



Evaluation of the Hepatoprotective and Antifibrotic Effects of *Moringa oleifera* in Carbon Tetrachloride (CCl₄) Induced Liver Fibrosis in Rats

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Abstract

Liver fibrosis is a significant global health concern characterized by excessive extracellular matrix protein deposition, leading to progressive hepatic dysfunction. Conventional treatments often present adverse effects, necessitating the exploration of safer, natural alternatives. Carbon tetrachloride (CCl₄) is a well-established hepatotoxin widely used to induce experimental liver fibrosis in animal models, as it closely mimics human hepatic injury and fibrotic progression. In humans, CCl₄ exposure primarily through inhalation or ingestion has been linked to severe liver damage, oxidative stress, and progressive fibrosis, making it a relevant model for studying hepatoprotective interventions. This study investigates the hepatoprotective effects of *Moringa oleifera* leaf powder against CCl₄-induced liver fibrosis in rats. Thirty-five albino rats (180–200 g) were divided into seven groups: a normal control, an injury control (CCl₄-induced), a standard drug treatment, placebo groups, and treatment groups receiving 1 g/kg and 2 g/kg *Moringa oleifera* leaf powder. Liver function and fibrosis progression were assessed through biochemical assays, gene expression analysis (P53, Bax, IL6, TOP2A), and histopathological evaluations. The results demonstrated that *Moringa oleifera* treatment significantly improved liver function, reduced ALT and AST levels, and preserved hepatic histoarchitecture compared to the CCl₄-injured group. Gene expression analysis revealed a regulatory effect on fibrosis-related markers, suggesting a protective role against fibrotic progression. These findings highlight the potent antifibrotic and hepatoprotective properties of *Moringa oleifera*, underscoring its potential as a natural therapeutic agent for liver fibrosis.

Introduction

Liver fibrosis is a pathological disorder that develops due to constant liver injury and is marked by abnormal accumulation of extracellular matrix components. It serves as a precursor to cirrhosis and hepatic failure, contributing significantly to global morbidity and mortality. The primary etiological factors include viral hepatitis (HBV and HCV), chronic alcohol consumption, metabolic disorders, and prolonged exposure to hepatotoxic agents such as carbon

tetrachloride (CCl₄) (Schuppan et al., 2018). The fibrotic process is driven by hepatic stellate cell (HSC) activation, oxidative stress, and pro-inflammatory cytokine release, leading to tissue remodeling and impaired liver function (Cordero-Espinoza and Huch, 2018). Without timely intervention, liver fibrosis can progress irreversibly, necessitating liver transplantation.

Current therapeutic strategies for liver fibrosis include pharmacological agents such as antifibrotic drugs and immunomodulators, alongside surgical interventions like

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liver transplantation. However, these treatments are often associated with high costs, limited availability, and severe side effects, underscoring the urgent need for alternative, cost-effective, and safer therapeutic options (Tan et al., 2021). Nutraceuticals, including plant-based bioactive compounds, have gained considerable attention for their hepatoprotective potential, offering antioxidant, anti-inflammatory, and antifibrotic effects that may attenuate liver fibrosis progression (Li et al., 2022, El-Tantawy and Temraz, 2022).

Moringa oleifera, commonly called the "miracle tree," is a medicinal plant widely recognized for its diverse antioxidant, anti-inflammatory, and hepatoprotective properties (Vergara-Jimenez et al., 2017). Its leaves are rich in polyphenols, flavonoids, vitamins, and essential minerals, which have been reported to modulate oxidative stress and inflammatory pathways in hepatic diseases (Falowo et al., 2018). Emerging evidence suggests that *Moringa oleifera* may exert protective effects against liver fibrosis by downregulating profibrotic gene expression, inhibiting hepatic stellate cell activation, and improving liver function biomarkers (Supriono et al., 2022). Despite these promising findings, the precise molecular mechanisms by which *Moringa oleifera* mitigates liver fibrosis remain largely unexplored, requiring further research. This study evaluates the hepatoprotective effects of *Moringa oleifera* leaf powder against CCl₄-induced liver fibrosis in rats, aiming to establish it as a natural, cost-effective alternative to conventional treatments while advancing plant-based therapeutics.

