RESEARCH PAPER

Cerastes vipera venom attenuates CCL4-induced liver cirrhosis through inhibition of oxidative stress and inflammatory cytokines in Rats

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ABSTRACT

Liver cirrhosis is a major health concern in Egypt due to the high prevalence of chronic viral hepatitis, driving the need for effective therapeutic alternatives. Snake venoms contain diverse bioactive molecules with antioxidant and anti-inflammatory properties, making them promising candidates for managing hepatic disorders. This study aimed to evaluate the potential protective effect of Cerastes vipera venom extract (CVE) against CCl_4-induced liver cirrhosis in rats. Adult male Wistar rats (160–200 g) were randomly assigned to four groups (n = 10): a healthy control, a healthy group treated with CVE (0.978 mg/kg/day, i.p.), a CCl_4-induced cirrhosis group receiving CCl_4 (0.5 mg/kg, i.p., twice weekly), and a cirrhotic group treated with CVE for six weeks. Serum biochemical markers, hepatic oxidative stress parameters, inflammatory cytokines, AFP, and liver histopathology were assessed to evaluate the extent of injury and the therapeutic efficacy of CVE. CCl_4 administration induced severe hepatic dysfunction, marked by significant elevations in liver enzymes, increased lipid peroxidation, depletion of GSH, and decreased SOD, CAT, and GPx activities, alongside increased inflammatory cytokines and AFP levels. CVE treatment significantly reversed these alterations by restoring total protein and albumin levels, lowering serum liver enzymes, reducing MDA concentrations, replenishing GSH, and enhancing antioxidant enzyme activities. CVE also suppressed inflammatory cytokines, normalized AFP levels, and markedly improved hepatic histoarchitecture and fibrosis scores. CVE exhibits strong antioxidant, anti-inflammatory, and hepatoprotective properties capable of mitigating CCl_4-induced chronic liver injury. These effects are likely mediated by its bioactive proteins and peptides, highlighting CVE as a promising natural candidate for further exploration in the management of toxic and fibrotic liver diseases

Keywords: Cirrhosis, CCl4, Cerastes vipera, Rat, Immunomodulation, Oxidative Stress.

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INTRODUCTION

Environmental pollutants and chemical carcinogens are major contributors to chronic liver disorders, including fibrosis and cirrhosis. The liver is extremely susceptible to toxic damage because it is the main organ for detoxification and xenobiotic metabolism. This can result in cirrhosis, fibrosis, steatosis, and hepatocellular cancer. Fibrosis represents a critical, potentially reversible stage in chronic liver disease, but without timely intervention, it often progresses to cirrhosis and cancer (Du et al. 2025). While genetic predisposition is important, modifiable factors such as diet, physical activity, body weight, alcohol intake, sleep quality, and mental health significantly influence disease risk (Hydes et al. 2024; Palupi et al. 2024). Early detection and targeted strategies, including lifestyle modification and minimizing environmental exposures, are essential to prevent progression (Yang et al. 2025). Global prevalence estimates of cirrhosis vary widely, ranging from 0.1–1% in population-based studies to 4.5–9.5% in autopsy/biopsy studies, and up to 22.7% when considering all chronic liver diseases (Han et al. 2022; Taheri et al. 2025). Alarmingly, the number of cases has surged from 988 million in 1990 to 1.7 billion in 2021, largely driven by non-alcoholic fatty liver disease (Du et al. 2025). Mortality remains substantial, with over one million deaths annually, accounting for 2–3.5% of global deaths (Abdelmageed and Güzelgül 2023; Dash et al. 2025).

Carbon tetrachloride (CCl_4) is a widely used hepatotoxin for inducing liver injury in experimental models. Its toxicity stems from reactive free radical generation, which impairs antioxidant defenses, triggers lipid peroxidation, and activates inflammatory pathways, leading to cytokine release (TNF-α, IL-1β, IL-6) (El-Kashef and Zaghloul 2022; Mitazaki et al. 2018). The interplay of oxidative stress and inflammation drives tissue damage and histological changes, including fatty degeneration, fibrosis, necrosis, cirrhosis, and carcinogenesis (Li et al. 2021). Due to its reproducibility and severity, the CCl_4-induced fibrosis model is a preferred tool for evaluating therapeutic agents such as curcumin, silymarin, and diosgenin (Abo-Zaid et al. 2020; Song and Yu 2022; Tsai et al. 2008). It enables studies on disease progression, biomarker identification (Fan et al.

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2015), and histopathology, with distinct lobular fibrosis patterns, most severe in the median and right lobes (Yorozu et al. 2004), providing insights into localized hepatic damage.

Oxidative stress and inflammatory cytokines play a significant role in both the progression and suppression of liver cirrhosis. Oxidative stress is a major contributor to liver cirrhosis, driving inflammation, fibrosis, and hepatocellular carcinoma (HCC) (Allameh et al. 2023). Excessive reactive oxygen species (ROS) disrupt redox homeostasis, leading to lipid peroxidation, mitochondrial dysfunction, and activation of inflammatory pathways (El-Sehrawy et al. 2025). These processes promote hepatocyte death, stimulate hepatic stellate cell activation, and increase extracellular matrix deposition, hallmarks of fibrosis (L. Lin et al. 2018). The interplay between oxidative stress and inflammation creates a self-perpetuating cycle that accelerates disease progression (J. Zhao et al. 2019). Emerging evidence suggests that antioxidants can counteract oxidative stress, preserve liver function, prevent apoptosis, and facilitate the clearance of damaged mitochondria, offering potential therapeutic benefits against cirrhosis (L. Lin et al. 2018; Turkseven et al. 2020). Pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-2 play a central role in liver cirrhosis by promoting inflammation, hepatocyte necrosis, and fibrogenesis (Mirodzhov and Pulatova 2023; Pratim Das and Medhi 2023). In contrast, anti-inflammatory mediators like IL-10 and adiponectin help suppress inflammatory responses and protect hepatic tissue from damage (He et al. 2021). Therefore, a comprehensive understanding of antioxidants and cytokine regulation is crucial for investigating their potential in the prevention and treatment of hepatic cirrhosis.

Snake venoms are among the most studied animal venoms and serve as a rich source for therapeutic research (El-Aziz et al. 2019). They comprise complex mixtures of proteins, peptides, and small molecules, including enzymes and non-enzymatic components, with over 42 identified protein families (Lopes Júnior et al. 2024). Metalloproteinases contribute to cytotoxic, neurotoxic, myotoxic, and hematotoxic effects. While other venoms, such as those from spiders, scorpions, and cone snails, also contain bioactive molecules like antimicrobial peptides (Yu et al. 2020), snake venoms are better characterized and have been utilized in both traditional medicine and modern drug development (Diniz-Sousa et al. 2023). Cerastes vipera, commonly known as the Saharan sand viper, belongs to the family Viperidae within the order Squamata, class Reptilia, and phylum Chordata. This small, nocturnal snake is highly adapted to desert environments and is widely distributed across Saharan regions, primarily inhabiting sandy and dune ecosystems (Mochales-Riaño et al. 2024). Snake venoms exhibit diverse pharmacological properties, including immunomodulatory (Yacoub et al. 2018), antioxidant (Karam and Mohamed 2021), and anticancer effects (Aziz et al. 2022). Several species have shown therapeutic potential in liver disease and cancer models, improving liver function, reducing oxidative stress, and lowering tumor burden. Venoms from vipers, cobras, and sea snakes have demonstrated benefits in experimental fibrosis, hepatocellular carcinoma, and ischemia-reperfusion injury, highlighting their promise as sources for novel therapeutic agents (Aziz et al. 2022; Feng-Tao et al. 2018; Karthikeyan et al. 2007; J.-Y. Kim et al. 2017; Park et al. 2011).

