Azhar Int J Pharm Med Sci 2025; Vol 5 (2):179-190.
Research Article

Open Access

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Hepatoprotective Effect of Quercetin against CCl_4 Induced Liver Fibrosis: miRNA-124-3p Regulation in Rat Model

Amira A. Atta[1, *], Mohamed A. Elkady[2, *], Ahmed M. Mansour[3], Heba Gamal Abd El-Aziz[4] and Gamil M. Abd-Allah[1,2].

[1] Department of Biochemistry, Faculty of Pharmacy, Egyptian Russian University, Badr City, 11829, Cairo, Egypt.
[2] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Boys), Al-Azhar University, Nasr City, 11651, Cairo, Egypt.
[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy (Boys), Al-Azhar University, Nasr City, 11651, Cairo, Egypt.
[4] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr City, 11765, Cairo, Egypt.

*** Correspondence**: amira-ahmed@eru.edu.eg and MohamedElkady1565.el@azhar.edu.eg

Article history: Received 09-11- 2024 **Revised** 07-01-2025 **Accepted** 13-02-2025

Abstract: Quercetin (QUE) is a natural flavonoid compound found in many herbal drugs. It has broad biopharmacological effects that are beneficial for treating liver diseases such as liver steatosis, liver fibrosis, and liver cancer. It can mitigate liver damage through its powerful antioxidative, anti-inflammatory, and antifibrotic properties. This study aimed to clarify the molecular regulatory effect of QUE on the expression levels of miRNA-124-3p in liver fibrosis induced by Carbon tetrachloride (CCl_4). Twenty-four adult, male albino rats were randomly allocated into 4 groups (n=6); group I (control group), group II (QUE group; 5mg/kg/day, p.o), group III (CCl_4 group; 2mL/kg, twice a week, SC), and group IV (QUE+CCl_4; received the same previous doses). After eight successive weeks, the rats were sacrificed and hepatic enzymes and oxidative stress biomarkers (MDA, SOD, and CAT) were evaluated using colorimetric methods, while IL-6, TNF-α, and TGF-β1 levels were measured using ELISA. In addition, the miRNA-124-3p expression levels were determined using qRT-PCR. Histopathological examination was performed. The results revealed that group IV (QUE+CCl_4) treated rats exhibited a significant decrease in serum liver enzyme activities, histopathological alterations, hepatic MDA, and inflammatory markers (IL-6 and TNF-α), as well as a significant increase in hepatic SOD and CAT compared to group III (CCl_4). Moreover, the hepatic levels of TGF-β1 were significantly decreased. In addition, there was an upregulation of miRNA-124-3p expression level. Furthermore, an inverse significant correlation was observed between miRNA-124-3p and TGF-β1. These results revealed that QUE exhibits hepatoprotective effects against CCl_4-induced liver fibrosis by regulating miRNA-124-3p/TGF-β1 molecular axis.

Keywords: Hepatoprotective; Liver Fibrosis; Quercetin; miRNA-124-3p; TGF-β1

1. INTRODUCTION

Liver fibrosis is an abnormal wound-healing process secondary to a progression of chronic liver diseases represented by parasitic and viral infections, autoimmune hepatitis, alcohol abuse, non-alcoholic steatohepatitis, metabolic ailment, and cholestatic liver injury [1]. Hepatic stellate cells (HSCs) are the central fibrogenic cell type implicated in liver fibrosis [2]. During liver injury, HSCs are differentiated into proliferative myofibroblasts-like cells, resulting in a massive gathering of extracellular matrix (ECM) proteins leading to distortion of the hepatic physiological architecture [3].

Interestingly, transforming growth factor-β (TGF-β) is a crucial fibrotic moderator tangled in liver fibrosis, significantly influencing its pathophysiology [4]. It acts as a potent activator of HSCs [5], triggers expression of genes that link to fibrosis, and subsequently increases the deposition of

Cite this article: Atta, A., Elkady, M., Mansour, A., Abd El-Aziz., H. and Abd-Allah, G. Hepatoprotective Effect of Quercetin against CCl_4 Induced Liver Fibrosis: miRNA-124-3p Regulation in Rat Model. Azhar International Journal of Pharmaceutical and Medical Sciences, *2025; Vol 5 (2):179-190.* doi: 10.21608/aijpms.2025.335012.1299.

DOI : 10.21608/aijpms.2025.335012.1299.

hepatic ECM components [6, 7]. It also simultaneously inhibits matrix turnover by suppressing matrix metalloproteinases [8]. Thus, attenuating TGF-β activity is an attractive target for combating liver fibrogenesis.

Importantly, specific treatments for hepatic fibrosis are lacking. Nevertheless, natural compounds are suggested to slow down or reduce the advancement of the condition. In this context, quercetin (QUE), a prominent flavonoid found abundantly in vegetables and fruits, exhibits a variety of potential pharmacological and biological actions [9, 10].

Recently several researchers have confirmed the effective role of microRNAs (miRNAs) in controlling liver fibrosis [11, 12]. MicroRNAs are conserved, small non-coding RNAs of approximately 22 nucleotides in length, that function as regulators of gene expression after transcription. In this regard, miRNAs are integral to a wide range of biological processes, and their aberrant profiles are tied to the genesis and advancement of diverse illnesses, including liver fibrosis [13]. Among these miRNAs, miRNA-124 has been shown to play varied functions in both normal and pathological processes, highlighting its importance in cellular regulation and disease mechanisms [14-16]. As well as it has also been reported that miRNA-124 controls renal and pulmonary fibrosis [17, 18].

In liver fibrosis, inflammation exhibits a principal role in the pathophysiology of the disease [19]. Numerous investigations have emphasized the potential effect of miRNA-124 in mitigating inflammation by modulating the expression pattern of key inflammatory target genes, thus regulating inflammatory responses [20-22]. Additionally, the recent study by Zhang in 2021 indicated that TGF-β1 was a target of miRNA-124 which can inhibit the upregulation of hypertrophic scar fibroblasts [23].

This work was designed to clarify the molecular regulatory effect of QUE on the expression pattern of miRNA-124-3p and to assess its association with the fibrotic marker (TGF-β1) in liver fibrosis induced by CCl_4.

2. METHODS

2.1. Chemicals and drugs

Carbon tetrachloride (CCl_4), QUE, and dimethyl sulfoxide (DMSO) were sourced from Sigma-Aldrich (Co. St. Louis, MO, USA). CCl_4 was dissolved in corn oil (50% v/v), while QUE was diluted in 10% DMSO (10mg/2ml). CCl_4 dose was (2mL/kg) [24], while the QUE dose was (5mg/kg) [25]. All the other compounds were of the highest available commercial quality.

2.2. Ethical approval

The experimental protocol and animal care procedures followed the guidelines established by Al-Azhar University's Research Ethics Committee - Faculty of Pharmacy (Girls) - Egypt (serial approval number: PhD 328/2022).

2.3. Animals

Twenty-four male albino rats, each weighing (200 ± 20 g), were obtained from the Nile Company for Pharmaceuticals & Chemical Industries, Cairo, Egypt. Rats had access to both pellets and tap water without restriction. Rats were adapted to the lab environment for two weeks. They were housed in plastic cages (6 rats/cage) in a standard environment of (12-12 h) light-dark cycle, (25 ºC temperature), and (60%-70% humidity).