Materials and Methods

Experimental Design

Thirty-five albino rats (180-200g) were obtained from the animal house facility of the Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan, and housed under standard laboratory conditions. The rats were divided into seven groups (n=5 per group) and housed in a stainless-steel cage Table 1.

Table 1. Division of rats in various groups

Sr.	Group type	Treatment
1.	Normal	No treatment
2.	Injured	CCl ₄ -induced fibrosis
3.	Standard drug	Omeprazole, 40mg/200g
4.	Placebo 1	Rice powder 1g/kg
5.	Placebo 2	Rice powder 2g/kg
6.	Treatment 1	<i>Moringa oleifera</i> powder, 1g/kg
7.	Treatment 2	<i>Moringa oleifera</i> powder 2g/kg

Liver fibrosis was induced by intraperitoneal administration of a 1:1 mixture of carbon tetrachloride and olive oil (0.2 mL per 160-200 g body weight) for four weeks (Fig. 1).



Fig. 1. Induction of CCL4 intraperitoneally in rat

Following the induction period, treatment groups were administered omeprazole (40 mg/200 g rat), placebo (rice powder), or *Moringa oleifera* leaf powder for an additional two weeks, as detailed in Table 1.

Moringa oleifera branches were air-dried in a shaded area after cleaning. The dried leaves were then ground into a fine powder, sifted to remove large particles, and stored in an airtight vacuum container for later use.

Sample collection

After four weeks, the rats were anesthetized with chloroform to collect blood and liver samples (Fig. 2). Blood was drawn directly from the heart's left ventricle to ensure accurate and uncontaminated samples. The serum was then separated by centrifugation at 2200-2500 rpm for subsequent biochemical analysis. The liver was carefully excised, and the tissue was divided into two portions: one half was preserved in formalin for histopathological examination, whereas the remaining portion was stored in Trizol for gene expression analysis.



Fig. 2. Dissection and collection of liver tissue

Biochemical Analysis

Liver function was assessed by measuring serum ALT and AST levels following supplementation with powdered

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Moringa oleifera leaves. The enzymatic assay kits (Roche, Germany) were used, and the measurements were conducted on a fully automated Cobas 6000 analyzer to evaluate hepatic injury and recovery (Zhang et al., 2023).

Gene Expression Analysis

RNA was extracted from liver tissues using the standard Trizol method, and cDNA was synthesized using the M-MLV reverse transcriptase kit (Invitrogen, USA). Real-time PCR (Applied BioSystems, CFX Connect) was conducted to evaluate the expression of fibrosis-related genes (P53, Bax, IL6, and TOP2A), while GAPDH was used as the internal control (Ujah et al., 2013).

Histopathological Examination

Liver tissue samples were preserved in formalin (10%) and subsequently stained with hematoxylin and eosin. Microscopic examination was performed to assess liver architecture, fibrosis severity, and cellular integrity.

Statistical Analysis

Quantitative data from various groups were statistically compared to evaluate treatment effects using One-Way ANOVA (GraphPad Prism 7.04; La Jolla, CA, USA). The results were expressed as mean ± SD, with a p-value ≤ 0.05 considered statistically significant.

Results

Growth

At the start of the experiment, all rats weighed between 180-200g. The body weight of the experimental groups showed significant differences compared to the control group (Fig 3).

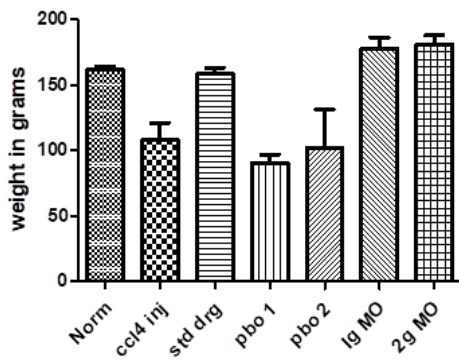


Fig. 3. Mean body weight determination in control and treated groups.