Liver cirrhosis remains a major global health challenge with limited effective therapies. Exploring bioactive compounds from natural sources offers a promising alternative to conventional treatments. Snake venoms, known for their diverse pharmacological properties, represent an underexplored resource in hepatoprotective research. This study aims to assess the potential of Cerastes vipera venom (CVE) as a novel anti-cirrhotic agent by evaluating its ability to mitigate carbon tetrachloride (CCl_4)-induced hepatic cirrhosis in male rats.

MATERIALS AND METHODS

Chemicals

Olive oil and carbon tetrachloride (CCl_4) were procured from Sigma-Aldrich (St. Louis, MO, USA). Following the procedure outlined by El-Fakharany et al. (2025), CCl_4 was diluted in olive oil and injected intraperitoneally twice a week at a dose of 0.5 mg/kg body weight to cause hepatic cirrhosis.

Crude Venom Collection:

Adult *Cerastes vipera* snakes were captured from their natural habitat by licensed Egyptian hunters. The procedure outlined by Willemse et al. (1979) was used to extract venom. In short, a rubberized synthetic membrane stretched over the mouth of a sterile glass beaker was used to induce the snake's fangs to bite while its head was gently restrained. The collected venom was subsequently filtered, lyophilized, and stored at 4 °C until use. A BCA Protein Assay Kit (Pierce Biotechnology, USA) was used to measure the protein concentration.

Animals

Adult male albino rats (160–200 g) have been sourced from the animal facility of the National Research Centre, Egypt. Animals were housed under controlled conditions (temperature: 25 ± 1 °C; light/dark cycle: 12/12 h) with ad libitum access to standard chow and water. A one-week acclimatization period was provided before the experiment. All procedures complied with institutional guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of the Faculty of Science, Al-Azhar University, Assiut.

Experimental Design

The effects of CCl_4 and Cerastes vipera venom (CVE) on oxidative stress, hepatic injury, liver enzyme activity, and antioxidant status have been assessed in male rats. Rats were randomly assigned into four groups (n = 10 per group): Group 1 (Control): Healthy rats without treatment. Group 2 (CVE): Healthy rats receiving intraperitoneal injections of Cerastes vipera venom (0.978 mg/kg) daily for six consecutive weeks. Group 3 (CCl_4): Rats with CCl_4-induced cirrhosis (0.5 mg/kg, intraperitoneally, twice

weekly) serving as positive controls. Group 4 (CCl_4 + CVE): Cirrhotic rats treated with CVE (0.978 mg/kg) for six weeks.

Blood and tissue sampling

Rats have been weighed and fasted for the entire night at the finale of the treatment duration (6 weeks). Sodium pentobarbital (9.1 mg/kg, diluted in sterile 0.9% NaCl, intramuscularly) was used to induce anesthesia. Sterile, heparinized glass capillaries were used to draw blood samples from the retro-orbital plexus. After centrifuging the samples at 100 RCF for 10 minutes at 4 °C, the separated sera became aliquoted and kept at -80 °C for further biochemical analyses. After blood collection, the animals had been sacrificed, and their livers were removed. Each liver was divided into two parts: one was preserved in 10% neutral buffered formalin for histopathological study, while the other was rinsed with saline, wrapped in aluminum foil, and stored at -80°C for biochemical assays.

Tissue homogenization

A portion of the liver tissue had been homogenized ultrasonically in ice-cold phosphate buffer (50 mM, pH 7.4) to prepare a 10% (w/v) homogenate. The homogenate had been centrifuged at 5000 rpm for 20 min at 4°C to remove nuclear and mitochondrial debris. The resulting supernatant was aliquoted and stored at −80 °C until biochemical analyses were performed.

Biochemical determinations

Spectrophotometric measurements were made of serum biochemical parameters. Following the manufacturer's instructions, commercially available reagent kits (DiaSys Diagnostic Systems GmbH, Germany) have been utilized to measure the activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT), as well as serum levels of albumin, total protein, and total bilirubin.

Oxidative stress markers of hepatic tissue

Rat-specific ELISA kits (SinoGeneClon Biotech Co., Ltd., Hangzhou, China) had been used to measure hepatic oxidative stress biomarkers, such as superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), nitric oxide (NO), and malondialdehyde (MDA). An ELISA microplate reader (Dynatech MR 5000, Midland, ON, Canada) was used to obtain absorbance readings, and all measurements were carried out in accordance with the manufacturer's instructions.

Cytokines and α-FP Assays

Alpha-fetoprotein (α-FP), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) have been detected in serum using rat-specific ELISA kits (SinoGeneClon Biotech Co., Ltd., Hangzhou, China). An ELISA microplate reader (Dynatech MR 5000, Midland, ON, Canada) was used to measure absorbance, and all assays were carried out in compliance with the manufacturer's guidelines.

Histopathology

To maintain the integrity of tissue structure, liver samples were promptly preserved in 10% neutral buffered formalin for a duration of 24 to 48 hours. Following fixation, the tissues underwent dehydration via a series of graded ethanol solutions, were subsequently cleared in xylene, and were then embedded in paraffin. A rotary microtome facilitated the sectioning of paraffin blocks at a thickness of 4–5 μm. For a preliminary histological examination, the sections were mounted on glass slides, deparaffinized, rehydrated, and subsequently stained with hematoxylin and eosin (H&E). The stained slides were then examined under a light microscope to assess hepatic architecture, inflammatory infiltration, cellular degeneration, fibrosis, and other pathological alterations.

Statistical analysis

Statistical analysis was performed using the statistical analysis system (SAS) software program (copyright 1998 by SAS Institute Inc., Cary, NC, USA). All data were expressed as mean ± standard error of the mean (SEM). The normality of data distribution was evaluated using the Shapiro-Wilk test. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

Serum levels of TNF-α, IL-1β, and α-fetoprotein (α-FP) have been markedly elevated in rats subjected to CCl_4 exposure relative to the control cohort, thereby suggesting robust inflammatory and hepatic injury responses. When compared to the CCl_4-only group, the concurrent administration of Cerastes vipera venom (CVE) with CCl_4 resulted in a significant reduction of these inflammatory cytokines and the tumor marker α-FP. These findings collectively demonstrate that CVE possesses anti-inflammatory and hepatoprotective characteristics in the context of CCl_4-induced liver cirrhosis (Figure 1).