2.4. Experimental design

Twenty-four rats were arbitrarily allocated into 4 groups (n= 6) as follows: I) Control group: subcutaneously (SC) injected with corn oil (2mL/kg, twice a week) and received oral 10% DMSO (0.1/day), 8 weeks, II) QUE group: received oral QUE (5mg/kg/day, 8 weeks), III) CCl_4 group: SC injected with CCl_4 (2mL/kg, twice a week for 8 weeks), IV) CCl_4 + QUE group: SC injected with CCl_4 (2mL/kg, twice a week) and received oral QUE (5mg/kg/day) for 8 weeks.

Afterward, animals received anesthesia using light ether, and blood samples were obtained from rat's retro-orbital sinuses. These samples were then centrifuged to isolate sera. These sera samples were then kept at -80 ◦C for subsequent biochemical analysis. The rat's livers were removed and subsequently divided into three portions. The first portion was used for biochemical investigations (oxidative stress biomarkers and TGF-β1), the second portion was used for microRNA analysis, and the third portion was assigned for histopathological evaluation.

2.5. Assessment of hepatotoxicity indices

Colorimetric determination of the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum was assayed with commercial kits (Biodiagnostics Company, Cairo, Egypt) following the manufacturers' instructions [26].

2.6. Assessment of oxidative stress levels

Spectrophotometric determination of hepatic malondialdehyde (MDA), Catalase (CAT), and superoxide dismutase (SOD) levels were evaluated following the protocol articulated by Ohkawa et al.

[27], Sinha [28], and Marklund and Marklund [29], respectively.

2.7. Assessment of pro-inflammatory cytokines

Tumor necrosis factor- α (TNF-α) and interleukin 6 (IL-6) levels were assessed following the manufacturers' instructions using a rat enzyme-linked immunosorbent (ELISA) kits (Bioassay Technology Laboratory, Shanghai, China, [Cat. # E0135Ra] & [Cat. # E0764Ra], respectively).

2.8. Assessment of fibrotic marker, TGF-β1:

TGF-β1 levels were measured in liver homogenates following the manufacturers' instructions using a rat sandwich ELISA kit (Cat. # MBS824788, MyBioSource, San Diego, California, USA.).

2.9. Assessment of miRNA-124 gene expressions:

Three steps were carried out to quantify miRNA-124-3p gene expressions:

2.9.1. Isolation of total RNA:

Total RNA from hepatic tissue lysate was extracted with miRNeasy Mini Kit (QIAGEN®, USA, Cat. # 217004). Then, nuclease-free water (Invitrogen, Germany) was utilized for the isolated RNA for further purification. All RNA aliquots' concentrations (ng/mL) and purities were measured using a NanoDrop®1000 spectrophotometer (ND®-1000, Thermo Scientific, Wilmington, DE, USA). The RNA samples were conserved at - 80 ºC [30].

2.9.2. Reverse transcription and quantification of miRNA-124-3p expressions using qRT-PCR:

The Complementary DNA (cDNA) synthesis and PCR amplification of miRNA-124 was performed in a single reaction tube using the miRCURY LNA™ miRNA PCR Starter kit (QIAGEN®, USA, Catalog no.: 339320). The cDNA was produced efficiently from the extracted RNA using miRCURY RT Enzyme Mix containing a unique thermostable reverse transcriptase. The relative expression profiles of miRNA-124 were determined by a miRCURY SYBR® Green master mix (2X), SYBR Green dye, Taq DNA polymerases, and primers in the presence of ROX dye. The miRNA-124 expression was standardized to a small nuclear RNA RNU6 (U6). The amplification curve was determined from 65˚C to 95 ˚C. The miRNA-124 quantification relative to values of U6 was measured using the $2^{-\Delta Ct}$ values, where ΔCt = (CtmiR-124 - CtU6). The fold changes of miRNA-124 were computed by using the $2^{-\Delta\Delta Ct}$ method. The primers utilized were defined in (Table 1).

Table 1. The qRT-PCR primer's sequences

Product	Primer's sequence (5′- 3′)
miRNA-124-3p (target)	**Forward: 5'-GCGGCCGTGTTCACAGCGGACC-3'** **Reverse: 5'-GTGCAGGGTCCGAGGT-3'**
RNU6 (U6) (reference)	**Forward: 5′-CTCGCTTCGGCAGCACA-3′** **Reverse: 5-AACGCTTCACGAATTTGCGT-3′**

2.10. Histopathological examination

Liver specimens were thoroughly rinsed and subsequently fixed in 10% neutral-buffered formalin. Following fixation, the samples were trimmed, dehydrated in graded alcohol solutions, cleared in xylene, and embedded in paraffin wax. Five µm sections were obtained using a rotary microtome. These sections were then subjected to staining with hematoxylin and eosin (H&E) before being examined under a light microscope [31].

2.11. Statistical Analysis:

The GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA) for Windows® was used for all statistical analysis. The quantitative alteration among the studied groups will be evaluated by a one-way analysis of variance (ANOVA) test followed by post hoc Tukey's test for parametric variables. The correlation between parameters has been assessed via Pearson's correlation coefficient. The criteria for statistical significance were at $p < 0.05$.

3. RESULTS

3.1. Effect of QUE on hepatic aminotransferases

The hepatotoxicity of CCl_4 was evidenced by a 3-fold and 4.5-fold increase in serum AST and ALT levels, respectively, compared to the control group. Interestingly, QUE (5mg/kg) co-treatment significantly decreased their levels by 38.1% and 49%, respectively, relative to the CCl_4 group (Fig. 1A and B).

3.2. Effect of QUE on oxidative stress markers

The effects of CCl_4 and/or QUE (5mg/kg) administration on various oxidative stress markers are illustrated in (Fig. 2A, B, and C). The hepatic MDA levels in the CCl_4 group exhibited a substantial elevation by 4.4-fold relative to the control group. Meanwhile, co-treatment with QUE (5mg/kg) significantly improved this surge by 36.4% in comparison to the CCl_4 group (Fig. 2A). Furthermore, the CCl_4 group exhibited a significant decrease in hepatic SOD and CAT activities by 46.2% and 81.3%, respectively, in comparison to the control group. Nevertheless, as compared to the CCl_4 group, QUE (5mg/kg) co-treatment led to a marked increase in their activities by 1.2-fold and 3.3-fold, respectively (Fig. 2B and C).

