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Investigating the Potential of Curcumin in Reducing Liver Fibrosis via Inhibition of TGF-β1 Gene Expression

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Authors:
Reyhane Ebrahimi [1]
Dr.Abbas Sahebghadam Lotfi [2*]

1. Department of Clinical Biochemistry, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran, reyhane_ebrahimi@modares.ac.ir
2. Department of Clinical Biochemistry, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran, lotfi_ab@modares.ac.ir

**** Corresponding author:***
Dr.Abbas Sahebghadam Lotfi

ABSTRACT

Introduction: Transforming growth factor (TGF)-β1 is crucial in developing liver fibrosis. Curcumin has been shown to effectively halt the advancement of liver fibrosis by inhibiting the TGF-β1/Smad signaling pathway. Nevertheless, curcumin's impact on liver fibrosis regression remains unclear. This study explored the involvement of curcumin and TGF-β1 in the regression of liver fibrosis.

Methods: An experimental male C57BL/6 mice model included 6 treatment groups. The treatment groups were injected with carbon tetrachloride (CCl4) for 4 and 6 weeks to induce liver fibrosis, negative controls were injected with olive oil. After cessation of injection, 2 of the treatment groups were given curcumin for 2 weeks. TGF-β1 expression in liver cells was analyzed by real-time PCR assay. ELISA analyzed hydroxyproline liver tissue levels. Values of $p<0.05$ were regarded as statistically significant.

Results: CCl4 injection induced liver fibrosis and significantly increased TGF-β1 expression and hydroxyproline levels in tissues. Curcumin administration decreased the expression of TGF-β1 and hydroxyproline levels in the liver and accelerated the regression of liver fibrosis.

Conclusion: Curcumin accelerates regression of liver fibrosis, likely through decreasing TGF-β1 expression in the liver.

Keywords:
Liver fibrosis, Carbon tetrachloride, Curcumin, TGF-β1, Hydroxyproline

INTRODUCTION

Liver diseases account for approximately 2 million deaths annually worldwide, with 1 million cases due to complications of cirrhosis and 1 million cases due to viral hepatitis and hepatocellular carcinoma [1].The most common causes of chronic liver diseases include hepatitis B virus, hepatitis C virus, alcohol-related liver disease (ALD), and non-alcoholic fatty liver disease (NAFLD) [2]. Liver fibrosis results from a prolonged response in the wound-healing process, triggered by various chronic liver injuries, such as viral infections, autoimmune diseases, metabolic disorders, drug toxicity, alcoholic liver disease, and non-alcoholic fatty liver disease [3]. No matter the cause, clinical evidence indicates that liver fibrosis can regress once the underlying cause is eliminated [4]. The signaling pathways of Transforming Growth Factor Beta1 (TGF-β1) play a critical role in regulating several cellular processes such as proliferation, differentiation, migration, and apoptosis, all of which are vital for maintaining balance in tissues and organs. TGF-β1 is a significant regulator of liver health, influencing all stages of disease development, from the initial liver injury through inflammation and fibrosis to cirrhosis and hepatocellular carcinoma [5]. The pro-fibrogenic effects of TGF-β1 in liver fibrosis primarily occur through the activation of hepatic stellate cells, which are the main producers of extracellular matrix during liver fibrosis. TGF-β1 triggers the activation, proliferation, and differentiation of HSCs into myofibroblasts while inhibiting their apoptosis. This leads to excessive synthesis of ECM proteins, including fibronectin and collagens types I, III, and IV. Additionally, TGF-β1 disrupts the production of matrix-degrading proteases and increases the levels of protease inhibitors, such as tissue inhibitors of metalloproteinases (TIMP) and plasminogen activator inhibitors [6, 7]. Therefore, inhibiting

the TGF-β1/Smad signaling pathway is crucial for restraining the activation and proliferation of HSCs and improving liver fibrosis [8].