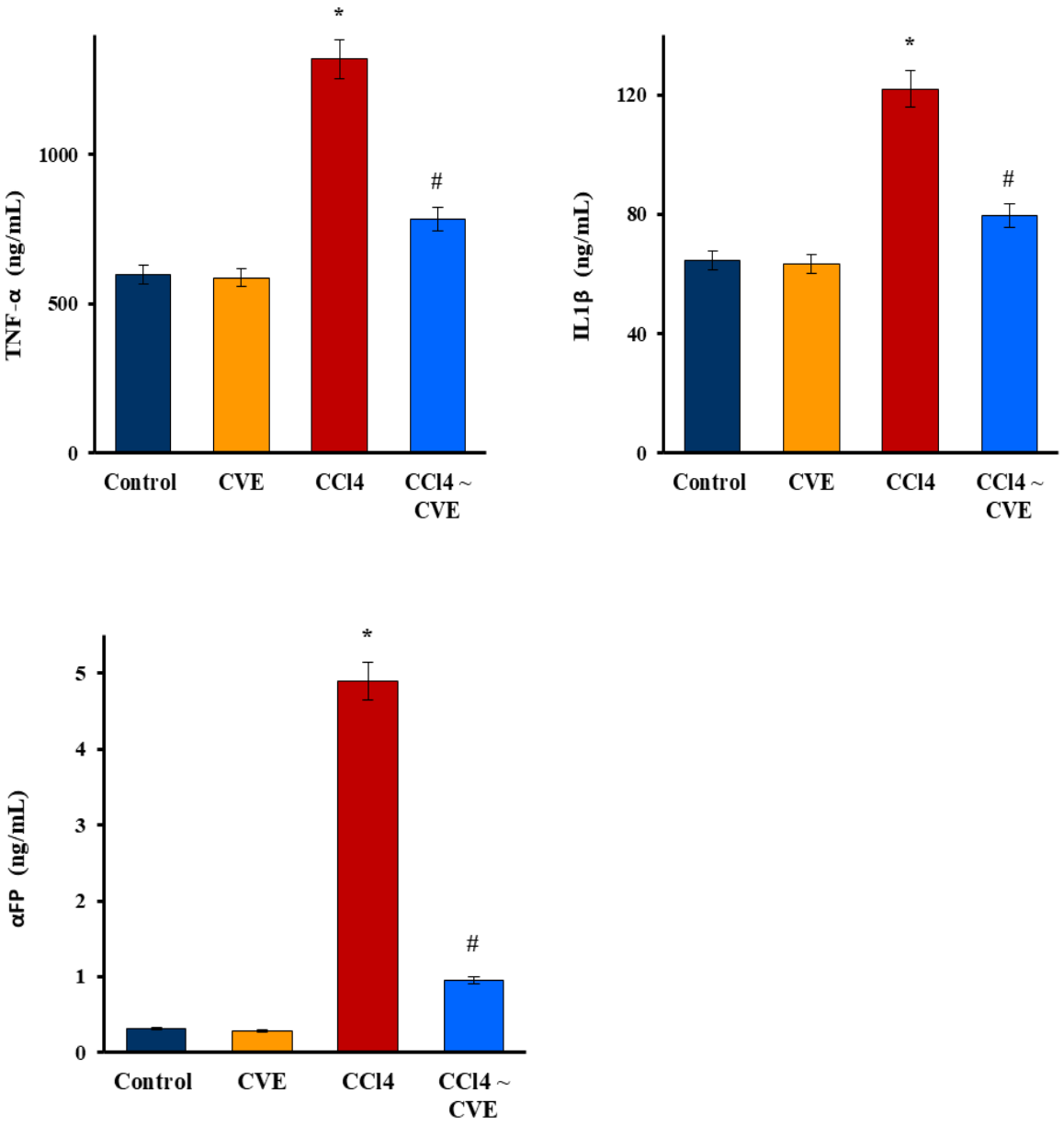

Figure 1. Serum levels of TNF-α, IL-1β, and α-fetoprotein (α-FP) in control rats, CCl_4-induced cirrhotic rats, and rats treated with CVE. Data are presented as mean ± SEM (n = 10). * p ≤ 0.05 vs. control group; # p ≤ 0.05 vs. CCl_4-induced cirrhosis group. CCl_4, carbon tetrachloride; CVE, Cerastes vipera venom extract.

When contrasted with the control group, the data in Table 1 reveal that the administration of CVE alone did not alter the serum activities of ASAT, ALAT, or ALP. Rats exposed to CCl_4, on the other hand, showed a marked increase in all four enzyme activities, indicating severe hepatic damage. The increases in these liver enzyme markers caused by CCl_4 were considerably lowered by co-treatment with CVE, indicating an obvious defense against CCl_4-mediated hepatic dysfunction. Similarly, Table 1 shows that CCl_4 administration caused a considerably lowered in serum total protein and albumin levels compared with the control group, indicating impaired hepatic synthetic function. In contrast, rats treated with CVE alongside CCl_4 exhibited a marked restoration of both total protein and albumin concentrations, approaching values observed in the normal control group. This improvement highlights the ability of CVE to counteract CCl_4-induced suppression of liver protein synthesis.

Table 1. Serum liver function markers in control, CCl_4-intoxicated, and CVE-treated male albino rats.

	Control	CVE	CCl_4	CCl_4 ~ CVE
ALAT (U/L)	41.2±2.8	41.3±5.1	89±6.31*	42.3±1.6#
ASAT (U/L)	113.8±6.6	109.3±8.9	209.2±14.6*	148.5±18.7#
ALP (U/L)	129.2±18.2	125.5±7.7	273.5±8.1*	142.4±7.2#
Albumin (g/dl)	3.29±0.09	3.31±0. 11	2.73±0.14*	3.08±0.06#
Total protein (g/dl)	5.86±0.08	5.87±0.057	4.54±0.13*	5.8±0.14#
Total bilirubin (mg/dl)	0.28±0.02	0.28±0.011	0.87±0.032*	0.28±0.024#
Direct bilirubin (mg/dl)	0.074±0.005	0.072±0.005	0.171±0.012*	0.081±0.007#

Data are presented as mean ± SEM (n = 10). * $p \leq 0.05$ vs. control group; # $p \leq 0.05$ vs. CCl_4-induced cirrhosis group. CCl_4, carbon tetrachloride; CVE, Cerastes vipera venom extract.

Table 2 demonstrates that CCl_4 intoxication resulted in a considerably increase in hepatic MDA and NO levels, accompanied by a pronounced reduction in GSH, SOD, and CAT activities compared with the control group, indicating severe oxidative stress. Notably, co-treatment with CVE considerably lowered the increases in MDA and NO caused by CCl_4 while significantly returning GSH content and SOD and CAT activities to normal levels. These results point to CVE's potent antioxidant and free-radical-scavenging capabilities in liver tissue under CCl_4 challenge.

Table 2. Hepatic levels of malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in control, CCl_4-intoxicated, and CVE-treated male albino rats.

	Control	CVE	CCl_4	CCl_4 ~ CVE
MDA (µmol/g tissue)	9.61±1.2	9.6±0.92	115.3±18.9*	34.9±2.0#
NO (µmol/g tissue)	143.3±3.05	138.08±9.1	316.72±5.22*	179.8±17.5#
GSH (nmol/g tissue)	2158.4±246.1	2486±465	667±33*	2375±260#
SOD (U/g tissue)	421.4±59.2	412.05±6.9	195.8±28.1*	399.7±85.92#
CAT (U/g tissue)	41.8±2.4	42.1±1.08	9.15±0.94*	26.9±3.0#

Data are presented as mean ± SEM (n = 10). * $p \leq 0.05$ vs. control group; # $p \leq 0.05$ vs. CCl_4-induced cirrhosis group. CCl_4, carbon tetrachloride; CVE, Cerastes vipera venom extract.