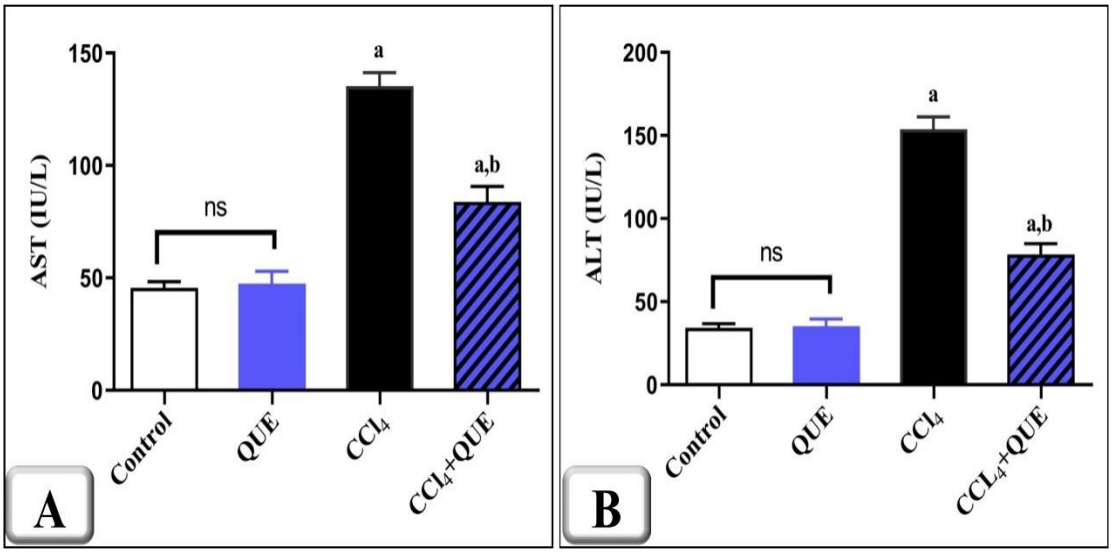

Figure1. Effect of co-treatment with QUE (5mg/kg) on hepatotoxicity markers in response to CCl_4-induced hepatic damage in rats. (A) AST and (B) ALT. All values are shown as mean ± SD. (a) and (b) Significance when compared to control and CCl_4 groups, respectively at $P < 0.05$, as determined by the ANOVA test. AST: aspartate transaminase, ALT: alanine transaminase, CCl_4: Carbon tetrachloride, QUE: Quercetin, ns: non-significant.

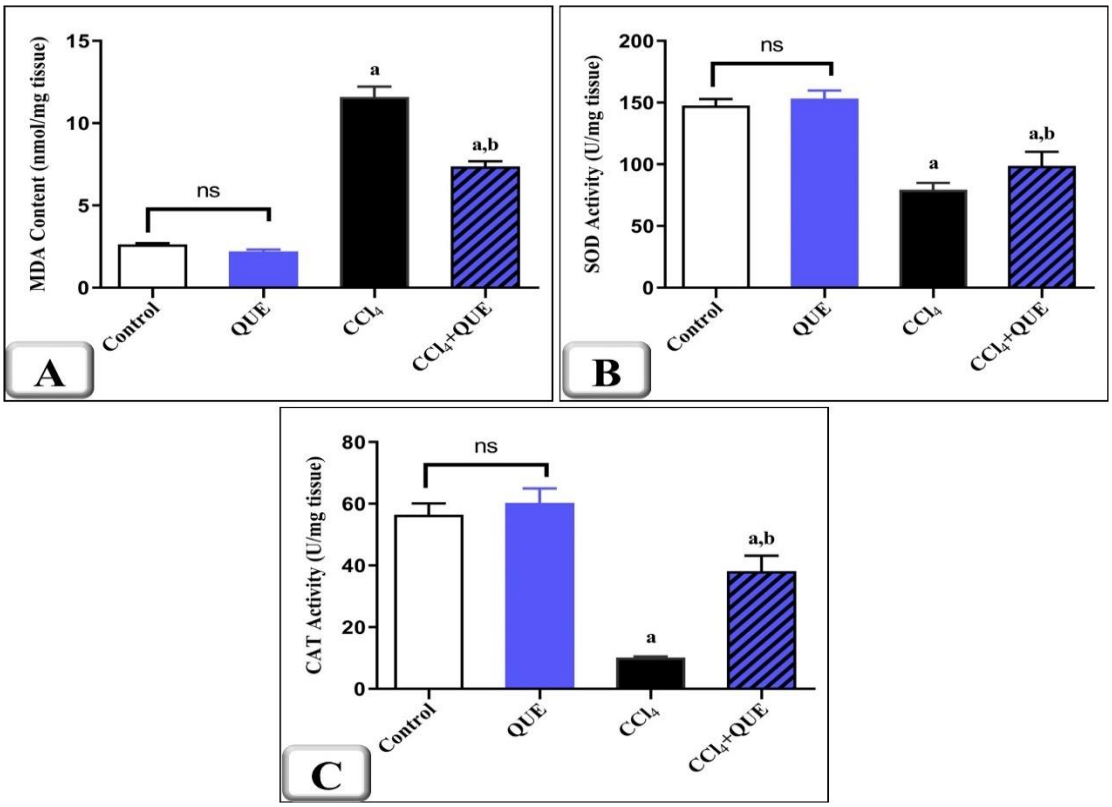

Figure 2. Effect of co-treatment with QUE (5mg/kg) on indicators of oxidative stress in response to CCl_4-induced hepatic damage in rats. (A) MDA, (B) SOD, and (D) CAT. All values are shown as mean ± SD. (a) and (b) Significance when compared to control and CCl_4 groups, respectively at $P < 0.05$, as determined by the ANOVA test. MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, CCl_4: Carbon tetrachloride, QUE: Quercetin, ns: non-significant.

3.3. Effect of QUE on pro-inflammatory indicators

A notable characteristic of liver damage attributed to CCl_4 is Inflammation. The CCl_4 group had markedly elevated levels of IL-6 and TNF-α by 2.1-fold and 2.5-fold, respectively, when compared to the control group. Conversely, QUE (5mg/kg) co-treatment significantly decreased the levels of these cytokines by 29.3% and 33.5%, respectively, when compared to the CCl_4 group (Fig. 3A and B).

3.4. Effect of QUE on fibrotic marker, TGF-β1

The CCl_4 group has a significant 3.1-fold elevation in TGF-β1 levels when compared to the control group. Alternatively, co-treatment with QUE (5mg/kg) significantly decreased its levels by 44.6% compared to the CCl_4 group (Fig. 4).

3.5. Effect of QUE on miRNA-124-3p expression

The expression of miRNA-124-3p decreased significantly by 80.5% in the CCl_4 group when compared to the control group. Conversely, QUE (5mg/kg) co-treatment significantly boosted miRNA-124-3p expression by 2.5-fold in comparison to the CCl_4 group (Fig. 5).

3.6. Correlation between miRNA-124-3p and TGF-β1 in the studied groups

The correlation between miRNA-124-3p level and TGF-β1 was evaluated using the Pearson correlation coefficient. The results revealed a strong negative correlation between TGF-β1 and miRNA-124-3p, $r= -0.9043$ at $p< 0.0001$ (Fig. 6). Therefore, miRNA-124-3p/TGF-β1 molecular axis may be effective as a therapeutic target for managing liver fibrosis.

3.7. Effect of QUE on liver histopathological assessment

Liver sections of the control and quercetin-only-treated groups showed normal histopathological features, with no signs of fibrosis (Fig. 7A and B). On the contrary, the CCl_4 group showed significant pathological alterations, including the presence of thick fibrous bands with moderate inflammatory infiltrate, multiple complete nodules with scattered apoptotic hepatocytes, and marked macro-vesicular steatosis (Fig. 7C). Notably, the CCl_4+QUE group showed significant improvement in general histological features showed as an average central vein with intact hepatocytes in peri-venular area, and a mild degree of steatosis (Fig. 7D).

