Original Article

Pre-clinical evaluation of *Fagonia cretica* L. extract against CCl_4-induced hepatotoxicity in male Wistar Rats

Fatima Arshad[1], Awais Altaf[1*], Ali Raza Arshad[2], Tahir Maqbool[1], Muhammad Abdul Basit Ashraf[3], Afsheen Akbar[4]

ABSTRACT

OBJECTIVE: To investigate the hepatoprotective effects of *Fagonia cretica* L. (*F. cretica*) extracts against CCl_4-induced hepatotoxicity in rats.

METHODOLOGY: The study evaluated the hepatoprotective effects of ethanol and hexane extracts of *F. cretica* against CCl_4-induced liver toxicity in 35 Wistar albino rats divided across five experimental groups. Hepatotoxicity was induced in groups II to V with CCl_4, while groups III and IV received plant extracts. Group V was treated with cisplatin, which was used as a standard drug. Alterations in liver damaging and oxidative stress biomarkers were assessed after treatment.

RESULTS: The results showed the hepatoprotective effects of EFC (ethanol extract of *F. cretica*) and HFC (hexane extract of *F. cretica*) in CCl_4 induced hepatotoxicity using male Wistar rats. The extracts demonstrated therapeutic efficacy by normalized liver enzyme levels (ALT, AST). Treatment with plant extracts significantly reduced oxidative stress markers (MDA, NO), while substantially restoring the antioxidative activity of GSH and CAT). Additionally, the extracts effectively downregulated immunological markers (MMP-9 and TGF-β). Histopathological analysis showed enhanced liver regeneration with *F. cretica* extracts, restoring near normal tissue architecture and minimizing hepatocyte damage and fibrosis. These findings suggest that *F. cretica* extracts possess potent hepatoprotective effects against chemical-induced liver injury.

CONCLUSION: The findings of the study revealed the promising antifibrotic potential of *F. cretica* extract on liver tissues. Further studies are needed to isolate specific antifibrotic phytochemicals and their cellular targets to advance the treatment options against liver fibrosis and hepatotoxicity.

KEYWORDS: Hepatoprotective potential, Medicinal plant, hepatotoxicity, anti-inflammatory, antioxidant activity

INTRODUCTION

Hepatic fibrosis is a multifaceted and progressive condition primarily driven by hepatocyte death and activation of hepatic stellate cells (HSCs)[1]. Globally, 3.3% of the population suffers from advanced liver fibrosis, reflecting its significant role in liver disorders[2]. The liver is the gatekeeper of various cell types and can biotransform and detoxify foreign substances, as its high metabolic rates make it susceptible to chemical and oxidative stress. Liver fibrosis, a common outcome of chronic hepatic injury, is closely associated with cellular damage caused by persistent oxidative stress[3]. Hepatic injury triggers a cascade of cellular and inflammatory responses encompassing fibrogenic cytokines like TGF-β and reactive oxygen species (ROS) from activated macrophages, platelets, and impaired hepatocytes. These factors collectively induce the stimulation of hepatic cells. The activation of quiescent hepatic cells leads to increased TIMP-1 expression, a key factor for hepatic fibrogenesis. Once activated, hepatic stellate cells can transform into myofibroblasts, producing significant amounts of extracellular matrix and releasing cytokines like TGF-β to maintain activity. Eventually, mature collagen fibers accumulate in the Disse gap, forming scars in the liver. This ongoing hepatocyte damage triggers the fibrotic cascade, which involves hepatic macrophages and NK cells that release growth-promoting factors and cytokines, such as TNF-alpha, IL-6, TGF-β, and PDGF. These mediators stimulate effector cells such as hepatic stellate cells. This ultimately activates HSC and forms a fibrotic scaffold, resulting in the excessive production of ECM proteins. To reverse liver fibrosis, it is essential to protect hepatocytes, restrain the activation of hepatic stellate cells, and prevent fibrotic lesion formation[4]. Many drugs induce hepatoxicity by targeting mitochondria; for example, isoniazid, amiodarone, and valproic acid contribute to oxidative stress and inhibit fatty acid metabolism, underscoring their potential to cause liver toxicity[5]. CCl_4 is a well-known hepatoxic agent that is widely used to induce

[1]*Institute of Molecular Biology and Biotechnology, Centre of Research in Molecular Medicine, The University of Lahore, Lahore, Pakistan*
[2]*Aziz Bhatti Shaheed Teaching Hospital, Gujrat, Pakistan*
[3]*Department of Physiology, Faisalabad Medical University, Faisalabad, Pakistan*
[4]*Department of Physiology, Avicenna Medical College, Lahore, Pakistan*
***Correspondence:** awaisaltaf362@yahoo.com*
doi: 10.22442/jlumhs.2025.01218
Received: 02-12-2024 *Revised: 27-01-2025*
Accepted: 03-02-2025 *Published Online: 19-05-2025*

liver injuries in experimental animals. Following administration of CCl_4, liver function tests (LFTs) may show elevated aspartate transaminase and alanine transaminase levels, which can cause abnormal deposition of ECM (extracellular matrix) proteins, inflammation, and cell necrosis, resulting in liver damage[6,7]. The potency of several natural phytochemicals like silymarin, caffeine, garlic, grape seeds, proanthocyanidins, and curcumin is used to prevent or decrease the progression of liver fibrosis via distinct ways by targeting the HSCs (hepatic stellate cells)[8]. Herbal plants are cheap and effective in treating various human diseases.