Histopathological examination:

Histopathological examination of liver sections from the control group showed normal hepatic architecture, with radiating hepatic cords, intact hepatocytes, and a clearly defined central vein (Figure 2A). The CVE-treated group had histological characteristics akin to the control animals, but there were no discernible degenerative or inflammatory alterations (Figure 2B and C). In contrast, liver tissues from CCl_4-intoxicated rats exhibited pronounced pathological alterations (Figure 2D). These included hepatocellular degeneration, ballooning of hepatocytes, inflammatory cell infiltration around the central vein, and disruption of the hepatic cords. Marked cytoplasmic vacuolation and increased nuclear abnormalities were also evident, indicating severe hepatocellular injury. Importantly, these histopathological lesions were significantly improved when CVE and CCl_4 were administered together (Figure 2E and F). In comparison to the CCl_4 group, the hepatic cords showed less inflammatory infiltration, fewer degenerative hepatocytes, and greater organization. The central vein structure was partially restored, and most hepatocytes showed nearly normal morphology. These enhancements show that CVE has a strong hepatoprotective effect against hepatic damage caused by CCl_4.

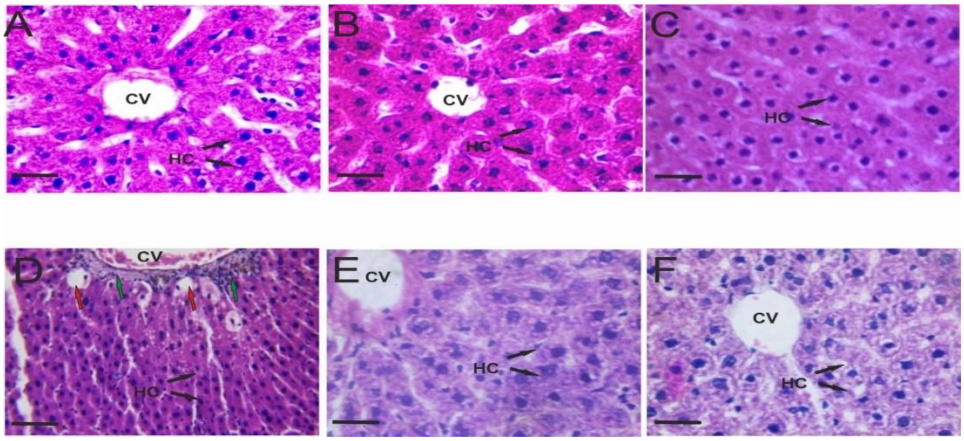

Figure 2. Histopathological examination of liver tissues in control, CVE-treated, CCl_4-intoxicated, and CVE + CCl_4-treated rats (H&E stain). (A-C) Liver sections from control and CVE-treated rats showing normal hepatic architecture with a well-defined central vein (CV) and regularly arranged hepatocytes (HC). (D) CCl_4-intoxicated rats exhibiting severe histopathological alterations, including hepatocellular degeneration, inflammatory cell infiltration, cytoplasmic vacuolation, and disruption of hepatic cords. (E–F) Liver tissues from rats co-treated with CVE and CCl_4 displayed marked improvement, with reduced degeneration and inflammation, more organized hepatic cords, and restoration of hepatocyte morphology. Scale bar =10 µm

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DISCUSSION

A common model for simulating the pathophysiology of human liver cirrhosis uses animals with hepatic cirrhosis caused by CCl_4 (Fortea et al., 2018). The findings of the current study clearly demonstrate that CVE venom therapy possesses substantial hepatoprotective, anti-inflammatory, and antioxidant activities against CCl_4-induced liver cirrhosis in rats. Serum TNF-α, IL-1β, and α-fetoprotein (α-FP) were all significantly elevated after CCl_4 administration, as was to be expected. ALT, AST, ALP, and GGT levels were also significantly elevated, indicating severe hepatocellular injury and inflammatory activation. These changes are in line with the well-established hepatotoxic mechanisms of CCl_4, which include disruption of hepatocyte membrane integrity, lipid peroxidation, and the production of free radicals. The validity of our findings is further supported by earlier research (El-Fakharany et al. 2025; El-Kashef and Zaghloul 2022; Mitazaki et al. 2018) that reported similar patterns of elevated liver enzymes and inflammatory mediators in CCl_4-intoxicated rats. CCl4-induced oxidative stress and inflammation may be the cause of increased serum enzyme levels (Ogaly et al. 2022).

The recognized mechanisms of CCl_4-induced hepatotoxicity are reflected in the disturbance of hepatic architecture in rats treated with CCl_4. Metabolic activation of CCl_4 by hepatic cytochrome P450 enzymes generates highly reactive trichloromethyl and trichloromethyl-peroxyl radicals, which initiate extensive lipid peroxidation and oxidative stress (Algefare et al. 2024; Mondal et al. 2023). Hepatocytes become structurally and functionally disrupted as a result of this oxidative burden, which destroys lipids, proteins, and DNA (Ismail et al. 2016). Major injury-related signaling pathways are also activated by CCl_4. While MAPK components (ERK, JNK, and p38) increase cellular stress and fibrogenic responses (Lee et al. 2023), NF-κB increases pro-inflammatory cytokines (Almohaimeed et al. 2023; X. Lin et al. 2022). Extracellular matrix production is further enhanced by TGF-β/Smad activation (Almohaimeed et al. 2023). Additionally, oxidative circumstances attract PI3K/Akt, which affects the results of inflammation and apoptosis (X. Lin et al. 2022). On the other hand, although it is first inhibited and then reactivated as a compensatory reaction, Nrf2 offers antioxidant defense (Lee et al. 2023). Toll-like receptors, particularly TLR4 and TLR5, enhance inflammation via the activation of NF-κB and MAPK pathways (Chen et al. 2021; Yan et al. 2019). These processes collectively contribute to the significant liver damage caused by CCl_4.

The marked reduction in pro-inflammatory cytokines and α-FP levels in rats treated with CVE demonstrates its strong anti-inflammatory and hepatoprotective effects against CCl_4-induced injury. This protection likely involves coordinated suppression of inflammatory mediators, attenuation of fibrogenic pathways, enhancement of antioxidant defenses, and improvement in hepatic function. These findings align with previous evidence showing that several snake venoms possess therapeutic potential; for instance, Agkistrodon halys venom reduced IL-1β and TNF-α in CCl_4-induced cirrhosis (Chang et al. 2005), while Crotalus helleri venom preconditioning inhibited the PLA2/COX-2 pathway in experimental brain injury (C. H. Kim et al. 2017). Additional studies report

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anti-inflammatory and immunomodulatory actions of C. cerastes venom in rheumatoid arthritis and schistosomiasis models (Mahdy et al. 2025; Soliman et al. 2023). Collectively, these results highlight the broad biological activity of snake venoms and support CVE as a promising source of bioactive molecules capable of mitigating toxic and inflammatory liver damage.

In the present study, CVE demonstrated a notable hepatoprotective effect against CCl_4-induced liver injury, as evidenced by the marked reduction in the elevated activities of AST, ALT, GGT, and ALP. In this context, the current work explored the potential of crude CVE as a candidate for mitigating cirrhosis-related liver injury. Although certain venom constituents have been proposed to modulate fibrogenic pathways and may hold promise for managing liver fibrosis or cirrhosis, such effects do not necessarily equate to direct normalization of liver enzyme markers (Al-Asmari et al. 2017; Chang et al. 2005; Ghosh et al. 2018). Nonetheless, the observed biochemical improvements in this study indicate that CVE possesses bioactive components capable of attenuating hepatocellular damage induced by CCl_4.