The primary curcuminoid found in turmeric is called curcumin, which is a polyphenol that was first isolated from the rhizome of Curcuma longa about two centuries ago. Its structure, known as diferuloylmethane, was identified in 1910 [9]. Curcumin has been extensively studied as an anti-fibrosis agent associated with TGF-β1. Additionally, several studies using animal models of chronic liver diseases have demonstrated that curcumin exhibits anti-fibrogenic properties by modulating various processes involved in liver damage; This includes targeting different cellular signaling pathways, such as TGF-β1 and Inhibition of hepatic stellate cell activation, cytokines, transcription factors, and genes regulating cell proliferation and apoptosis [10, 11].

We have consequently assessed the effectiveness of curcumin in treating liver fibrosis induced by CCl4 in animal models and examined the levels of TGF-β1 expression and hydroxyproline within this model.

MATERIALS AND METHODS

Experimental Animals

This research was conducted as an in vivo experimental study employing male C57BL/6 mice. A total of 30 male C57BL/6 mice, aged 6-8 weeks and weighing an average of 20 ± 2 g, were utilized in the study. The research was approved and registered by the Ethics Committee of Tarbiat Modarres University of Medical Sciences under a specific code IR.MODARES.AEC.1403.003.

Animal Grouping

1. Control Groups (4 and 6 Weeks): Each group consisted of five mice. These mice received olive oil twice a week for either 4 or 6 weeks.
2. Fibrosis Groups (4 and 6 Weeks): Each group comprised five mice. The mice were treated with carbon tetrachloride (CCl_4) diluted in olive oil at a concentration of 0.5 ml/g BW, administered intraperitoneally.
3. Curcumin Treatment Groups: Each group included five mice with fibrosis. After cessation of CCl4 injection, These mice were given curcumin(8203540002, Sigma-Aldrich Chemie GmbH, Germany) at a dosage of 100 mg/kg BW every other day for two weeks via gavage.

RNA isolation and real-time PCR

The total RNA was extracted from the mouse liver using TRIzol. Subsequently, cDNA was synthesized using a cDNA Synthesis Kit (A101161,parstous, Iran). Real-time PCR was performed to TGF gene expression (normalized to GAPDH) using specific primers (Table 1) and SYBR Green Master Mix.

Table1. Primers applied for Real-time PCR assays

Target gene	Forward primer (5'-3')	Reverse primer (5'-3')
TGF-β1	CAACGCCATCTATGAGAAAACCA	AAAGCCCTGTATTCCGTCTCC
GAPDH	GAGAGTGTTTCCTCGTCCCGTA	TGCCGTGAGTGGAGTCATACT

ELISA

The concentration of hydroxyproline in liver tissue was measured using a kit(KHPO96, Kiazist, Iran) Tissue samples were homogenized and then digested with a strong acid before oxidizing. The resulting samples were then reacted with a chromogen. The optical density (OD) of each well was measured at a wavelength of 560 nm. The OD values are directly proportional to the collagen concentration in the samples.

Histopathological Evaluation

The liver tissues were fixed in 10% formalin for 24 hours, dehydrated using an alcohol series from 70% to 100% alcohol, and embedded in paraffin. Thin sections measuring 4-5 micrometers were then cut using a microtome and mounted on glass slides. These sections were stained with Hematoxylin to visualize nuclei and Eosin to highlight cytoplasmic and extracellular matrix components, enabling examination of tissue structure. Slides were observed under a light microscope after being stained with hematoxylin and eosin (H&E).

Statistical Analysis

The data were analyzed using GraphPad Prism, version 9.5.1 for Windows, a statistical analysis program. A 95% confidence interval (α=0.05) was used as a decision threshold for significance. Statistical analyses were performed

using one-way ANOVA followed by Tukey's post hoc test. Results are expressed as mean ± SD.

RESULT

Results Histopathological analysis

The staining of Hematoxylin and eosin (H&E) showed normal liver architecture in control mice. Fibrotic mice showed disrupted architecture with cell death and infiltration of mononuclear inflammatory cells around vessels and liver parenchyma. Curcumin-treated mice showed reduced inflammation, cell death, and partial tissue repair