F. cretica (Zygophyllaceae) is found in the arid grasslands of Pakistan, Afghanistan, and Mediterranean Africa and is locally known as Dhamasa. It is widely available in dry areas with rocky or sandy soils in Pakistan and grows primarily from late spring to early summer. This plant contains several bioactive compounds, including flavonoids, alkaloids, terpenoids, saponins, tannins, sterols, anthraquinones, and coumarin. These compounds provide antioxidant, anti-inflammatory, and antibacterial potential. This plant has various therapeutic properties, such as antipyretic and antidiabetic, and treats various inflammatory conditions, including liver problems[9,10]. Studies have revealed that *F. cretica* possesses remarkable anticancer properties; its aqueous extract has a substantial inhibitory effect on cell viability in MCF-7 and MD A-MB-21 cell lines[11].

In this study, the antifibrotic potential of *F. cretica* extracts is comprehensively evaluated for the first time. The study emphasized the importance of conducting additional research to identify and isolate the most potent compounds, establishing a foundation for novel plant-based approaches to liver disease management.

METHODOLOGY

The aerial parts of *F. cretica* leaves were freshly collected during the summer season from the Cholistan desert in Bahawalpur district, Punjab, Pakistan. The plant identification was authenticated by Prof. Zaheer-ud-din Khan at Government College University (GCU), Lahore, Pakistan, and a voucher ID (GC.Herb.Bot.3785) was deposited in the herbarium of GC University, Lahore, Pakistan. After collection, the medicinal plant underwent shade drying and was subsequently pulverized into a fine powder using an electromechanical grinder. For the extract preparation, 400 g of powdered *F. cretica* was macerated separately using ethanol and hexane solvents. The dried powder was soaked in 1000 mL of ethanol and hexane solvents for two weeks at room temperature. After the filtration process, the filtrate from each extract was concentrated using a rotary evaporator at 40^0C, dried further in a freeze dryer (lyophilizer), and stored at 4^0C for further experimentation.

Chemical reagent

Analytical-grade chemicals and solvents were used, including Ethanol (LOT number # 19J154016), Hexane (LOT number # STBJ9587), and CCl_4 (LOT number # 10460). The solvents and chemicals used in this study were obtained from Sigma Aldrich (St. Louis, MO, USA).

In vivo studies

Place of study

All experiments were performed at the Institute of Molecular Biology and Biotechnology (IMBB), Center for Research in Molecular Medicine (CRiMM) at The University of Lahore.

Research Animal Model/ Ethical Frameworks

Thirty-five Wistar albino rats weighing between 150 and 250 g were purchased from the animal house at the Institute of Molecular Biology and Biotechnology (IMBB) at The University of Lahore. The rats were divided into five groups, each with seven rats (n = 7), and were acclimated for one week on a standard rat diet before experimentation. They were housed in ventilated stainless-steel cages in a sterile environment with 12 h light-dark cycles, and the room temperature was maintained at approximately 35 ± 2 [12]. The study followed ethical guidelines approved by the Institute of Molecular Biology and Biotechnology (IMBB) Departmental Bioethical, Biosafety, and Biosecurity Review Committee under reference number IMBB/UOL/23/488-A.

Inclusion and exclusion criteria

Inclusion criteria for male albino rats consisted of healthy male Wistar albino rats with a weight range of 150 to 250 grams. Exclusion criteria encompassed any male albino rats that showed abnormal behavior or physical deformities before treatment, which were also excluded from research studies.

Experimental Design

A hepatotoxicity study using carbon tetrachloride (CCl_4) was conducted using a rat model at the Institute of Molecular Biology and Biotechnology (IMBB), Center for Research in Molecular Medicine (CRiMM) at The University of Lahore in 2023. Group I, the negative control group, was given only normal saline and a normal diet. Group II received an intraperitoneal (i.p.) dose of CCl_4 mixed with olive oil as a vehicle in a 1:1 ratio (1 mL/kg of body weight) for 14 consecutive days. Groups III and IV received EFC and HFC extracts at a dosage of 300 mg/kg of body weight for 28 days. Group V received cisplatin at a dosage of 4 mg/kg and was administered intraperitoneal (i.p) once a day/week for the same duration. Following the 28th day treatment regimen, rats from all groups were sacrificed using an intraperitoneal injection (300 µL) of xylazine/Ketamine (50 mg/kg of ketamine and 5 mg/kg of xylazine).