Exposure to CCl_4 substantially decreases serum albumin and total protein levels, indicating its severe hepatotoxic effects and the ensuing impairment of hepatic synthetic capacity (Hsu et al. 2022; Wei et al. 2022). The decrease in total protein results from oxidative stress and lipid peroxidation brought on by CCl_4, which damages hepatocytes and obstructs normal protein synthesis, upsetting cellular homeostasis (Wong et al. 2011). Similarly, because albumin synthesis is extremely sensitive to hepatocellular damage, the liver's reduced capacity to produce this vital plasma protein is responsible for the notable drop in serum albumin (Nakatsuka et al. 2007). Together, these biochemical changes show significant hepatic dysfunction and demonstrate how sensitive protein-synthesis markers are to oxidative and inflammatory liver damage. The present findings suggest that CVE may beneficially modulate serum albumin and total protein levels, indicating a potential therapeutic role in liver cirrhosis, where protein synthesis is impaired. This aligns with previous evidence demonstrating that C. cerastes venom improved hepatic function in a rat model of hepatocellular carcinoma, where treatment elevated total protein and albumin levels, enhanced antioxidant enzyme activity, and ameliorated liver histopathology (Aziz et al. 2022). In experimental models, Agkistrodon halys pallas venom preparation significantly improved liver biochemistry, reduced fibrosis indices, increased bile secretion, and restored hepatic microcirculation, key processes essential for maintaining effective protein synthesis (Chang et al. 2005). Collectively, these studies support the potential of specific snake venom components, including CVE, as promising candidates for improving liver function and counteracting the protein-synthetic deficits associated with chronic hepatic injury.

CCl_4 exposure markedly elevated MDA levels, and a marked decrease in the activities of important antioxidant enzymes, such as SOD and CAT, is indicative of this oxidative challenge (Algefare et al. 2024; Xu et al. 2021). Because its biotransformation by cytochrome P450, especially CYP2E1, produces trichloromethyl and trichloromethyl peroxy radicals that start extensive lipid peroxidation, exposure to CCl_4 significantly impairs hepatic antioxidant defenses (Al-Dalaen et al. 2016; Algefare et al. 2024). The liver's ability to neutralize reactive oxygen species is compromised by the decrease in these enzymatic activities, which exacerbates cellular damage. Reduced GSH, a key non-enzymatic antioxidant necessary for preserving redox homeostasis and assisting GPx-mediated detoxification of hydrogen peroxide, is significantly depleted concurrently with CCl_4 intoxication (Login et al. 2015). The severity of CCl_4-induced oxidative stress and its critical role in hepatocellular damage are highlighted by the combined suppression of enzymatic and non-enzymatic antioxidant components.

On the other hand, the administration of CVE venom extract significantly increased the activities of major antioxidant enzymes, such as SOD, GPx, and catalase. It restored hepatic GSH concentrations, indicating a strengthened endogenous defense system that can counteract damage caused by free radicals. The current results show that CVE has an opposing, protective effect under CCl_4-induced toxicity, as demonstrated by the significant reduction in lipid peroxidation and stabilization of hepatic cellular structure, even though some snake venoms can cause oxidative stress by increasing ROS generation and lipid peroxidation (Al-Quraishy et al. 2014; Aziz et al. 2022). This increase in antioxidants is in line with previous findings that CVE contains strong bioactive proteins and peptides that contribute to its hepatoprotective effectiveness (El-Bitar 2018). Furthermore, research on other species supports the antioxidant-modulating potential of snake venoms. For instance, in a myocardial injury model, *Agkistrodon halys* venom extract was shown to increase SOD activity and suppress oxidative biomarkers, further demonstrating the capacity of venom-derived compounds to modify redox homeostasis (Wang et al. 2019). All these results highlight CVE's ability to boost hepatic antioxidant defenses and validate its potential as a treatment option for oxidative liver damage caused by CCl_4.

The biochemical and inflammatory data are structurally confirmed by histopathological findings. Rats that were intoxicated with CCl_4 showed signs of cirrhosis, such as ballooned hepatocytes, central venous congestion, significant inflammatory infiltration, and hepatic cord disruption. These findings are consistent with previous research (Al-Quraishy 2014; Li et al. 2021; Abo-Zaid et al. 2020; Song and Yu 2022). CVE treatment significantly ameliorated these lesions, resulting in improved hepatic architecture, reduced inflammatory infiltration, and restoration of normal hepatocyte morphology. Numerous investigations have examined the impact of snake venom and its constituent elements on hepatic damage and oxidative stress (Fu et al. 2024; Lian et al. 2022). Certain components of snake venom, like phospholipase A_2 (PLA_2) inhibitors, have been shown in some studies to reduce liver damage. Varespladib, a PLA_2 inhibitor, for example, has

been demonstrated to mitigate acute liver injury caused by Naja atra by lowering oxidative stress and blocking pathways that result in ferroptosis and mitochondrial dysfunction (Liu et al. 2025; W. Zhao et al. 2024). Similarly, it has been discovered that natural inhibitors from Sinonatrix annularis prevent liver damage caused by venom by lowering apoptosis and oxidative stress (Fu et al. 2024; Lian et al. 2022). These studies may offer valuable perspectives regarding the potential protective mechanisms against carbon tetrachloride (CCl_4)-induced oxidative injury.

When considered collectively, the results of this study demonstrate how CVE can counteract important pathological processes such as oxidative stress, inflammation, hepatocellular damage, and impaired protein synthesis that are involved in CCl_4-induced liver cirrhosis. The hepatoprotective potential of CVE is strongly supported by the convergence of biochemical, molecular, and histological evidence. The current evidence indicates that the venom contains substances with potential therapeutic value against chemically induced liver injury, even though the specific bioactive components causing these effects are still unknown.

CONCLUSION

The present study provides an initial and compelling indication of the therapeutic potential of Cerastes vipera venom extract in ameliorating hepatic cirrhosis. The findings of this study demonstrate that Cerastes vipera venom extract (CVE) exerts significant hepatoprotective effects against CCl_4-induced liver cirrhosis through its potent antioxidant, anti-inflammatory, and antifibrotic activities. CVE markedly improved biochemical markers of liver function, restored hepatic antioxidant capacity, suppressed lipid peroxidation and inflammatory mediators, and enhanced histological architecture. These protective actions are likely attributed to the bioactive proteins and peptides within the extract, which collectively counteract oxidative stress and support tissue repair. Overall, CVE shows promising therapeutic potential as a natural candidate for mitigating toxic and fibrotic liver injury, warranting further investigation into its active constituents and clinical applicability.