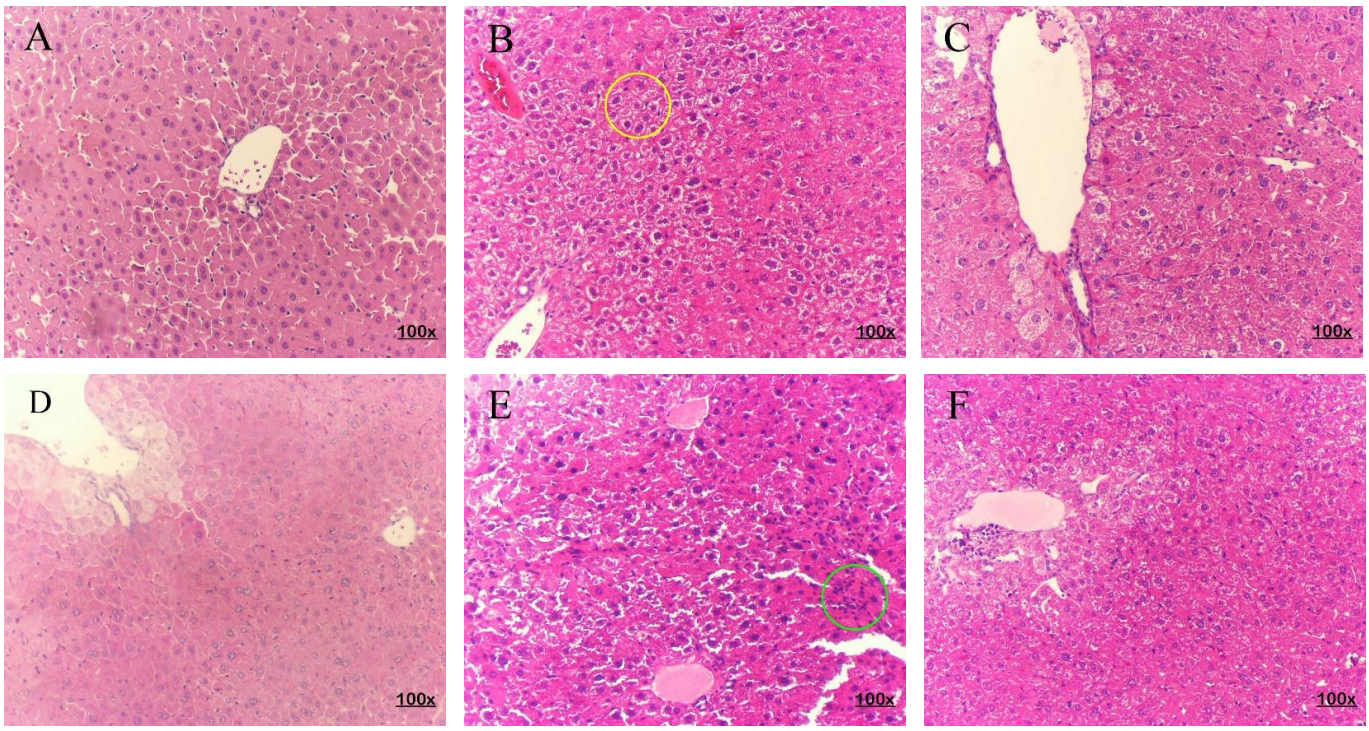

Figure 1. Representative liver histopathology of H&E-stained mice livers in different experimental groups. A: Control, treated with olive oil for 4 weeks; B: Liver fibrosis, treated with CCl4 for 4 weeks (The circle indicates necrotic cells.); C: Curcumin-treated liver fibrosis, treated with CCl4 4 weeks + curcumin 2 weeks; D: Control, treated with olive oil 6 weeks; E: Liver fibrosis, treated with CCl4 6 weeks (The circle indicates mononuclear inflammatory cells.) ; F: Curcumin-treated liver fibrosis, treated with CCl4 4 weeks + curcumin 2 weeks.