Evaluation of biochemical markers

Following the sacrifice, 5 mL of blood was drawn from the rat heart and centrifuged at 14000 rpm for 10 minutes to separate the serum. After the separation of serum, it was stored at -20 ^{o}C for further biochemical

analysis. Each liver lobe was preserved in 7% formalin for histopathological studies, and the remaining liver tissue was cryopreserved at -80 ^{o}C for subsequent molecular and biochemical assays.

Liver function markers analysis

The serum samples were analyzed to measure the liver function markers, including AST (aspartate aminotransferase) and ALT (alanine aminotransferase), using an automated biochemical analyzer (COBAS-C311, USA) at the University of Lahore, Lahore, Pakistan.

Estimation of Oxidative stress and Antioxidative biomarker

Estimation of Malondialdehyde (MDA)

Lipid peroxidation levels were estimated calorimetrically following the methodology established by[13] with minor modifications. A 0.2 mL serum sample was combined with 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), followed by adding 1.5 mL of 20% acetic acid (pH 3.8) and 1.5 mL of 0.8% TBA solution. After centrifuging at 300 rpm for ten minutes, 5 mL of the supernatant (n-butanol) was collected for spectrophotometric analysis. The sample absorbance was measured at 532 nm against a blank solution using a BioTek-ELx800 microplate reader.

Determination of Nitric oxide (NO)

The NO level is measured using well-known methods, such as the calorimetric Griess assay, as outlined in reference [14]. The Griess reagent consisted of 0.33% sulfanilamide and 1% N-(1-naphthyl) ethylenediamine hydrochloride in a phosphate saline buffer solution. In this assay, a 100 µL serum sample was mixed with 100 µL Griess reagent and 2.5 mL deionized water in a spectrophotometer cuvett. Incubate the reaction mixture for thirty minutes at room temperature. Prepare a blank sample by mixing 100 µL Griess reagent and 2.9 mL deionized water. Measure the absorbance of the nitrite-containing sample at 546 nm relative to the reference samples using a BioTek-ELx800 microplate reader.

Estimation of Glutathione (GSH)

The antioxidant biomarker was quantified using the methodology described by [15], with minor adjustments. To quantify the GSH level, 1 mL of serum sample, 1 mL of 10% trichloroacetic acid and 4 mM ethylenediaminetetraacetic acid were added in a test tube. The resulting mixture was centrifuged at 3000 rpm for 5 minutes. After centrifugation, 0.5 mL of the supernatant was mixed with 1 mL of 0.6 mM Ellman reagents (DTNB) solution and 1.5 mL of sodium phosphate buffer (0.2 M at pH 8) in a test tube and incubated for 3 minutes. The estimation was done by measuring the sample absorbance at 412 nm.

Estimation of catalase (CAT)

Serum catalase level was estimated using the colorimetric assay reported by [16]. In a test tube, a 0.1 mL serum sample was mixed with 1.5 mL of phosphate buffer (150 M at pH 7.0) and 1.0 mL of H_2O_2 (0.2 M). The mixture was incubated for 15 minutes. Afterwards, 2 mL of 5% solution of dichromate acetic acid reagent was added, and the resulting absorbance was measured at 240 nm using a microplate reader (BioTek-ELx800).

Estimation of immunological biomarkers

Determination of matrix metalloproteinase -9 (MMP-9)

The level of MMP-9 in the serum sample was measured with the help of an ELISA kit according to the Invitrogen Thermofisher manufacturer's protocol (CAT#EEL130). The assay was conducted on a microplate using a pre-coated monoclonal antibody. The methods involve pouring 100 µL of the rat serum sample and 100 µL of standard into the wells using micropipettes, followed by a 2-hour incubation at room temperature. Following this, the washing solution removed the unbound substances from the wells. Afterwards, 100 µL biotinylated anti-rat detection antibodies were added to the wells and incubated for 1 hour. Then, repeat the washing steps to remove unnecessary substances. Then, 100 µL horseradish peroxide (HRP)-conjugated streptavidin antibody was added to the wells for 30 minutes and rewashed. The reaction was stopped, and measured the intensity at 450 nm. The MMP-9 level was determined employing the standard calibration curve and represented in ng/mL.

Estimation of Tumor Growth Factor-β (TGF-β)

The serum levels of TGF-β were analyzed using an enzyme-linked immunosorbent assay kit (CAT#BMS608-4) from Invitrogen and following the manufacturer's guidelines. The serum samples and standards were transferred to a pre-coated 96-well plate and kept at room temperature. After this step, the wells were washed with an anti-TGF-β detection antibody diluted in 10% PBS. Subsequently, horse radish peroxidase-conjugated streptavidin was added and incubated for half an hour at 25 °C. After washing again, the horseradish peroxidase (HRP) substrate and 3,3,5,5-tetramethylbenzidine (TMB) solution were added to facilitate color development. The absorbance was measured at 450 nm, allowing for the quantification of serum TGF-β levels using a calibration curve expressed in ng/mL.