REFERENCE

1. Abdelmageed, M., & Güzelgül, F. (2023). Copeptin: Up-to-date diagnostic and prognostic role highlight. Analytical Biochemistry, 673. https://doi.org/10.1016/j.ab.2023.115181

2. Abo-Zaid, M. A., Shaheen, E. S., & Ismail, A. H. (2020). Immunomodulatory effect of curcumin on hepatic cirrhosis in experimental rats. Journal of Food Biochemistry, 44(6). https://doi.org/10.1111/jfbc.13219

3. Al-Asmari, A. K., Osman, N. M. A., Abbasmanthiri, R., & Al-Asmari, S. A. (2017). The effect of ascorbic acid on Bitis arietans venom induced toxicity in rats. Journal of the Association of Arab Universities for Basic and Applied Sciences, 24. https://doi.org/10.1016/j.jaubas.2016.07.004

4. Al-Dalaen, S., Alzyoud, J., & Al-Qtaitat, A. (2016). The effects of L-arginine in modulating liver antioxidant biomarkers within carbon tetrachloride induced hepatotoxicity: Experimental study in rats. Biomedical and Pharmacology Journal, 9(1), 293–298. https://doi.org/10.13005/bpj/938

5. Al-Quraishy, S., Dkhil, M. A., & Abdel Moneim, A. E. (2014). Hepatotoxicity and oxidative stress induced by Naja hajecrude venom. Journal of Venomous Animals and Toxins Including Tropical Diseases, 20(1). https://doi.org/10.1186/1678-9199-20-42

6. Algefare, A. I., Alfwuaires, M., Famurewa, A. C., Elsawy, H., & Sedky, A. (2024). Geraniol prevents CCl4-induced hepatotoxicity via suppression of hepatic oxidative stress, pro-inflammation and apoptosis in rats. Toxicology Reports, 12, 128–134. https://doi.org/10.1016/j.toxrep.2024.01.007

7. Allameh, A., Niayesh-Mehr, R., Aliarab, A., Sebastiani, G., & Pantopoulos, K. (2023). Oxidative Stress in Liver Pathophysiology and Disease. Antioxidants, 12(9). https://doi.org/10.3390/antiox12091653

8. Almohaimeed, H. M., Aggad, W. S., & Assiri, R. (2023). Hepatoprotective role of emodin in chemical-induced liver injury histopathological study in mice model. Rendiconti Lincei, 34(4), 1231–1239. https://doi.org/10.1007/s12210-023-01200-1

9. Aziz, M. M., Hassan, M. K., Abbas, O. A., Mohammed, E. A., & Rahman, M. A. A. (2022). Viper snake (Cerastes cerastes) venom overcomes the hepatocellular carcinoma in experimental rats: histological evidence. Journal of Biotech Research, 13, 189–198. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85140015619&partnerID=40&md5=8f33dcdd41e11ef74725fb879994145c

10. Chang, P., Hung, D. Y., Siebert, G. A., Bridle, K., & Roberts, M. S. (2005). Therapeutic effects and possible mechanisms of a snake venom preparation in the fibrotic rat liver. Digestive Diseases and Sciences, 50(4), 745–752. https://doi.org/10.1007/s10620-005-2567-z

11. Chen, L., Ji, X., Wang, M., Liao, X., Liang, C., Tang, J., et al. (2021). Involvement of TLR4 signaling regulated-COX2/PGE2 axis in liver fibrosis induced by Schistosoma japonicum infection. Parasites and Vectors, 14(1). https://doi.org/10.1186/s13071-021-04790-7

12. Dash, D., Kesharwani, P., & Koiri, R. K. (2025). Comprehensive approach to liver cirrhosis treatment: Integrating ayurveda, homeopathy, and allopathy. Pharmacological Research - Natural Products, 6. https://doi.org/10.1016/j.prenap.2025.100184

13. Diniz-Sousa, R., Caldeira, C. A. D. S., Pereira, S.

S., Da Silva, S. L., Fernandes, P. A., Teixeira, L. M. C., et al. (2023). Therapeutic applications of snake venoms: An invaluable potential of new drug candidates. International Journal of Biological Macromolecules, 238. https://doi.org/10.1016/j.ijbiomac.2023.124357

14. Du, X., Zhang, S., Liu, S., Gao, Y., Wang, Z., Dong, N., et al. (2025). Involvement of ductal reaction in Di-(2-ethylhexyl)-phthalate-caused hepatic fibrosis: Molecular mechanisms and potential intervention strategies. Chemico-Biological Interactions, 421. https://doi.org/10.1016/j.cbi.2025.111738

15. El-Aziz, T. M. A., Soares, A. G., & Stockand, J. D. (2019). Snake venoms in drug discovery: Valuable therapeutic tools for life saving. Toxins, 11(10). https://doi.org/10.3390/toxins11100564

16. El-Bitar, A. M. H. (2018). Antiviral Activity of Egyptian Snake, Cerastes vipera Venom Against Hepatitis C Virus. Egyptian Academic Journal of Biological Sciences, G. Microbiology, 10(2), 51–64.

17. El-Fakharany, E. M., El-Sayed, M. H., Ali, H. M., Omar, S. M., Sayed, A. M., Atwa, A., et al. (2025). Ameliorative efficacy of Syzygium aromaticum ethanolic extract and Silymarin® upon carbon tetrachloride-induced liver fibrosis: antioxidant, antibacterial and anti-inflammatory activities. Medical Oncology, 43(1), 37. https://doi.org/10.1007/s12032-025-03100-w

18. El-Kashef, D. H., & Zaghloul, R. A. (2022). Ameliorative effect of montelukast against carbon tetrachloride-induced hepatotoxicity: Targeting NLRP3 inflammasome pathway. Life Sciences, 304. https://doi.org/10.1016/j.lfs.2022.120707

19. El-Sehrawy, A. A. M. A., Mohammed, A. N., Gupta, J., Mohammed, J. S., Roopashree, R., Kashyap, A., et al. (2025). Combating oxidative stress in non-alcoholic fatty liver disease: From mechanisms to therapeutic strategies. Pathology, research and practice, 272, 156053. https://doi.org/10.1016/j.prp.2025.156053

20. Fan, H.-N., Chen, N.-W., Shen, W.-L., Zhao, X.-Y., & Zhang, J. (2015). Endogenous hydrogen sulfide is associated with angiotensin II type 1 receptor in a rat model of carbon tetrachloride-induced hepatic fibrosis. Molecular Medicine Reports, 12(3), 3351–3358. https://doi.org/10.3892/mmr.2015.3873

21. Feng-Tao, C., Xue-Rong, Z., Lin, S., Xiu-Nan, W., Ming, L., Jian-Dong, B., et al. (2018). Effect of cobra venom NGF on inducing apoptosis of LX2 cells by inhibiting Akt signaling pathway and its mechanism. Chinese Pharmacological Bulletin, 34(1), 23–27. https://doi.org/10.3969/j.issn.1001-1978.2018.01.007

22. Fu, K., Zhao, J., Zhong, L., Xu, H., Yu, X., Bi, X., & Huang, C. (2024). Dual therapy with phospholipase and metalloproteinase inhibitors from Sinonatrix annularis alleviated acute kidney and liver injury caused by multiple snake venoms. Biomedicine and Pharmacotherapy, 177. https://doi.org/10.1016/j.biopha.2024.116967

23. Ghosh, R., Mana, K., & Sarkhel, S. (2018). Ameliorating effect of Alstonia scholaris L. bark extract on histopathological changes following viper envenomation in animal models. Toxicology Reports, 5. https://doi.org/10.1016/j.toxrep.2018.10.004

24. Han, X., Wang, J., Gu, H., Liao, X., & Jiang, M. (2022). Predictive value of liver cirrhosis using metabolite biomarkers of bile acid in the blood. Medicine (United States), 101. https://doi.org/10.1097/MD.0000000000028529

25. He, Y., Hwang, S., Ahmed, Y. A., Feng, D., Li, N., Ribeiro, M., et al. (2021). Immunopathobiology and therapeutic targets related to cytokines in liver diseases. Cellular and Molecular Immunology, 18(1), 18–37. https://doi.org/10.1038/s41423-020-00580-w