4. DISCUSSION

Liver fibrosis represents a major global health issue caused by various chronic liver diseases. Hepatocellular carcinoma and end-stage cirrhosis, the two primary contributors to liver-related mortality, are caused by liver fibrosis [11]. CCl_4 is recognized as a hepatotoxic agent that induces liver fibrosis in experimental models, and its effects closely resemble the clinical features of human liver cirrhosis, making it a preferred model among the different experimental approaches to studying liver fibrosis [32]. Upon CCl_4 administration, significant hepatic injury and dysfunction are observed, as evidenced by an elevation of serum liver transaminases due to the leaking of these enzymes from damaged hepatocytes into the bloodstream, a decrease in protein synthesis, and an alteration in lipid profile [33, 34].

In the current study, serum ALT and AST were substantially augmented in the CCl_4 group compared to the controls. These findings were significantly resolved by the co-administration of QUE. The inhibitory effect of QUE on aminotransferases aligns with prior investigations by **Wang et al. and Ekpo et al**., who reported that QUE preserves hepatic enzymes homeostasis by functioning as a membrane-stabilizing agent that attenuates liver cell injury [25, 35].

Oxidative stress has emerged as a vital determinant in the pathogenesis of liver fibrosis. It initiates the fibrotic process through several mechanisms: it induces hepatocellular injury, stimulates the release of profibrogenic mediators, promotes recruitment of inflammatory cells, and directly activates HSCs, which collectively contribute to the advancement of fibrotic liver disease [36, 37]. The hepatotoxic effects of CCl_4 are primarily attributed to its metabolism by hepatic cytochrome P450 enzymes, which convert CCl_4 into highly reactive trichloromethyl radicals. These radicals initiate a cascade of oxidative processes, including lipid peroxidation, leading to cellular membrane damage and subsequent release of inflammatory mediators [38, 39]. MDA serves as a key lipid peroxidation product and a prominent biomarker for cellular oxidative damage, and an increase in MDA concentration reflects an accelerated rate of lipid peroxidation [35]. In the current study, MDA level was markedly elevated in the CCl_4 group. This observation aligns with recent research by **Abdelghffar et al**., who reported a surge in MDA content in CCl_4-intoxicated mice [40].

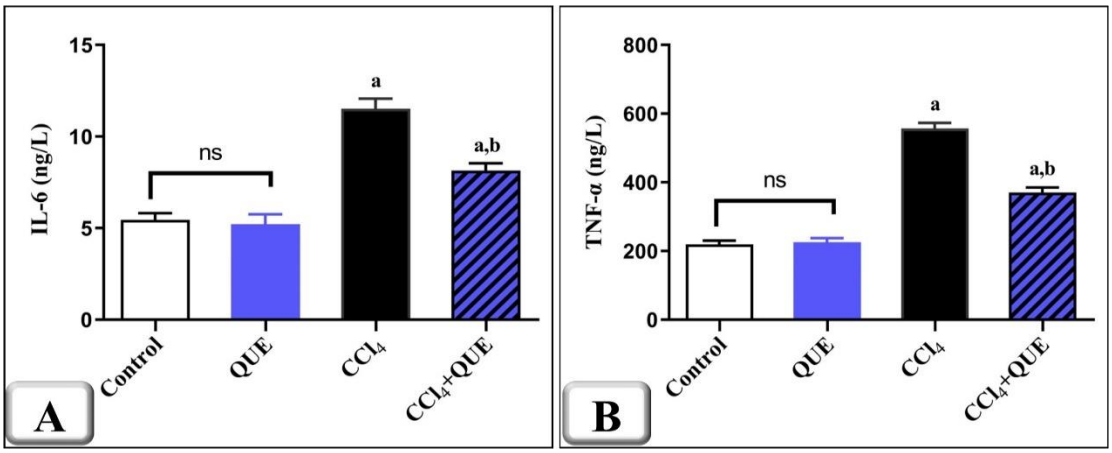

Figure 3. Effect of co-treatment with QUE (5mg/kg) on inflammatory markers in response to CCl_4-induced hepatic damage in rats. (A) IL-6 and (B) TNF-α. All values are shown as mean ± SD. (a) and (b) Significance when compared to control and CCl_4 groups, respectively at $P < 0.05$, as determined by the ANOVA test. IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha, CCl_4: Carbon tetrachloride, QUE: Quercetin, ns: non-significant.

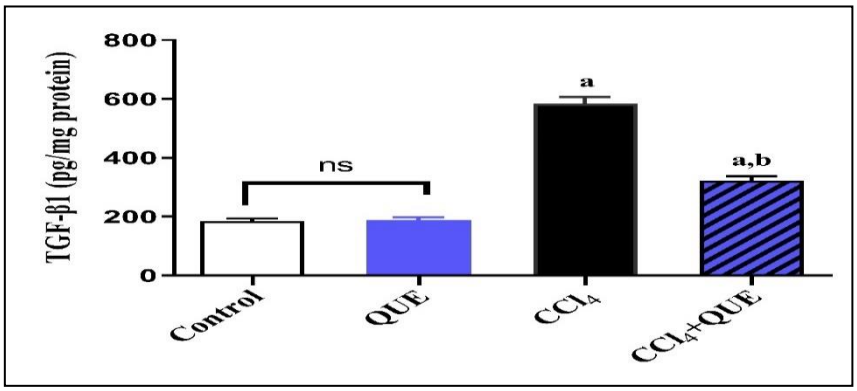

Figure 4. Effect of co-treatment with QUE (5mg/kg) on TGF-β1 in response to CCl_4-induced hepatic damage in rats. All values are shown as mean ± SD. (a) and (b) Significance when compared to control and CCl_4 groups, respectively at $P < 0.05$, as determined by the ANOVA test. TGF-β1: Transforming growth factor-βeta 1, CCl_4: Carbon tetrachloride, QUE: Quercetin, ns: non-significant.

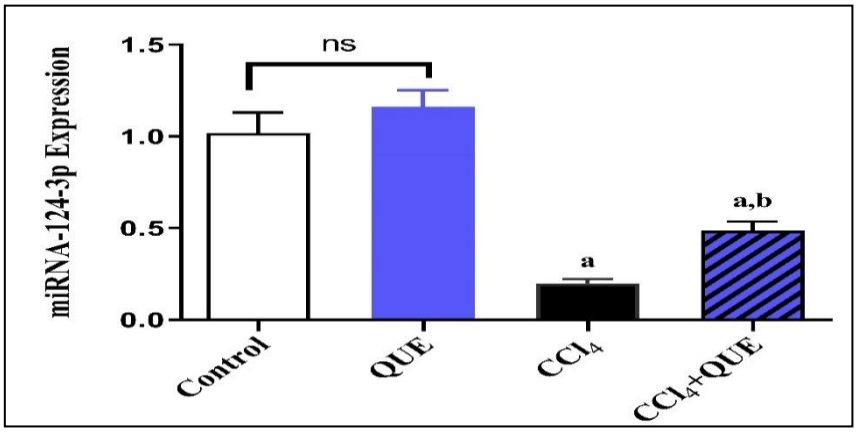

Figure 5. Effect of co-treatment with QUE (5mg/kg) on miRNA-124-3p expression in response to CCl_4-induced hepatic damage in rats. The expression of miRNA-123-3p is based on qRT-PCR analysis. All values are shown as mean ± SD. (a) and (b) Significance when compared to control and CCl_4 groups, respectively at $P < 0.05$, as determined by the ANOVA test. CCl_4: Carbon tetrachloride, QUE: Quercetin, ns: non-significant.