Histopathological examination

Liver tissues from dissected rats were meticulously excised and cleansed with a standard saline solution. The liver samples were subsequently preserved in 7% formalin. After thorough fixation, grossing is conducted. Following this, the tissues were re-fixed in formalin. Liver tissues were embedded in paraffin to prepare paraffin blocks. These paraffin blocks were sectioned into 4-5 micrometers thin slices for further analysis. These sections were stained using hematoxylin and eosin (H and E) dye to assess CCl_4-induced liver damage. The stained samples were then analyzed through microscopic examination.

Statistical analysis

Results were expressed as mean ± SD. For statistical analysis of the *in-vivo* experiment, one-way ANOVA was applied, followed by the Bonferroni post-hoc test

for multiple comparisons among treatment groups. GraphPad Prism version 8.0 (Inc. USA) was used for data analysis, and statistical significance was set at a *p*-value of less than 0.05.

RESULTS

Evaluation of liver function biomarker in CCl_4-induced hepatotoxicity

The serum ALT and AST levels increased in the CCl_4-treated group compared to the control group. These enzymes are a critical marker of liver function, and their increased level indicates that CCl_4 administration led to impaired liver function. The lower levels of aminotransferases were reinstated in the group treated with EFC, HFC, and cisplatin relative to the diseased group affected by CCl_4 (**Figures IA, IB**).

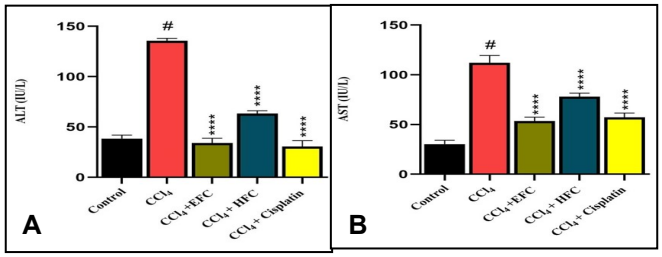

Figure I: Effect of ethanol and hexane extracts of *F. cretica* on liver functions in CCl_4 intoxicated rat groups. The liver function markers include (A) Alanine transaminase (ALT) and (B) Aspartate aminotransferase (AST). All data are represented as mean ± SD (n=7). One-way ANOVA was applied using the Bonferroni multiple comparison test. All extract-treated groups and standard drugs are compared to the diseased CCl_4 group. EFC ethanol extract of *F. cretica*; HFC= hexane extract of *F. cretica*; CCl_4= carbon tetrachloride.

Evaluation of Oxidative stress and Antioxidative biomarkers in CCl_4-intoxicated rats

In CCl_4-induced hepatotoxicity, the *in vivo* study demonstrated that *F. cretica* and cisplatin extracts significantly restore biochemical parameters, such as MDA, NO, GSH, and CAT. The *in vivo* study variables were all elevated in the CCl_4 group and eventually decreased with cisplatin and plant extract administration. Co-administration of *F. cretica* extracts (EFC and HFC) with CCl_4 reduced the level of MDA and NO, thereby reducing oxidative stress. Cisplatin also demonstrated comparable effects by reestablishing the equilibrium of free radicals in the liver (**Figure II A, II B**). Furthermore, the antioxidant enzymes, such as glutathione (GSH), and Catalase (CAT), aid in neutralizing oxidative stress. The administration of CCl_4, which suppressed the activities of these antioxidants, exacerbated liver injury. Conversely, the antioxidant defense system was strengthened, and liver cells were protected from CCl_4 -induced oxidative damage by treating *F. cretica* (EFC, HFC) extracts and cisplatin, significantly enhancing GSH and CAT activities. (**Figure II C and II D**).

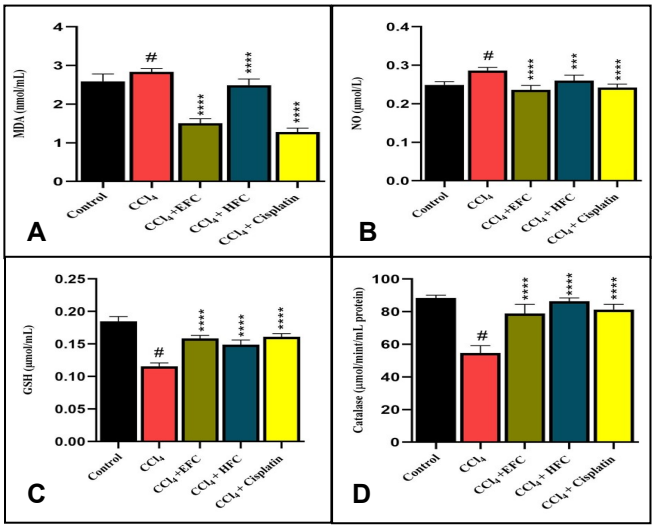

Figure II: Effect of *F. cretica* extracts on the oxidative and antioxidative biomarker in CCl_4-induced liver toxicity in rats. (A) MDA (B) NO (C) GSH, (D) Catalase. Data expressed as mean ± SD (n= 7). One-way ANOVA was applied using the Bonferroni multiple comparison test. All extract-treated groups and standard drugs are compared to the diseased CCl_4 group. The # indicates the significant difference between disease and control, while (***$p < 0.001$) (****$p < 0.0001$) indicates the level of significance of the treatment group compared to the CCl_4 carbon tetrachloride group; EFC= ethanol extract of *F. cretica*; HFC= hexane extract of *F. cretica*

Evaluation of immunological biomarkers in CCl_4 intoxicated rats

The serum levels of immunological biomarkers such as MMP-9 and TGF-β were significantly elevated in the CCl_4 group, indicating increased inflammatory response when compared to the control group. Treatment with extracts from EFC and HFC effectively lowered the levels of these inflammatory markers. Furthermore, the anticancer drug cisplatin effectively reduced the MMP-9 and TGF-β levels, as shown in Figures **III A** and **III B**.