26. Hsu, Y.-W., Chen, Y.-Y., & Tsai, C.-F. (2022). Protective effects of chlorogenic acid against carbon tetrachloride-induced hepatotoxicity in mice. Processes, 10(1). https://doi.org/10.3390/pr10010031

27. Hydes, T. J., Kennedy, O. J., Glyn-Owen, K., Buchanan, R., Parkes, J., Cuthbertson, D. J., et al. (2024). Liver Fibrosis Assessed Via Noninvasive Tests Is Associated With Incident Heart Failure in a General Population Cohort. Clinical Gastroenterology and Hepatology, 22. https://doi.org/10.1016/j.cgh.2024.03.045

28. Ismail, A. F. M., Salem, A. A. M., & Eassawy, M. M. T. (2016). Hepatoprotective effect of grape seed oil against carbon tetrachloride induced oxidative stress in liver of γ-irradiated rat. Journal of Photochemistry and Photobiology B: Biology, 160, 1–10. https://doi.org/10.1016/j.jphotobiol.2016.03.027

29. Karam, H., & Mohamed, M. (2021). Beneficial effect of low dose gamma irradiation or quercetin on Cerastes cerastes snake venom induced toxicity in male rats. Toxin Reviews, 40(1), 35–47. https://doi.org/10.1080/15569543.2019.1580746

30. Karthikeyan, R., Karthigayan, S., Sri Balasubashini, M., Vijayalakshmi, S., Somasundaram, S. T., & Balasubramanian, T. (2007). Antitumor effect of snake venom (Hydrophis spiralis) on Ehrlich Ascites Carcinoma bearing mice. International Journal of Cancer Research, 3(4), 167–173. https://doi.org/10.3923/ijcr.2007.167.173

31. Kim, C. H., McBride, D. W., Sherchan, P., Person, C. E., Gren, E. C. K., Kelln, W., et al. (2017). Crotalus helleri venom preconditioning reduces postoperative cerebral edema and improves neurological outcomes after surgical brain injury. Neurobiology of Disease, 107. https://doi.org/10.1016/j.nbd.2017.03.001

32. Kim, J.-Y., An, H.-J., Kim, W.-H., Park, Y.-Y., Park, K. D., & Park, K.-K. (2017). Apamin suppresses biliary fibrosis and activation of hepatic stellate cells. International Journal of Molecular Medicine, 39(5), 1188–1194. https://doi.org/10.3892/ijmm.2017.2922

33. Lee, C., Yoon, S., & Moon, J.-O. (2023). Kaempferol Suppresses Carbon Tetrachloride-Induced Liver Damage in Rats via the MAPKs/NF-κB and AMPK/Nrf2 Signaling Pathways. International Journal of Molecular Sciences, 24(8). https://doi.org/10.3390/ijms24086900

34. Li, X., Liu, X., Zhang, Y., Cheng, C., Fan, J., Zhou, J., et al. (2021). Hepatoprotective effect of apolipoprotein A4 against carbon tetrachloride induced acute liver injury through mediating hepatic antioxidant and inflammation response in mice. Biochemical and Biophysical Research Communications, 534. https://doi.org/10.1016/j.bbrc.2020.11.024

35. Lian, Q., Zhong, L., Fu, K., Ji, Y., Zhang, X., Liu, C., & Huang, C. (2022). Hepatic inhibitors expression profiling of venom-challenged Sinonatrix annularis and antidotal activities. Biomedicine and Pharmacotherapy, 156. https://doi.org/10.1016/j.biopha.2022.113900

36. Lin, L., Zhou, F., Shen, S., & Zhang, T. (2018). Fighting Liver Fibrosis with Naturally Occurring Antioxidants. Planta Medica, 84(18), 1318–1333. https://doi.org/10.1055/a-0757-0008

37. Lin, X., Wei, Y., Li, Y., Xiong, Y., Fang, B., Li, C., et al. (2022). Tormentic Acid Ameliorates Hepatic Fibrosis in vivo by Inhibiting Glycerophospholipids Metabolism and PI3K/Akt/mTOR and NF-κB Pathways: Based on Transcriptomics and Metabolomics. Frontiers in Pharmacology, 13. https://doi.org/10.3389/fphar.2022.801982

38. Liu, J., Wang, L., Xie, M., Zhao, W., Sun, J., Jin, Y., et al. (2025). Varespladib attenuates Naja atra-induced acute liver injury via reversing Nrf2 signaling-mediated ferroptosis and mitochondrial dysfunction. Redox Report, 30(1). https://doi.org/10.1080/13510002.2025.2507557

39. Login, C. C., Nagy, A.-L., Mureșan, A., Moldovan, R., Decea, N., Daicoviciu, D., & Clichici, S. (2015). Antioxidant and hepatoprotective effect of chitosan versus vitamin E in experimental carbon tetrachloride-induced liver injuries. Studia Universitatis Babes-Bolyai Chemia, 60(2), 389–397. https://www.scopus.com/inward/record.uri?eid=2-s2.0-84938310401&partnerID=40&md5=5aed77f258a995557d3d1877ea703830

40. Lopes Júnior, C. A., Mendes, M. K. de A., Sousa, M. da S., Vieira, E. C., Andrade, T. de A., & de Jesus, J. R. (2024). Chapter Seventeen - Exploring metalloproteins found in the secretion of venomous species: Biological role and therapeutical applications. In R. B. T.-A. in P. C. and S. B. Donev (Ed.), Metalloproteins and Motor Proteins (Vol. 141, pp. 539–562). Academic Press. https://doi.org/https://doi.org/10.1016/bs.apcsb.2024.03.002

41. Mahdy, A., Mostafa, O. M. S., Aboueldahab, M. M., & Nigm, A. H. (2025). Antiparasitic activity of Cerastes cerastes venom on Schistosoma mansoni infected mice. Experimental Parasitology, 268. https://doi.org/10.1016/j.exppara.2024.108866

42. Mirodzhov, G. K., & Pulatova, S. D. (2023). Cytokines in Liver Cirrhosis (Their Importance in Assessing Activity and Decompensation). Russian Journal of Gastroenterology, Hepatology, Coloproctology, 33(4), 24–29. https://doi.org/10.22416/1382-4376-2023-33-4-24-29

43. Mitazaki, S., Kotajima, N., Matsuda, S., Ida, N., Iide, M., Honma, S., et al. (2018). Dimethylthiourea ameliorates carbon tetrachloride-induced acute liver injury in ovariectomized mice. Biomedicine & Pharmacotherapy, 104. https://doi.org/10.1016/j.biopha.2018.05.065

44. Mochales-Riaño, G., Burriel-Carranza, B., Barros, M. I., Velo-Antón, G., Talavera, A., Spilani, L., et al. (2024). Hidden in the sand: Phylogenomics unravel an unexpected evolutionary history for the desert-adapted vipers of the genus Cerastes. Molecular Phylogenetics and Evolution, 191. https://doi.org/10.1016/j.ympev.2023.107979

45. Mondal, M., Bala, J., Mondal, K. R., Afrin, S., Saha, P., Saha, M., et al. (2023). The protective effects of nerol to prevent the toxicity of carbon tetrachloride to the liver in Sprague-Dawley rats. Heliyon, 9(12). https://doi.org/10.1016/j.heliyon.2023.e23065

46. Nakatsuka, R., Taniguchi, M., Hirata, M., Shiota, G., & Sato, K. (2007). Transient expression of bone morphogenic protein-2 in acute liver injury by carbon tetrachloride. Journal of Biochemistry, 141(1), 113–119. https://doi.org/10.1093/jb/mvm012

47. Ogaly, H. A., Aldulmani, S. A. A., Al-Zahrani, F. A. M., & Abd-Elsalam, R. M. (2022). D-Carvone attenuates CCl4-induced liver fibrosis in rats by inhibiting oxidative stress and TGF-ß 1/SMAD3 signaling pathway. Biology, 11(5), 739.