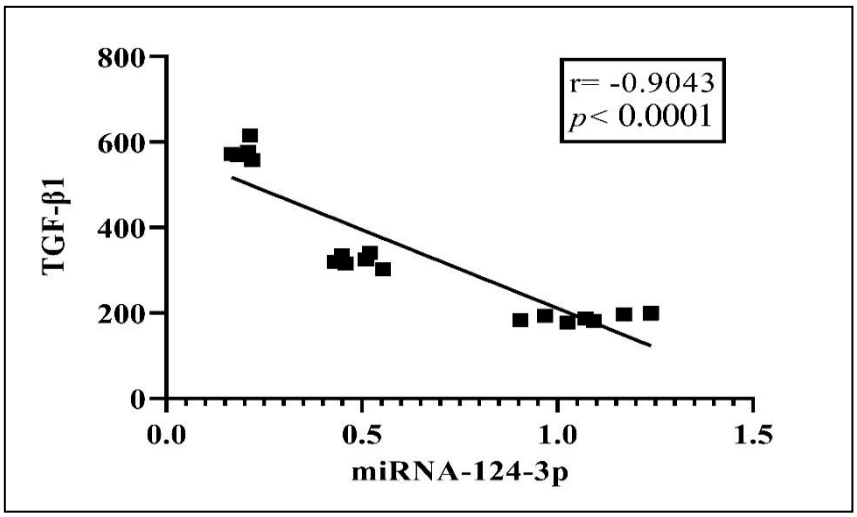

Figure 6. Association of miRNA-124-3p with TGF-β1

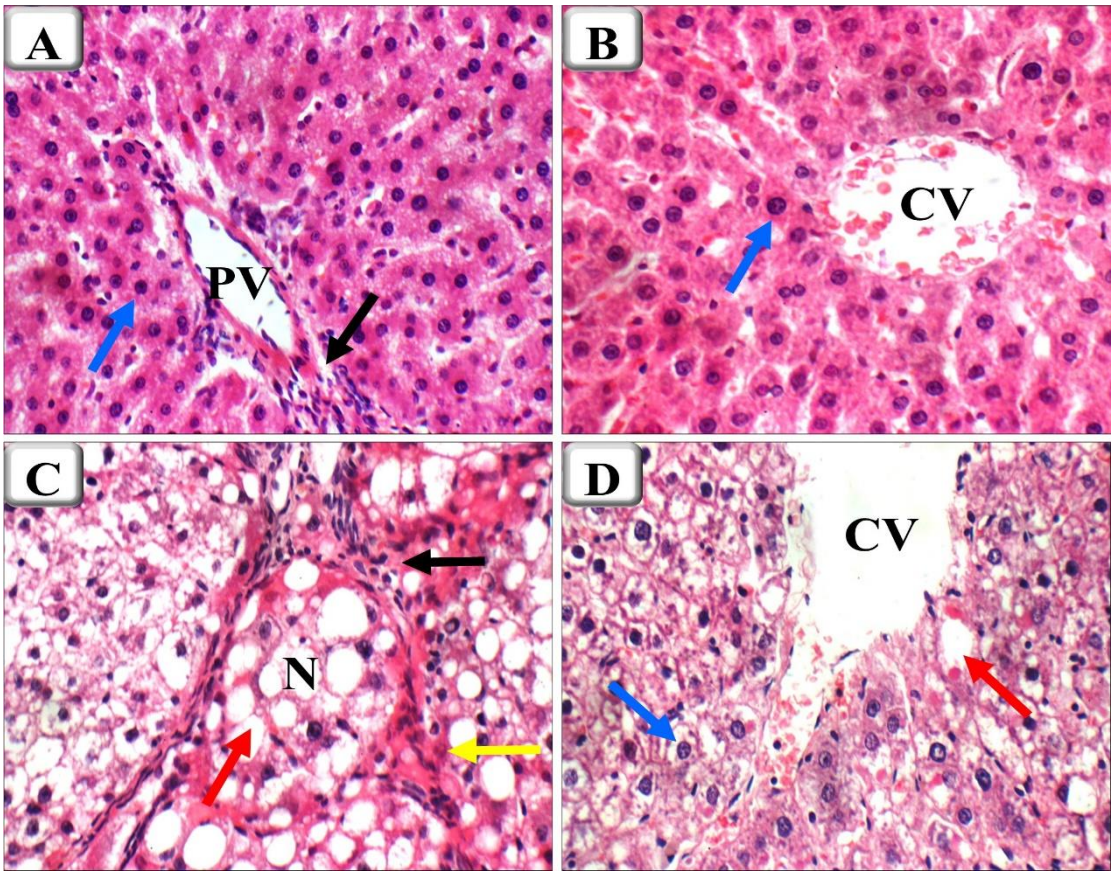

Figure 7. Representative photomicrographs of liver sections stained with hematoxylin and eosin (H&E X400). **(A)** Liver tissues obtained from rats in the control group showing an average portal vein (PV) with an average portal tract (black arrow) and normal intact hepatocytes in the peri-portal area (blue arrow). **(B)** Liver tissues obtained from rats in the QUE-only-treated group showing an average central vein (CV) with normal intact hepatocytes in the peri-venular area (blue arrow). **(C)** Liver tissues obtained from rats in the CCl_4 group showing thick fibrous bands with moderate inflammatory infiltrate (black arrow), multiple complete nodules (N) with scattered apoptotic hepatocytes (yellow arrow), and marked macro-vesicular steatosis (red arrow). **(D)** Liver tissues obtained from rats in the QUE+CCl_4 group showing an average central vein (CV) with normal intact hepatocytes in the peri-venular area (blue arrow) and mild macro-vesicular steatosis (red arrow).

Additionally, CCl_4 administration is strongly associated with a marked deficiency in antioxidant capacity. This correlation is observed by significant reductions in key antioxidant molecules and enzymes, including reduced glutathione, CAT, and SOD [41, 42]. Our results revealed a substantial reduction in the hepatic profile of SOD and CAT following CCl_4 administration. QUE co-treatment notably ameliorated these alterations, consistent with findings from a previous study by **El-Nekeety et al.**, who demonstrate QUE's potential to restore redox homeostasis due to its ability to reduce lipid peroxidation (MDA) and boost antioxidants production (glutathione peroxidase and SOD) [43].

The interplay between oxidative stress and cytokine-mediated inflammation is a crucial step in the progress of liver fibrosis [44]. Elevated oxidative stress leads to the secretion of TNF-α, IL-6, and IL-1β [45]. In particular, IL-6 has been linked to the severity of liver fibrosis, it can increase HSC survival, proliferation, and activity [45]. Additionally, TNF-α exacerbates hepatocyte injury, promotes the stellate cell's activation and proliferation, enhances the deposition of ECM components, amplifies inflammatory and fibrotic responses, and hence, contributes to liver fibrosis [46-49]. In this research, the CCl_4 group presented a notable inflammatory response confirmed by the upsurge of both IL-6 and TNF-α profiles. However, QUE displayed a distinctive anti-inflammatory function via lowering their levels, similar to previous studies [50, 51].