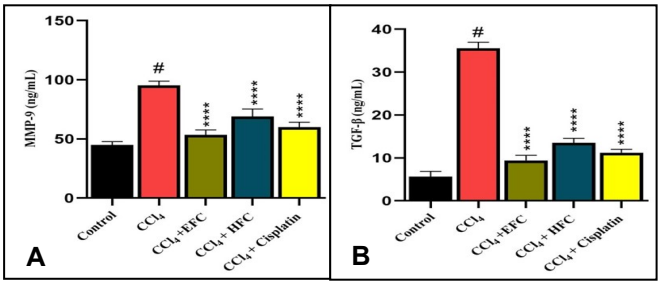

Figure III: Effect of *F. cretica* extracts on hepatic inflammatory markers in CCl_4-induced rat model. (A) Matrix metalloproteinase (MMP-9) (B) Transforming growth factor-beta (TGF-β). Results expressed as mean± SD (n= 7). One-way ANOVA was applied using the Bonferroni multiple comparison test. All extract-treated groups and standard drugs are compared to the diseased CCl_4 group. The (****p<0.0001) indicates

the significance level of improved results compared to the diseased group. The # indicates the significant difference between disease and control. EFC = ethanol extract of *F. cretica*; HFC = hexane extract of *F. cretica*; CCl_4= carbon tetrachloride.

Histopathological analysis of the liver tissues

Histopathological analysis of hematoxylin-eosin (H and E) stained liver section revealed distinct differences among experimental groups. **Figure IV** (**A**) shows that the control group showed intact hepatic architecture, well-structured hepatocyte cords, unremarkable portal triads, and central veins. In contrast, the CCl_4-induced toxic group displayed significant pathological changes, including fatty changes, sinusoidal congestion, and widespread fibrosis, particularly in periportal regions throughout the hepatic tissue, as shown in **Figure IV (B)**. Rats treated with *F. cretica* ethanol and hexane extracts (EFC and HFC) at a dose of 300 mg/kg body weight showed significant restoration of liver structure with improved hepatocyte morphology, reduced fatty changes, mild fibrosis, normal-appearing portal triads and the presence of a few benign hepatocytes, and adjacent sinusoids (**Figure IV C and IV D**). Moreover, the most pronounced recovery was observed in cisplatin at a dose of 4 mg/kg body weight, the results exhibiting nearly normal hepatocyte structure and liver fragments with the portal triads displaying minimal cytoplasmic vacuolation and no evidence of fibrosis (**Figure IV E**).

These results indicate that *F. cretica* provides substantial protection against CCl_4-induced hepatic damage, whereas cisplatin treatment exhibited superior efficacy in reversing CCl_4-induced liver injury.

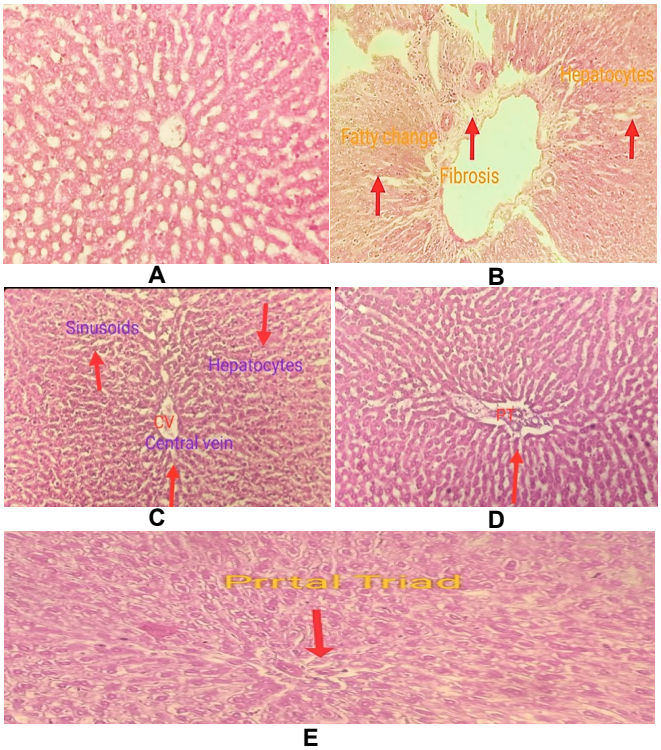

Figure IV: Histopathological results of the liver sample. (A) Normal control group; (B) CCl_4-induced rat liver; (C) liver of CCl_4-induced rats treated with ethanol extract of *F. cretica* (EFC); (D) liver of CCl_4-intoxicated rats treated with hexane extract of *F. cretica* (HFC); (E) liver from CCl_4-exposed rats receiving cisplatin. Arrow represents mononuclear cell infiltration, congested vascular sinusoids, and fibrous tissues.

