.3478>
- Hirao H, Dery KJ, Kageyama S, Nakamura K, Kupiec-Weglinski JW (2020) Heme oxygenase-1 in liver transplant ischemia-reperfusion injury: from bench-to-bedside. *Free Radic Biol Med* 157:75–82. <https://doi.org/10.1016/j.freeradbiomed.2020.02.012>
- Islam MR, Rauf A, Rahaman MS, El-Imran MIH, Fakir MNH et al. (2025) Clinical and Mechanistic Exploration of Ellagic Acid in Neurodegenerative Diseases: Targeted Neuroprotection Through Cellular and Molecular Mechanisms. *Chem Biodivers* 22:e00105. <https://doi.org/10.1002/cbdv.202500105>

- Jalouli M, Rahman MA, Biswas P, Rahman H, Harrath AH et al. (2025) Targeting natural antioxidant polyphenols to protect neuroinflammation and neurodegenerative diseases: a comprehensive review. *Front Pharmacol* 16:1492517. <https://doi.org/10.3389/fphar.2025.1492517>
- Jayakumar AR, Rama Rao KV, Norenberg MD (2015) Neuroinflammation in hepatic encephalopathy: mechanistic aspects. *J Clin Exp Hepatol* 5:S21-S28. <https://doi.org/10.1016/j.jceh.2014.07.006>
- Leke R, de Oliveira DL, Mussulini BHM, Pereira MS, Kazlauskas V et al. (2012) Impairment of the Organization of Locomotor and Exploratory Behaviors in Bile Duct-Ligated Rats. *PLoS ONE* 7:e36322. <https://doi.org/10.1371/journal.pone.0036322>
- Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J (2016) Role of Nrf2/HO-1 system in development, oxidative stress response, and diseases. *Cell Mol Life Sci* 73:3221–3247. <https://doi.org/10.1007/s00018-016-2223-0>
- Mamun AA, Shao C, Geng P, Wang S, Xiao J (2024) Polyphenols targeting NF- κ B pathway in neurological disorders: what we know so far? *Int J Biol Sci* 20:1332–1355. <https://doi.org/10.7150/ijbs.90982>
- Mazrooei Z, Dehkordi HT, Shahraki MH, Lorigooini Z, Zarean E et al. (2023) Ellagic acid through attenuation of neuro-inflammatory response exerted antidepressant-like effects in socially isolated mice. *Heliyon* 9:e15550. <https://doi.org/10.1016/j.heliyon.2023.e15550>
- Najibi A, Rezaei H, Manthari RK, Niknahad H, Jamshidzadeh A et al. (2022) Cellular and mitochondrial taurine depletion in bile duct ligated rats: a justification for taurine supplementation in cholestasis/cirrhosis. *Clin Exp Hepatol* 8:195-210. <https://doi.org/10.5114/ceh.2022.119216>
- Pavlidis ET, Pavlidis TE (2018) Pathophysiological consequences of obstructive jaundice and perioperative management. *Hepatobiliary Pancreat Dis Int* 17:17-21. <https://doi.org/10.1016/j.hbpd.2018.01.008>
- Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA et al. (2022) Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. *Front Pharmacol* 13:806470. <https://doi.org/10.3389/fphar.2022.806470>
- Ryter SW, Alam J, Choi AMK (2006) Heme oxygenase-1/carbon monoxide pathway in cellular stress. *Physiol Rev* 86:583–650. <https://doi.org/10.1152/physrev.00011.2005>
- Simicic D, Cudalbu C, Pierzchala K (2022) Overview of oxidative stress findings in hepatic encephalopathy: From cellular and ammonium-based animal models to human data. *Anal Biochem* 654:114795. <https://doi.org/10.1016/j.ab.2022.114795>
- Sivakumaran MH, Mackenzie AK, Callan IR, Ainge JA, O'Connor AR (2018) Discrimination Ratio from Novel Object Recognition tasks as a measure of Recognition Memory Sensitivity rather than Bias. *Sci Rep* 8:11579. <https://doi.org/10.1038/s41598-018-30030-7>
- Wang H, Miao F, Ning D, Shan C (2022) Ellagic acid alleviates hepatic ischemia-reperfusion injury in C57 mice via the Caspase-1-GSDMD pathway. *BMC Vet Res* 18:229. <https://doi.org/10.1186/s12917-022-03326-0>
- Weiskirchen R, Weiskirchen S, Tacke F (2021) Organ crosstalk in liver cirrhosis: central and peripheral manifestations. *J Hepatol* 75:112–124. <https://doi.org/10.1016/j.jhep.2021.03.023>

Widyawati R, Yuniarti WM, Lukiswanto BS (2023) Ellagic acid from whole pomegranate fruit reduces liver injury in a rat model of hepatic cholestasis. *Open Vet J* 13:466-472. <https://doi.org/10.5455/OVJ.2023.v13.i4.8>

Wójcik-Borowska K, Wójciak W, Żuk M, Luchowski P, Skalska-Kamińska A et al. (2025) Oxidative stress protection and anti-inflammatory activity of polyphenolic fraction from *Urtica dioica*: in vitro study using human skin cells. *Molecules* 30:2515. <https://doi.org/10.3390/molecules30122515>

Wojtunik-Kulesza K, Niziński P, Krajewska A, Oniszczyk T, Combrzyński M et al. (2025) Therapeutic potential of ellagic acid in liver diseases. *Molecules* 30:2596. <https://doi.org/10.3390/molecules30122596>

Xu D, Xu M, Jeong S, Qian Y, Wu H et al. (2019) The role of Nrf2 in liver disease: novel molecular mechanisms and therapeutic approaches. *Front Pharmacol* 9:1428. <https://doi.org/10.3389/fphar.2018.01428>

Zhang X, Liu Y, Liu M, Wang X, Zhang H et al. (2024) Ellagic acid supplementation ameliorates cisplatin-induced liver injury in mice by inhibiting NF- κ B pathway and activating Nrf2/HO-1 pathway. *One Health Adv* 2:20. <https://doi.org/10.1186/s44280-024-00056-w>

Zia B, Alshehhi MHA, Sheikh A, Mirza S, Subramanya SB et al. (2026) Pharmacological Mechanisms of Phytochemicals and Pharmaceutical Agents in Protecting Against Methotrexate-Induced Liver Injury. *Oxid Med Cell Longev* 2026:9407932. <https://doi.org/10.1155/2026/9407932>

Statements and Declarations

Acknowledgements

The authors acknowledge Üsküdar University for providing the research facilities and technical support necessary for conducting this study.

Author Contributions

Conceptualization: C.K. and E.S.; Methodology: C.K. and E.S.; Formal analysis: C.K. and E.S.; Investigation: C.K.; Resources: C.K. and E.S.; Writing – original draft: C.K.; Writing – review and editing: C.K. and E.S.; Supervision: E.S. All authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding

The authors declare that no specific grant or financial support was received from any funding agency for this study.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: The study protocol was approved by the Üsküdar University Animal Experiments Local Ethics Committee (Approval No. 76, September 5, 2025).

Consent to Participate: Not applicable.

Consent for Publication: Not applicable.