TGF-β1 is a major contributor to liver fibrosis, which induces the transdifferentiate of stellate cells into fibrogenic myofibroblasts. It plays a role in all phases of disease progression, from the initiation of liver damage via inflammation and fibrosis to excessive hepatocyte apoptosis, cirrhosis, and cancer [2, 38, 52]. In this work, it was found that the level of TGF-β1 was substantially elevated in the rats that were injected with CCl_4 only and its level was decreased with QUE co-treatment, protecting against hepatic fibrosis. These results align with the prior research findings indicating that QUE can attenuate liver fibrosis in LX-2 cells (human immortalized HSC line) by reducing TGF-β1 expression [53].

MicroRNAs represent another significant factor in hepatic fibrosis pathogenesis. The expression patterns of miRNAs offer a further precise reflection of pathophysiological conditions compared to mRNA expression patterns. Consequently, miRNAs have the potential to function as valuable diagnostic and prognostic biomarkers in hepatic fibrosis, while concurrently acting as targets for antifibrotic agents [54, 55]. Several recent research have displayed that QUE has beneficial effects on miRNA expression in fibrotic models [11, 56, 57]. Therefore, we proceeded with our investigation by assessing the expression pattern of miRNA-124-3p in CCl_4-induced liver fibrosis and QUE co-administration. MiRNA-124 was found to be downregulated in different fibrotic models [58, 59], as well as in hepatocellular carcinoma (HCC) [14]. Moreover, miRNA-124 is known as an anti-inflammatory miRNA and recently, it was reported that miRNA-124 overexpression can diminish HSCs cytokines secretion including TNF-α, IL-6, and IL-1β, consequently decreasing the inflammatory responses [60].

This finding demonstrated the anti-inflammatory role of miRNA-124-3p in liver fibrosis. Furthermore, a recent research study by Mahmoudi et al**.** verified the impact of miRNA-124 overexpression on the TGF-β1 expression in the pathogenesis of non-alcoholic fatty liver disease. Real-time PCR results revealed that overexpression of miRNA-124 after transfection significantly reduced TGF-β1 expression [61]. Another recent supportive investigation demonstrated that miRNA-124-3p-enriched exosomes (ExomiRNA-124-3p) treatment reduces collagen accumulation, attenuates inflammation, and inhibits TGF-β1 activity [45].

In line with these studies, our findings showed that miRNA-124-3p expression decreased in CCl_4-intoxicated rats, but co-treatment with QUE leads to upregulation of its expression. Moreover, it should be noted that an inverse substantial association was observed between the expression of miRNA-124-3p and TGF-β1 level, showing that miRNA-124-3p can potentially prevent the progress of hepatic fibrosis via modulating the TGF-β1. As a result, therapeutic strategies focused on restoring the inappropriate expression of miRNAs are considered novel treatments for liver fibrosis [62, 63]. Thus, the restoration of miRNA-124-3p expression can be a promising strategy for ameliorating liver fibrosis.

5. CONCLUSION

QUE alleviates liver fibrosis by inhibiting TGF-β1 expression through overexpression of miRNA-124-3p. Therefore, the regulation of the miRNA-124-3p/TGF-β1 axis is a promising therapeutic approach for liver fibrosis.

Acknowledgment: NA

Funding: NA

Conflicts of Interest: NA

Author Contribution:

Amira A. Atta: Conceptualization, Methodology, Resources, Formal Analysis, Writing – original draft. Mohamed **A. Elkady:** Conceptualization, Methodology, Supervision, Formal analysis, Writing – review & editing. **Ahmed M. Mansour**: Conceptualization, Supervision, Data curation, Methodology, Writing – review & editing. **Heba Gamal Abd El-Aziz**: Conceptualization, Investigation, Supervision, Data curation, Writing – review & editing. **Gamil M. Abd-Allah:** Conceptualization, Methodology, Investigation, Supervision, Writing – review & editing.

REFERENCES

1. Li H, Liu T, Yang Y, Cho WC, Flynn RJ, Harandi MF, et al. Interplays of liver fibrosis-associated microRNAs: Molecular mechanisms and implications in diagnosis and therapy. Genes Dis. 2023;10(4):1457-69. https://doi.org/10.1016/j.gendis.2022.08.013

2. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol. 2017;14(7):397-411. https://doi.org/10.1038/nrgastro.2017.38

3. Ezhilarasan D. MicroRNA interplay between hepatic stellate cell quiescence and activation. Eur J Pharmacol. 2020;885:173507. https://doi.org/10.1016/j.ejphar.2020.173507

4. Brenner DA. Molecular pathogenesis of liver fibrosis. Trans Am Clin Climatol Assoc. 2009;120:361-8. PMCID: PMC2744540

5. Vázquez-Flores LF, Casas-Grajales S, Hernández-Aquino E, Vargas-Pozada EE, Muriel P. Antioxidant, Antiinflammatory, and Antifibrotic Properties of Quercetin in the Liver. Liver Pathophysiology: Elsevier; 2017. p. 653-74. https://doi.org/ 10.1016/B978-0-12-804274-8.00047-3

6. Casas-Grajales S, Vazquez-Flores LF, Ramos-Tovar E, Hernandez-Aquino E, Flores-Beltran RE, Cerda-Garcia-Rojas CM, et al. Quercetin reverses experimental cirrhosis by immunomodulation of the proinflammatory and profibrotic processes. Fundam Clin Pharmacol. 2017;31(6):610-24. https://doi.org/10.1111/fcp.12315

7. Wu L, Zhang Q, Mo W, Feng J, Li S, Li J, et al. Quercetin prevents hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing autophagy via the TGF-beta1/Smads and PI3K/Akt pathways. Sci Rep. 2017;7(1):9289. https://doi.org/10.1038/s41598-017-09673-5

8. Kim KK, Sheppard D, Chapman HA. TGF-beta1 Signaling and Tissue Fibrosis. Cold Spring Harb Perspect Biol. 2018;10(4):a022293. https://doi.org/10.1101/cshperspect.a022293

9. Miltonprabu S, Tomczyk M, Skalicka-Wozniak K, Rastrelli L, Daglia M, Nabavi SF, et al. Hepatoprotective effect of quercetin: From chemistry to medicine. Food Chem Toxicol. 2017;108(Pt B):365-74. https://doi.org/10.1016/j.fct.2016.08.034

10. Zhao X, Wang J, Deng Y, Liao L, Zhou M, Peng C, et al. Quercetin as a protective agent for liver diseases: A comprehensive descriptive review of the molecular mechanism. Phytother Res. 2021;35(9):4727-47. https://doi.org/10.1002/ptr.7104

11. Nozari E, Moradi A, Samadi M. Effect of Atorvastatin, Curcumin, and Quercetin on miR-21 and miR-122 and their correlation with TGFbeta1 expression in experimental liver fibrosis. Life Sci. 2020;259:118293. https://doi.org/10.1016/j.lfs.2020.118293

12. Zeng C, Wang YL, Xie C, Sang Y, Li TJ, Zhang M, et al. Identification of a novel TGF-beta-miR-122-fibronectin 1/serum response factor signaling cascade and its implication in hepatic fibrogenesis. Oncotarget. 2015;6(14):12224-33. https://doi.org/10.18632/oncotarget.3652

13. He Y, Huang C, Zhang SP, Sun X, Long XR, Li J. The potential of microRNAs in liver fibrosis. Cell Signal. 2012;24(12):2268-72. https://doi.org/10.1016/j.cellsig.2012.07.023