Biochemical analysis

The serum ALT and AST levels were analyzed to assess liver function in control and treated groups. The CCl4-injured group exhibited significantly elevated AST (Fig. 4) and ALT levels (Fig. 5), indicating liver damage. However, moringa-treated groups (1g/kg and 2g/kg) showed a substantial reduction in these enzyme levels, suggesting hepato-protective effects.

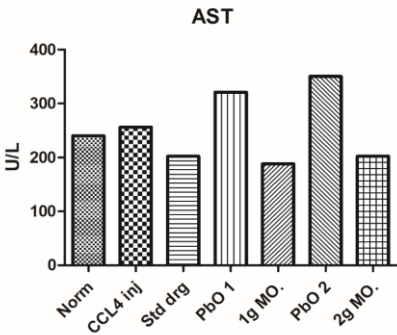


Fig. 4. The AST levels in experimental and control groups.

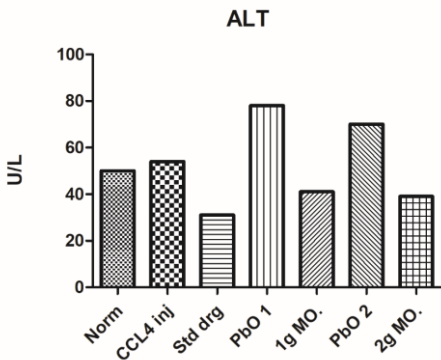


Fig. 5. ALT levels exhibited a significant difference between the control and treated groups.

Gene Expression:

The relative expression of fibrosis-related genes *P53*, *Bax*, *IL6* and *TOP2A* genes revealed distinct patterns among different groups.

P53 (tumor suppressor protein): Significantly upregulated in the placebo groups, indicating a stress response, whereas its expression decreased in Moringa-treated groups, suggesting reduced apoptosis (Fig. 6).

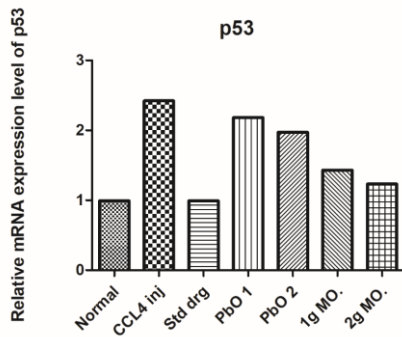


Fig. 6. *P53* gene expression levels in experimental groups.

Bax (BCL2-associated X, apoptosis regulator): High expression in the CCL4-injured group indicated increased apoptosis. Treatment with Moringa significantly lowered Bax expression, suggesting a protective effect against cellular damage (Fig. 7).

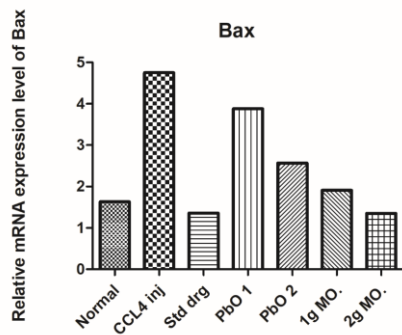


Fig. 7. *Bax* expression levels in control and treated groups.

IL6 (Interleukin 6): CCL4-induced inflammation led to elevated IL6 expression. Moringa-treated groups exhibited reduced IL6 levels, indicating anti-inflammatory effects (Fig. 8).

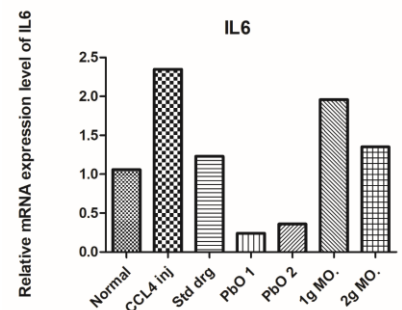


Fig. 8. *IL6* expression levels in control and treated groups.