48. Palupi, P. D., Wei, C.-Y., Chou, W.-H., Lin, M.-R., Wan, Y.-J. Y., & Chang, W.-C. (2024). Dietary contributions in the genetic variation of liver fibrosis: a genome-wide association study of fibrosis-4 index in the liver fibrosis development. Cell and Bioscience, 14. https://doi.org/10.1186/s13578-024-01321-6

49. Park, J.-H., Kum, Y.-S., Lee, T.-I., Kim, S.-J., Lee, W.-R., Kim, B.-I., et al. (2011). Melittin attenuates liver injury in thioacetamide-treated mice through modulating inflammation and fibrogenesis. Experimental Biology and

Medicine, 236(11), 1306–1313. https://doi.org/10.1258/ebm.2011.011127

50. Pratim Das, P., & Medhi, S. (2023). Role of inflammasomes and cytokines in immune dysfunction of liver cirrhosis. Cytokine, 170. https://doi.org/10.1016/j.cyto.2023.156347

51. Soliman, N. S., Kandeil, M. A., & Khalaf, M. M. (2023). "Cerastes snake venom as a promising approach in the management of complete Freund's adjuvant-induced rheumatoid arthritis in rats: Involvement of RANKL and JAK/STAT pathway." Journal of Ethnopharmacology, 314. https://doi.org/10.1016/j.jep.2023.116577

52. Song, W.-S., & Yu, Y. (2022). Diosgenin Reduces Liver Fibrosis Induced by Carbon Tetrachloride and its Mechanism. Chinese Journal of Pharmaceutical Biotechnology, 29(3), 261–266. https://doi.org/10.19526/j.cnki.1005-8915.20220308

53. Taheri, M., Dargah, A. H., Ramezani, P., Anafje, M., Nasrollahizadeh, A., Ebrahimi, P., & Mandegar, M. H. (2025). Immediate management of a cirrhosis-induced severe pericardial effusion: a case report and review of the literature. Journal of Medical Case Reports, 19. https://doi.org/10.1186/s13256-024-05016-x

54. Tsai, J. H., Liu, J. Y., Wu, T. T., Ho, P. C., Huang, C. Y., Shyu, J. C., et al. (2008). Effects of silymarin on the resolution of liver fibrosis induced by carbon tetrachloride in rats. Journal of Viral Hepatitis, 15(7), 508–514. https://doi.org/10.1111/j.1365-2893.2008.00971.x

55. Turkseven, S., Bolognesi, M., Brocca, A., Pesce, P., Angeli, P., & Di Pascoli, M. (2020). Mitochondria-targeted antioxidant mitoquinone attenuates liver inflammation and fibrosis in cirrhotic rats. American Journal of Physiology - Gastrointestinal and Liver Physiology, 318(2), G298–G304. https://doi.org/10.1152/AJPGI.00135.2019

56. Wang, Q.-H., Li, W., Jiang, Y.-X., Lu, X.-H., & Wang, G.-G. (2019). The extract from Agkistrodon halys venom protects against lipopolysaccharide (LPS)-induced myocardial injury. BMC Complementary and Alternative Medicine, 19(1). https://doi.org/10.1186/s12906-019-2595-4

57. Wei, Y., Ma, D., Fan, Y., Gao, C., Wang, Q., Yuan, Y., et al. (2022). Environmental carbon tetrachloride exposure disrupts the liver structure and metabolic detoxification function in mice via p38MAPK/NF-κB/NLRP3 pathway. Food and Agricultural Immunology, 33(1), 235–251. https://doi.org/10.1080/09540105.2022.2060192

58. Willemse, G. T., Hattingh, J., Karlsson, R. M., Levy, S., & Parker, C. (1979). Changes in composition and protein concentration of puff adder (Bitis arietans) venom due to frequent milking. Toxicon, 17(1), 37–42.

59. Wong, L. L. Y., Fan, S. T., Man, K., Sit, W.-H., Jiang, P. P., Jor, I. W.-Y., et al. (2011). Identification of liver proteins and their roles associated with carbon tetrachloride-induced hepatotoxicity. Human and Experimental Toxicology, 30(9), 1369–1381. https://doi.org/10.1177/0960327110391388

60. Xu, W., Wang, P., Wang, D., Liu, K., Zhang, S., Zhao, W., & Liu, G. (2021). S-ketamine alleviates carbon tetrachloride-induced hepatic injury and oxidative stress by targeting the nrf2/ho-1 signaling pathway. Canadian Journal of Physiology and Pharmacology, 99(12), 1308–1315. https://doi.org/10.1139/cjpp-2020-0763

61. Yacoub, T., Rima, M., Sadek, R., Hleihel, W., Fajloun, Z., & Karam, M. (2018). Montivipera bornmuelleri venom has immunomodulatory effects mainly up-regulating pro-inflammatory cytokines in the spleens of mice. Toxicology Reports, 5, 318–323. https://doi.org/10.1016/j.toxrep.2018.02.011

62. Yan, J., Shen, S., He, Y., & Li, Z. (2019). TLR5 silencing reduced hyperammonaemia-induced liver injury by inhibiting oxidative stress and inflammation responses via inactivating NF-κB and MAPK signals. Chemico-Biological Interactions, 299, 102–110. https://doi.org/10.1016/j.cbi.2018.11.026

63. Yang, H., Atak, D., Yuan, M., Li, M., Altay, O., Demirtas, E., et al. (2025). Integrative proteo-transcriptomic characterization of advanced fibrosis in chronic liver disease across etiologies. Cell Reports Medicine, 6. https://doi.org/10.1016/j.xcrm.2025.101935

64. Yorozu, K., Fujii, E., Teruya, S., Ogawa, Y., Ito, H., Suzuki, M., & Sugimoto, T. (2004). Lobular difference in fibrotic changes in rat cirrhosis model induced by carbon tetrachloride. Journal of Toxicologic Pathology, 17(4), 267–274. https://doi.org/10.1293/tox.17.267

65. Yu, C., Yu, H., & Li, P. (2020). Highlights of animal venom research on the geographical variations of toxin components, toxicities and envenomation therapy. International Journal of Biological Macromolecules, 165, 2994–3006. https://doi.org/10.1016/j.ijbiomac.2020.10.190

66. Zhao, J., Qi, Y., & Yu, Y. (2019). Research advances in the role of oxidative stress in the development and progression of liver fibrosis. Journal of Clinical Hepatology, 35(9), 2067–2071. https://doi.org/10.3969/j.issn.1001-5256.2019.09.040

67. Zhao, W., Liu, J., Wang, S., Tao, Q., Lei, Q., & Huang, C. (2024). Varespladib mitigates acute liver injury via suppression of excessive mitophagy on Naja atra envenomed mice by inhibiting PLA2 . Toxicon, 242. https://doi.org/10.1016/j.toxicon.2024.107694..