14. Long HD, Ma YS, Yang HQ, Xue SB, Liu JB, Yu F, et al. Reduced hsa-miR-124-3p levels are associated with the poor survival of patients with hepatocellular carcinoma. Mol Biol Rep. 2018;45(6):2615-23. https://doi.org/10.1007/s11033-018-4431-1

15. Yan G, Li Y, Zhan L, Sun S, Yuan J, Wang T, et al. Decreased miR-124-3p promoted breast cancer proliferation and metastasis by targeting MGAT5. Am J Cancer Res. 2019;9(3):585-96. PMID: 30949412

16. Liang Y, Xie J, Che D, Zhang C, Lin Y, Feng L, et al. MiR-124-3p helps to protect against acute respiratory distress syndrome by targeting p65. Biosci Rep. 2020;40(5):BSR20192132. https://doi.org/10.1042/BSR20192132

17. Zhang S, Xu L, Liang R, Yang C, Wang P. Baicalin suppresses renal fibrosis through microRNA-124/TLR4/NF-kappaB axis in streptozotocin-induced diabetic nephropathy mice and high glucose-treated human proximal tubule epithelial cells. J Physiol Biochem. 2020;76(3):407-16. https://doi.org/10.1007/s13105-020-00747-z

18. Wang D, Zhang H, Li M, Frid MG, Flockton AR, McKeon BA, et al. MicroRNA-124 controls the proliferative, migratory, and inflammatory phenotype of pulmonary vascular fibroblasts. Circ Res. 2014;114(1):67-78. https://doi.org/10.1161/CIRCRESAHA.114.301633

19. Luedde T, Schwabe RF. NF-kappaB in the liver--linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2011;8(2):108-18. https://doi.org/10.1038/nrgastro.2010.213

20. Sun Y, Li Q, Gui H, Xu DP, Yang YL, Su DF, et al. MicroRNA-124 mediates the cholinergic anti-inflammatory action through inhibiting the production of pro-inflammatory cytokines. Cell Res. 2013;23(11):1270-83. https://doi.org/10.1038/cr.2013.116

21. Ma C, Li Y, Li M, Deng G, Wu X, Zeng J, et al. microRNA-124 negatively regulates TLR signaling in alveolar macrophages in response to mycobacterial infection. Mol Immunol. 2014;62(1):150-8. https://doi.org/10.1016/j.molimm.2014.06.014

22. Han J, Pu CX, Xiao QX, Tang LJ, Liu T, He L, et al. miRNA-124-3p targeting of LPIN1 attenuates inflammation and apoptosis in aged male rats cardiopulmonary bypass model of perioperative neurocognitive disorders. Exp Gerontol. 2021;155:111578. https://doi.org/10.1016/j.exger.2021.111578

23. Zhang S, Pan S. miR-124-3p targeting of TGF-beta1 inhibits the proliferation of hypertrophic scar fibroblasts. Adv Clin Exp Med. 2021;30(3):263-71. https://doi.org/10.17219/acem/131753

24. Hassan MH, Edfawy M, Mansour A, Hamed AA. Antioxidant and antiapoptotic effects of capsaicin against carbon tetrachloride-induced hepatotoxicity in rats. Toxicol Ind Health. 2012;28(5):428-38. https://doi.org/10.1177/0748233711413801

25. Wang R, Zhang H, Wang Y, Song F, Yuan Y. Inhibitory effects of quercetin on the progression of liver fibrosis through the regulation of NF-small ka, CyrillicB/Ismall ka, CyrillicBalpha, p38 MAPK, and Bcl-2/Bax signaling. Int Immunopharmacol. 2017;47:126-33. https://doi.org/10.1016/j.intimp.2017.03.029

26. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56-63. https://doi.org/10.1093/ajcp/28.1.56

27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. https://doi.org/10.1016/0003-2697(79)90738-3

28. Sinha AK. Colorimetric assay of catalase. Analytical biochemistry. 1972;47(2):389-94. https://doi.org/10.1016/0003-2697(72)90132-729.

Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

30. Elkady MA, Doghish AS, Elshafei A, Elshafey MM. MicroRNA-567 inhibits cell proliferation and induces cell apoptosis in A549 NSCLC cells by regulating cyclin-dependent kinase 8. Saudi J Biol Sci. 2021;28(4):2581-90. https://doi.org/10.1016/j.sjbs.2021.02.001

31. Culling CFA. Handbook of histopathological and histochemical techniques: including museum techniques: Butterworth-Heinemann; 2013: p.671-681.

32. Chen Y, Zhao C, Liu X, Wu G, Zhong J, Zhao T, et al. Plumbagin ameliorates liver fibrosis via a ROS-mediated NF-small ka, CyrillicB signaling

pathway in vitro and in vivo. Biomed Pharmacother. 2019;116:108923. https://doi.org/10.1016/j.biopha.2019.108923

33. Lu Y, Hu D, Ma S, Zhao X, Wang S, Wei G, et al. Protective effect of wedelolactone against CCl4-induced acute liver injury in mice. Int Immunopharmacol. 2016;34:44-52. https://doi.org/10.1016/j.intimp.2016.02.003

34. Amer MA, Othman AI, El-Missiry MA, Farag AA, Amer ME. Proanthocyanidins attenuated liver damage and suppressed fibrosis in CCl4-treated rats. Environ Sci Pollut Res Int. 2022;29(60):91127-38. https://doi.org/10.1007/s11356-022-22051-7

35. Ekpo GI, Ofem Effiom E, Ndodo O, Nseobong B, Obinaju Effiong B, Anietie Eyo R, et al. Quercetin and hesperidin protection against hepato-renal damage occasioned by carbon tetrachloride (CCL4) in a rat model. Pharmacological Research - Modern Chinese Medicine. 2024;11:100429. https://doi.org/10.1016/j.prmcm.2024.100429

36. Sanchez-Valle V, Chavez-Tapia NC, Uribe M, Mendez-Sanchez N. Role of oxidative stress and molecular changes in liver fibrosis: a review. Curr Med Chem. 2012;19(28):4850-60. https://doi.org/10.2174/092986712803341520

37. Roehlen N, Crouchet E, Baumert TF. Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives. Cells. 2020;9(4):875. https://doi.org/10.3390/cells9040875

38. El-Ashmawy NE, Al-Ashmawy GM, Fakher HE, Khedr NF. The role of WNT/beta-catenin signaling pathway and glutamine metabolism in the pathogenesis of CCl(4)-induced liver fibrosis: Repositioning of niclosamide and concerns about lithium. Cytokine. 2020;136:155250. https://doi.org/10.1016/j.cyto.2020.155250

39. Heindryckx F, Colle I, Van Vlierberghe H. Experimental mouse models for hepatocellular carcinoma research. Int J Exp Pathol. 2009;90(4):367-86. https://doi.org/10.1111/j.1365-2613.2009.00656.x

40. Abdelghffar EA, Obaid WA, Alamoudi MO, Mohammedsaleh ZM, Annaz H, Abdelfattah MAO, et al. Thymus fontanesii attenuates CCl(4)-induced oxidative stress and inflammation in mild liver fibrosis. Biomed Pharmacother. 2022;148:112738. https://doi.org/10.1016/j.biopha.2022.112738