TOP2A (DNA topoisomerase II alpha): The expression was highest in the standard drug and Moringa 1g/kg groups, supporting liver regeneration (Fig. 9).

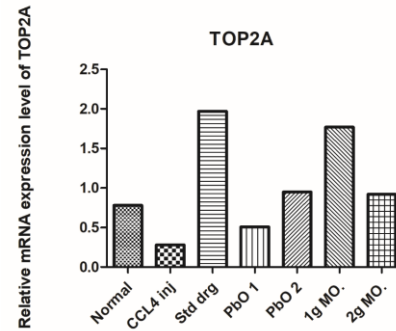


Fig. 9. TOP2A expression levels in various groups.

Histopathological Findings

The control group exhibited intact hepatic architecture with no pathological changes (Fig. 10a). The CCL4 injury group showed severe liver damage, including post-necrotic cirrhosis, mononuclear infiltration, vacuolated hepatocytes, apoptotic changes, and degeneration (Fig. 10b). The Placebo I group displayed structural disruption with vascular alterations, inflammation, fatty changes, and fibrosis (Fig. 10c), while the Placebo II group had pronounced hydropic degeneration, periportal fibrosis, and lymphocytic infiltration (Fig. 10d). The treatment II group showed mild degeneration with minimal structural damage and lymphocyte presence (Fig. 10e). The treatment I group maintained a well-preserved hepatic structure, with only focal central vein dilation, indicating the most favorable outcome (Fig. 10f).

Discussion

Liver fibrosis remains a critical pathological consequence of chronic liver injury and represents a major challenge due to its progressive nature and impact on hepatic function. The findings of this study align with previous research on the hepatoprotective properties of *Moringa oleifera*, demonstrating its potential as a natural remedy for liver fibrosis (Bataller and Brenner, 2005).

The reduction in ALT and AST levels in Moringa-treated groups indicates improved liver function and reduced hepatic injury. These results are consistent with prior studies suggesting that *Moringa oleifera* can mitigate liver enzyme elevation caused by hepatotoxic agents (Wilujeng et al., 2021). The comparable efficacy of Moringa to standard

pharmacological treatment further supports its therapeutic potential.

The modulation of fibrosis-related genes in Moringa-treated groups suggests multiple protective mechanisms. The decrease in Bax expression indicates a reduction in apoptosis, while the downregulation of IL6 suggests anti-inflammatory effects. Furthermore, the increased expression of TOP2A in Moringa 1g/kg-treated rats supports the hypothesis that Moringa enhances liver regeneration (Ujah et al., 2013).

Histological examination confirmed the biochemical and molecular findings. Severe fibrosis, necrosis, and inflammation observed in the CCl₄-injured group were significantly reduced in the Moringa-treated groups. The preservation of liver architecture suggests that Moringa protects against hepatic fibrosis and promotes tissue repair (Supriono et al., 2020).

Moringa oleifera contains a rich profile of bioactive compounds, including flavonoids, polyphenols, and alkaloids, which contribute to its hepatoprotective effects. Its antioxidant properties may help neutralize reactive oxygen species, thereby preventing oxidative damage. Additionally, its anti-inflammatory compounds can inhibit pro-inflammatory cytokines, reducing liver inflammation and fibrosis progression (George, 2021).

The findings of this study highlight the potential of Moringa oleifera as an alternative or adjunctive treatment for liver fibrosis. However, further studies are needed to determine optimal dosages, long-term safety, and efficacy in human subjects. Future research should also investigate the specific bioactive compounds responsible for its hepato-protective effects and explore its potential synergistic effects with existing antifibrotic drugs.

Conclusion

The present study concludes that Moringa oleifera possesses significant hepato-protective effects against CCl₄-induced liver fibrosis. The administration of Moringa oleifera powder resulted in improved biochemical markers, modulated gene expression, and preserved liver architecture. These findings support the use of Moringa oleifera as a potential natural therapeutic agent for liver fibrosis. Future research should focus on clinical trials to validate its efficacy in humans and explore its potential in combination with conventional treatments for liver diseases.