DISCUSSION

Natural compounds have been used in the development of new drugs due to their potential antioxidant activity, which could be beneficial in treating fetal diseases. So, herbal extracts with strong anti-inflammatory and antioxidant characteristics are recognized to be therapeutic in treating liver diseases and toxic lesions because hepatotoxicity is linked to inflammation and oxidative stress. As a result, herbal extracts could be a source of novel chemical metabolites with significant potential in xenobiotic-induced liver diseases.

Medicinal herbs contain many active compounds that are proven to modulate several therapeutic activities, such as fibrotic, inflammation, and cellular oxidation, promoting wellness and stability in the living system[17]. *Fagonia cretica* is a traditional medicinal herb that enhances liver function and mitigates oxidative stress. The overall study plan involves the hepatoprotective effects of *F. cretica* extracts on liver biomarkers in CCl_4-induced hepatotoxicity models. Assessing the extracts' antioxidant capabilities, the levels of oxidative stress parameters, such as GSH, MDA, NO, and CAT, were evaluated (**Figure II**). The standard and plant treatment groups displayed downregulation in the levels of oxidative stress biomarkers. The findings revealed a tendency for decreased levels of GSH and CAT, accompanied by an upward trend in MDA and NO levels in the intoxicated group. These results are consistent with prior research that has documented comparable impacts caused by CCl_4 [18]. The results revealed that groups receiving *F. cretica* and cisplatin doses showed significant reductions in MDA and NO levels, while increasing GSH and CAT levels. These results are consistent with demonstrating that *F. cretica* extract effectively counteracted Bisphenol A-induced oxidative damage[19]. The study revealed that extracts protect against oxidative stress by restoring the oxidant-antioxidant balance and systemically scavenging reactive oxygen and nitrogen species in rat tissues. The effect of CCl_4-induced hepatotoxicity indicated severe liver cell damage compared with CCl_4-treated rats. Treatment with cisplatin and plant extracts (EFC and HFC) restored protein levels, demonstrating their hepatoprotective action and decreased levels of liver enzymes (ALT and AST), suggesting a potential

defense against liver injury (**Figure I**). Our findings align with the observation reported by [20] who demonstrated that mulberry leaf extracts (MLE) markedly decreased liver enzyme levels of ALT and AST and improved hepatic pathological status in rats.

As a chronic liver injury, liver fibrosis is mediated by hepatic satellite cells (HSCs) through their activation of type 1 collagen and excessive ECM deposition in the liver. Therapeutic strategies for hepatic fibrosis often target HSC activation and respond to various stimuli, including oxidative stress and inflammation. Therefore, targeting MMP-9 expression and disrupting the TGF-β/Smad or TGF-β1/JAK1/STAT3 signaling to suppress HSC activation is emerging as a promising strategy in liver fibrosis treatment. In liver fibrosis, HSCs are the primary profibrotic factor, activated primarily by the upregulation of TGF-β. TGF-β not only regulates Smad signaling pathways but also promotes liver fibrosis through non-Smad pathways such as mTOR, PI3K/Akt, IKK, Wnt/β-catenin, MAPK, and Rho GTPase[21]. In our study, the level of inflammatory markers such as MMP-9 and TGF-β was upregulated in the CCl_4 intoxicated group (**Figure III**). Their levels were subsequently reduced following the administration of cisplatin and extract of *F. cretica* (EFC and HFC).

The results suggest that cisplatin and *F. cretica* extract may mitigate these adverse effects and lower the level of liver markers, thereby reducing oxidative stress associated with hepatic injury. Our results corroborate previous investigation[22-24] revealing the hepatoprotective potential of natural extracts (corcin and Phoenix dactylifera L. extracts) against cisplatin-induced inflammatory markers in male rats, effectively decreasing proinflammatory cytokines and ameliorating hepatic inflammation. The histological analysis of the control group revealed normal central veins and hepatocytes, no areas with fibrosis, hemorrhage, or fatty alterations, and no signs of inflammation or dysplasia. However, the CCl_4-intoxicated group was observed for evident cellular changes, including lipid deposition, congestion of sinusoidal, central hepatic vein, and areas with severe fibrosis (**Figure IV**). Extracts of EFC and HFC expedited liver regeneration by reducing hepatocyte disruption, moderate fibrosis and lipid changes. No fibrosis, inflammation, or aberrant hepatocytes were seen in the cisplatin group. Our findings are more consistent with [25] demonstrating that the water extracts of ashwagandha effectively improve liver damage induced by CCl_4 in Wistar albino rats using a dose of 300 mg/kg. The results of our study reveal that *F. cretica* extract exhibits significant hepatoprotective effects by reducing inflammatory factors, enhancing antioxidant defenses, and enhancing immune surveillance.