41. Mahmoud AM, Hozayen WG, Hasan IH, Shaban E, Bin-Jumah M. Umbelliferone Ameliorates CCl(4)-Induced Liver Fibrosis in Rats by Upregulating PPARgamma and Attenuating Oxidative Stress, Inflammation, and TGF-beta1/Smad3 Signaling. Inflammation. 2019;42(3):1103-16. https://doi.org/10.1007/s10753-019-00973-8

42. Alamri ZZ. Effect of Luteolin and Quercetin on Thioacetamide Induced Hepatic Fibrosis in Rats. International Journal of Pharmacology. 2019;15(7):863-71. https://doi.org/10.3923/ijp.2019.863.871

43. El-Nekeety AA, Abdel-Azeim SH, Hassan AM, Hassan NS, Aly SE, Abdel-Wahhab MA. Quercetin inhibits the cytotoxicity and oxidative stress in liver of rats fed aflatoxin-contaminated diet. Toxicol Rep. 2014;1:319-29. https://doi.org/10.1016/j.toxrep.2014.05.014

44. Pomacu MM, Trasca MD, Padureanu V, Buga AM, Andrei AM, Stanciulescu EC, et al. Interrelation of inflammation and oxidative stress in liver cirrhosis. Exp Ther Med. 2021;21(6):602. https://doi.org/10.3892/etm.2021.10034

45. Niknam B, Baghaei K, Mahmoud Hashemi S, Hatami B, Reza Zali M, Amani D. Human Wharton's jelly mesenchymal stem cells derived-exosomes enriched by miR-124 promote an anti-fibrotic response in an experimental model of liver fibrosis. Int Immunopharmacol. 2023;119:110294. https://doi.org/10.1016/j.intimp.2023.110294

46. Nathwani R, Mullish BH, Kockerling D, Forlano R, Manousou P, Dhar A. A review of liver fibrosis and emerging therapies. EMJ Hepatology 44 2019. 2020;4(4):105-16. https://doi.org/10.33590/emj/10310892.

47. Lu S, Wang Y, Liu J. Tumor necrosis factor-alpha signaling in nonalcoholic steatohepatitis and targeted therapies. J Genet Genomics. 2022;49(4):269-78. https://doi.org/10.1016/j.jgg.2021.09.009

48. Vachliotis ID, Polyzos SA. The Role of Tumor Necrosis Factor-Alpha in the Pathogenesis and Treatment of Nonalcoholic Fatty Liver Disease. Curr Obes Rep. 2023;12(3):191-206. https://doi.org/10.1007/s13679-023-00519-y

49. Osawa Y, Hoshi M, Yasuda I, Saibara T, Moriwaki H, Kozawa O. Tumor necrosis factor-

alpha promotes cholestasis-induced liver fibrosis in the mouse through tissue inhibitor of metalloproteinase-1 production in hepatic stellate cells. PLoS One. 2013;8(6):e65251. https://doi.org/10.1371/journal.pone.0065251

50. Mashaly MA, Saleh IG, Ashour AA, Mansour AM. The potential role of febrile condition in reversing the hepatoprotective effects of quercetin in the livers of LPS-presensitized mice. Life Sci. 2022;302:120647. https://doi.org/10.1016/j.lfs.2022.120647

51. Li X, Jin Q, Yao Q, Xu B, Li L, Zhang S, et al. The Flavonoid Quercetin Ameliorates Liver Inflammation and Fibrosis by Regulating Hepatic Macrophages Activation and Polarization in Mice. Front Pharmacol. 2018;9:72. https://doi.org/10.3389/fphar.2018.00072

52. Fabregat I, Moreno-Caceres J, Sanchez A, Dooley S, Dewidar B, Giannelli G, et al. TGF-beta signalling and liver disease. FEBS J. 2016;283(12):2219-32. https://doi.org/10.1111/febs.13665

53. Shakerian E, Afarin R, Akbari R, Mohammadtaghvaei N. Effect of Quercetin on the fructose-activated human hepatic stellate cells, LX-2, an in-vitro study. Mol Biol Rep. 2022;49(4):2839-45. https://doi.org/10.1007/s11033-021-07097-z

54. Kumar V, Mahato RI. Delivery and targeting of miRNAs for treating liver fibrosis. Pharm Res. 2015;32(2):341-61. https://doi.org/10.1007/s11095-014-1497-x

55. Chavali S, Bruhn S, Tiemann K, Saetrom P, Barrenas F, Saito T, et al. MicroRNAs act complementarily to regulate disease-related mRNA modules in human diseases. RNA. 2013;19(11):1552-62. https://doi.org/10.1261/rna.038414.113

56. Alshammari GM, Al-Qahtani WH, AlFaris NA, Alzahrani NS, Alkhateeb MA, Yahya MA. Quercetin prevents cadmium chloride-induced hepatic steatosis and fibrosis by downregulating the transcription of miR-21. Biofactors. 2021;47(3):489-505. https://doi.org/10.1002/biof.1724

57. Toker C, Kuyucu Y, Saker D, Kara S, Guzelel B, Mete UO. Investigation of miR-26b and miR-27b expressions and the effect of quercetin on fibrosis in experimental pulmonary fibrosis. J Mol Histol. 2024;55(1):25-35. https://doi.org/10.1007/s10735-023-10168-z

58. Li X, Li H, Zhang S, Zhang R, Li J, Wei Y, et al. Protective effect of Idelalisib on carbon tetrachloride-induced liver fibrosis via microRNA-124-3P/phosphatidylinositol-3-hydroxykinase signalling pathway. J Cell Mol Med. 2021;25(24):11185-97. https://doi.org/10.1111/jcmm.17039

59. Xiao Y, Wang J, Yan W, Zhou Y, Chen Y, Zhou K, et al. Dysregulated miR-124 and miR-200 expression contribute to cholangiocyte proliferation in the cholestatic liver by targeting IL-6/STAT3 signalling. J Hepatol. 2015;62(4):889-96. https://doi.org/10.1016/j.jhep.2014.10.033

60. Yang J, Xu C, Wu M, Wu Y, Jia X, Zhou C, et al. MicroRNA-124 inhibits hepatic stellate cells inflammatory cytokines secretion by targeting IQGAP1 through NF-kappaB pathway. Int Immunopharmacol. 2021;95:107520. https://doi.org/10.1016/j.intimp.2021.107520

61. Mahmoudi A, Jalili A, Aghaee-Bakhtiari SH, Oskuee RK, Butler AE, Rizzo M, et al. Analysis of the therapeutic potential of miR-124 and miR-16 in non-alcoholic fatty liver disease. J Diabetes Complications. 2024;38(4):108722. https://doi.org/10.1016/j.jdiacomp.2024.108722

62. Zhu S, Chen X, Chen Y, Li XF, Chen SY, Li JJ, et al. Role of microRNAs in Hepatic Stellate Cells and Hepatic Fibrosis: An Update. Curr Pharm Des. 2021;27(27):3000-11. https://doi.org/10.2174/1381612826666201023143542

63. Hyun J, Jung Y. MicroRNAs in liver fibrosis: Focusing on the interaction with hedgehog signaling. World J Gastroenterol. 2016;22(29):6652-62. https://doi.org/10.3748/wjg.v22.i29.6652