Contributions

SH conceptualized and designed the study. GN and HN were responsible for sample collection. SMA conducted the experiments and drafted the manuscript. AH reviewed and finalized the manuscript.

Source of funding

N/A

Conflict of interest

The authors affirm that they have no conflicts of interest.

Ethical statement

All animal handling and experimental procedures were approved by the Institute of Molecular Biology and Biotechnology, University of Lahore, Punjab, Pakistan.

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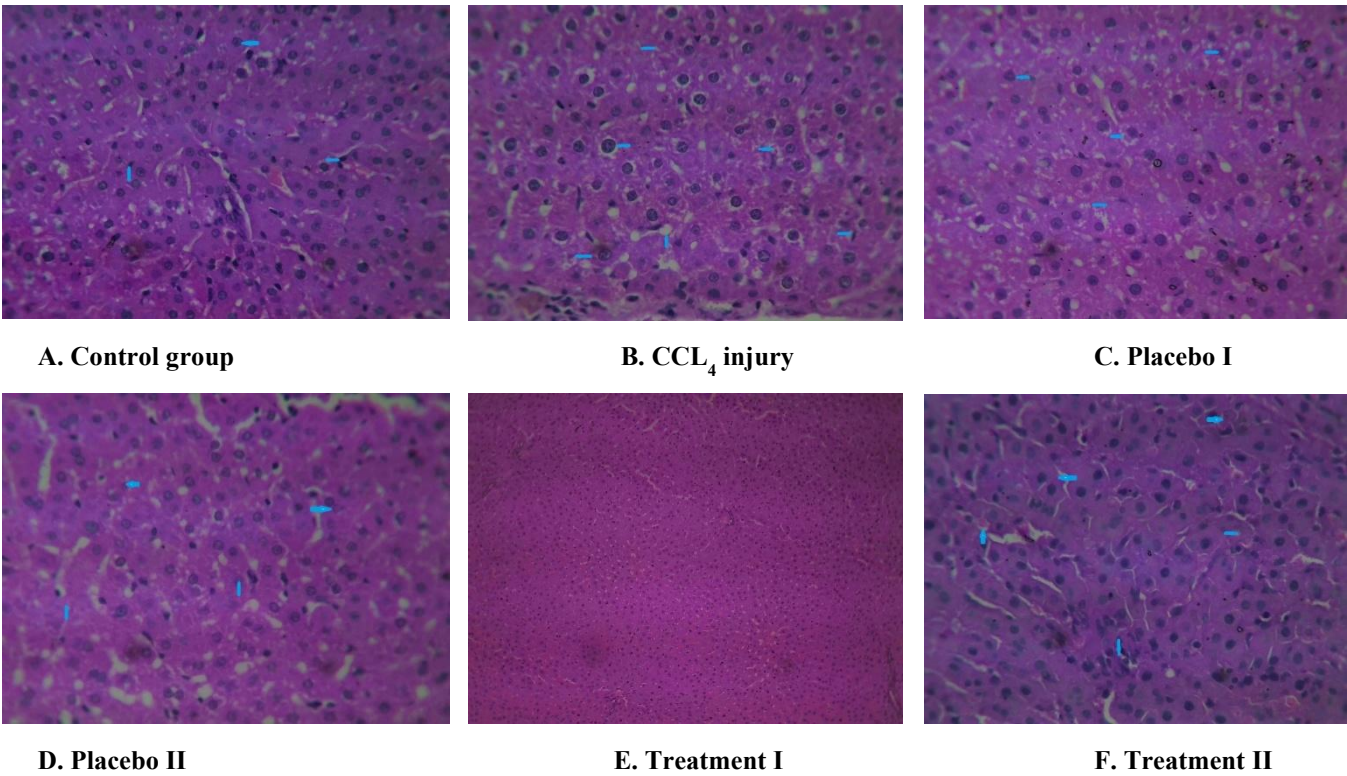


Fig. 10. Histological examination of Liver tissue in control and experimental groups.