CONCLUSION

The findings of our study indicate that the *F. cretica* extract may have significant potential to protect the liver from damage caused by CCl_4 exposure. The extract demonstrated remarkable antioxidant potential by substantially reducing oxidative stress levels, specifically decreasing MDA and NO levels, and significantly elevating GSH and CAT activities, effectively restoring the oxidant-antioxidant balance and protecting against cellular oxidative damage. The hepatic enzyme profiles, such as ALT and AST, indicate high hepatocyte integrity. The immunological markers TGF-β and MMP-9 were overexpressed during liver fibrosis progression, with *F. cretica* extract effectively reducing their expression levels and restoring molecular homeostasis. Improvements in histopathology results imply the potential reversal of fibrotic changes in liver tissues, presenting a promising strategy for the treatment of liver disease. This research represents a pivotal advancement in plant-based therapeutic intervention, systematically elucidating their protective capabilities against liver fibrosis and establishing a robust scientific foundation for future clinical investigation.

Ethical permission: University of Lahore, Pakistan**,** IRB letter No. IMBB/UOL/23/488-A.

Conflict of interest: There is no conflict of interest between the authors.

Financial Disclosure / Grant Approval: No Funding agency was involved in the research

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Arshad F: Performed all the experimental work, analyzed the data, and drafted the manuscript
Altaf A: Designed & conceptualized the entire research and guided in data analysis, Supervisor
Arshad AR: Financial support and assist in formatting the manuscript
Maqbool T: Data validation and visualization, guided in formal analysis and data collection
Ashraf MAB: Data validation and visualization, guided in formal analysis and data collection
Akbar A: Visualize the data and manuscript formatting

REFERENCES

1. Liedtke C, Nevzorova YA, Luedde T, Zimmermann H, Kroy D, Strnad Pet al. Liver fibrosis from mechanisms of injury to modulation of disease. Front Med. 2022; 8: 814496. doi: 10.3389/fmed.2021.814496.
2. Zamani M, Alizadeh-Tabari S, Ajmera V, Singh S, Murad MH, Loomba R. Global prevalence of

advanced liver fibrosis and cirrhosis in the general population: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2024: 1542-3565. doi: 10.1016/j.cgh.2024.08.020.
3. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative stress in liver pathophysiology and disease. Antioxidants. 2023; 12(9): 1653. doi: 10.3390/antiox12091653.
4. Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol hepatol. 2021; 18(3):151-166. doi: 10.1038/s41575-020-00372-7.
5. Alrawi DI, Khalaf M, Jamaludeen MK. Drug-Induced Liver Injury: Mechanisms and Counteracting Herbal-Derived Products. Iraqi J Pharm. 2024; 21(2): 45-56. https://doi.org/10.33899/iraqij.p.2024.145444.1078.
6. Broermann A, Schmid R, Gabrielyan O, Sakowski M, Eisele C, Keller S, Wolff M, Baum P, Stierstorfer B, Huber J, Krämer BK. Exosomal miRNAs as potential biomarkers to monitor phosphodiesterase 5 inhibitor induced antifibrotic effects on CCl_4 treated rats. Int J Mol Sci. 2020; 22(1): 382. doi: 10.3390/ijms22010382.
7. Chang SN, Kim SH, Dey DK, Park SM, Nasif O, Bajpai VK, Kang SC, Lee J, Park JG. 5-O-Demethylnobiletin alleviates CCl_4-induced acute liver injury by equilibrating ROS-mediated apoptosis and autophagy induction. Int J Mol Sci. 2021; 22(3): 1083. doi: 10.3390/ijms22031083.
8. Almatroodi SA, Anwar S, Almatroudi A, Khan AA, Alrumaihi F, Alsahli MA, Rahmani AH. Hepatoprotective effects of garlic extract against carbon tetrachloride (CCl_4)-induced liver injury via modulation of antioxidant, anti-inflammatory activities and hepatocyte architecture. Appl Sci. 2020; 10(18): 6200. doi: 10.3390/app10186200.
9. Rafiq M, Tunio AA, Qureshi AS, Rehman T, Bhutto MA, Lasharı Z. Determination of Phytochemicals, Antimicrobial, Antioxidant and Allelopathic Effects of *Fagonia cretica* L., collected from Jamshoro, Pakistan. J Agric Sci. 2022; 32(4): 785-794. doi: 10.29133/yyutbd.1122798.
10. Ali K, Khan H. *Fagonia indica*; A review on chemical constituents, traditional uses and pharmacological activities. Curr Pharm Des. 2021; 27(22): 2648-60. doi: 10.2174/1381612826666201210105941.
11. Lam M, Carmichael AR, Griffiths HR. An aqueous extract of *Fagonia cretica* induces DNA damage, cell cycle arrest and apoptosis in breast cancer cells via FOXO3a and p53 expression. PloS one. 2012; 7(6): e40152. doi.org/10.1371/journal.pone.0040152.
12. Abdel-Hamid M, Osman A, El-Hadary A, Romeih E, Sitohy M, Li L. Hepatoprotective action of papain-hydrolyzed buffalo milk protein on carbon tetrachloride oxidative stressed albino rats. J dairy sci. 2020; 103(2): 1884-1893. doi: 10.3168/jds.2019-17355.
13. Okoro IO, Okoro EO, Isoje FE, Oyubu G. Protective effects of Alstonia congensis Methanolic extract against CCl_4 induced liver damage in Wistar rats. Sci Afr. 2022; 17: e01315. doi: 10.1016/j.sciaf.2022.e01315.
14. Alkinani KB, Ali EM, Al-Shaikh TM, Awlia Khan JA, Al-Naomasi TM, Ali SS, Abduljawad AA, Mosa OF, Zafar TA. Hepatoprotective Effects of Epicatechin in CCl_4-Induced Toxicity Model Are Mediated via Modulation of Oxidative Stress Markers in Rats. Evid Based Complement Alternat Med. 2021: 4655150. doi: 10.1155/2021/4655150.
15. Shaban NZ, El-Kot SM, Awad OM, Hafez AM, Fouad GM. The antioxidant and anti-inflammatory effects of Carica Papaya Linn. seeds extract on CCl_4-induced liver injury in male rats. BMC Complement Med Ther. 2021; 21: 1-5. doi: 10.1186/s12906-021-03479-9.
16. Shameenii A, Thanebal PP, Vun-Sang S, Iqbal M. Hepatoprotective effects of *Pandanus amaryllifolius* against carbon tetrachloride (CCl_4) induced toxicity: A biochemical and histopathological study. Arab J Chem. 2021; 14 (10): 103390. doi: 10.1016/j.arabjc.2021.103390.
17. Agbor GA, Dell'Agli M, Kuiate JR, Ojo O. The role of medicinal plants and natural products in modulating oxidative stress and inflammatory related disorders. Front Pharmacol. 2022; 13: 957296. doi: 10.3389/fphar.2022.957296.
18. Ahmed AA, Omar ZM, El-Bakry MH, Ahmed MA. Hepatoprotective effect of dipeptidyl peptidase-4 inhibitor sitagliptin against carbon tetrachloride-induced liver fibrosis in mice. Al-Azhar Assiut Med J. 2021; 19(3): 459-68. doi: 10.4103/AZMJ.AZMJ_161_20.
19. Abd-El-Moneim OM, Abd El-Rahim AH, Mohamed AA, Farag IM, Mohamed Abdalla A. Enhancement effects of ethanolic extract of *Fagonia cretica* on Bisphenol A (BPA)-induced genotoxicity and biochemical changes in rats. Bull Natl Res Cen. 2020; 44: 1-13. doi: 10.1186/s42269-020-00295-y.
20. Yu Y, Chen Y, Shi X, Ye C, Wang J, Huang J, Zhang B, Deng Z. Hepatoprotective effects of different mulberry leaf extracts against acute liver injury in rats by alleviating oxidative stress and inflammatory response. Food Funct. 2022; 13(16): 8593-8604. doi: 10.1039/d2fo00282e.
21. Zhang J, Liu Q, He J, Li Y. Novel therapeutic targets in liver fibrosis. Front Mol Biosci. 2021; 8: 766855. doi: 10.3389/fmolb.2021.766855.
22. Khedr LH, Rahmo RM, Farag DB, Schaalan MF, Hekmat M. Crocin attenuates cisplatin-induced hepatotoxicity via TLR4/NF-κBp50 signaling and BAMBI modulation of TGF-β activity: Involvement

of miRNA-9 and miRNA-29. Food Chem Toxicol. 2020; 140: 111307. doi: 10.1016/j.fct.2020. 111307.
23. Gad El-Hak HN, Mahmoud HS, Ahmed EA, Elnegris HM, Aldayel TS, Abdelrazek HM, Soliman MT, El-Menyawy MA. Methanolic *Phoenix dactylifera* L. extract ameliorates cisplatin -induced hepatic injury in male rats. Nutrients. 2022; 14(5): 1025. doi: 10.3390/nu14051025.
24. Kiran A, Altaf A, Sarwar M, Maqbool T, Ashraf MAB, Naveed M, Aziz T, Alharbi M. Exploring the phytochemical profile, antioxidant activity, and anticancer potential of *Achillea millefolium* extracts: *In-vitro* and *in-silico* investigation. S Afr J Bot. 2025; 177: 684-698. doi: 10.1016/j.sajb. 2024.12.038.
25. Aboelhassan DM, Ibrahim NE, Elnasharty MM, Elwan AM, Elhadidy ME, Mohamed MA, Radwan HA, Ghaly IS, Farag IM. Biological and physical studies on the protective and therapeutic roles of ashwagandha seed extract against the potential toxic effect of amoxicillin in rats. Egypt Pharm J. 2024; 23(2): 251-263. doi: 10.4103/epj.epj_ 221_23.