Measurement of Hydroxyproline Levels in Liver Tissue

Hydroxyproline levels in the 4-week CCl4-induced liver fibrosis group significantly increased compared to the 4-week control group that received olive oil($P < 0.01$). In the group with 4-week CCl4-induced liver fibrosis, followed by two weeks of curcumin treatment, hydroxyproline levels significantly decreased($P < 0.01$). Similarly, hydroxyproline levels in the 6-week CCl4-induced liver fibrosis group significantly increased compared to the 6-week control group that received olive oil($P < 0.001$). In the 6-week CCl4-induced liver fibrosis group, followed by two weeks of curcumin treatment, hydroxyproline levels also significantly decreased($P < 0.01$). Additionally, hydroxyproline levels were significantly higher in the 6-week fibrosis group compared to the 4-week fibrosis group($P < 0.001$). Although curcumin treatment significantly reduced hydroxyproline levels in the 6-week fibrosis group, they did not return to control levels(Figure 2).

mRNA Expression of the TGF-β1 Gene

In the 4-week CCl_4-induced liver fibrosis group, TGF-β1 gene expression significantly increased compared to the 4-week control group that received olive oil ($P < 0.001$). However, following two weeks of curcumin treatment, expression levels significantly decreased ($P < 0.01$). A similar trend was observed in the 6-week CCl_4-induced liver fibrosis group, where gene expression markedly increased compared to the corresponding 6-week control group ($P < 0.0001$), but was significantly reduced after two weeks of curcumin treatment ($P < 0.05$). Furthermore, the TGF-β1 expression level in the 6-week fibrosis group was notably higher than that in the 4-week fibrosis group ($P < 0.05$) (Figure 3).

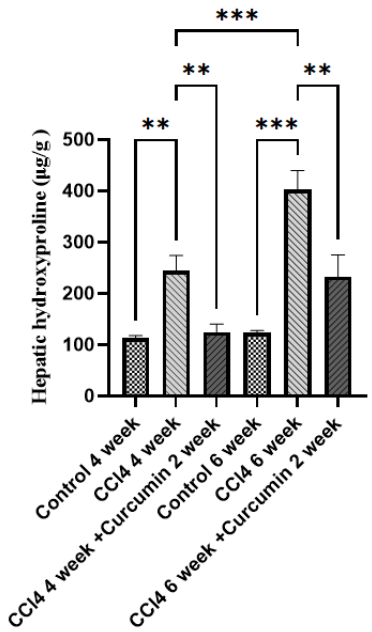

Figure 2: Changes in liver tissue hydroxyproline levels across the study groups. Data are presented as Mean ±SD. *P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001, **** P-value <0.0001, compared to the respective control groups.

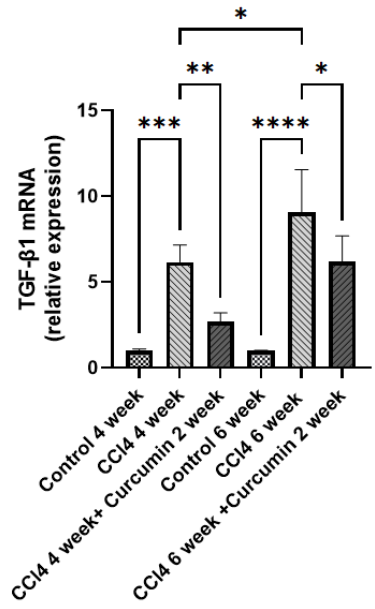

Figure 3: Expression of the TGF-β1 gene in the study groups. Data are presented as mean ± SD. * P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001, **** P-value < 0.0001.

DISCUSSION

Liver fibrosis is a consequence of various chronic conditions and frequently progresses to cirrhosis. Cirrhosis is linked to severe and life-threatening complications, such as liver failure, portal hypertension, and hepatocellular carcinoma, and is a leading cause of both morbidity and mortality globally.[12, 13] However, clinical and experimental evidence has shown that even advanced fibrosis and cirrhosis can be reversed [12-15]. Thus, preventing the progression from fibrosis to cirrhosis is regarded as a key goal in the management of patients with liver diseases [16].

This research examined the therapeutic potential of curcumin in treating liver fibrosis after its etiology has been removed using a CCl4-induced mouse model. Histopathological analysis showed a significant improvement in the liver structure. Also, the level of hydroxyproline and TGF beta 1 gene expression showed a significant decrease in the group treated with curcumin compared to the fibrotic group.

The CCl4 liver fibrosis model is the most widely used toxic model for inducing liver fibrosis globally. The mechanism of liver fibrosis induced by CCl4 has been extensively studied, and the most effective administration route is through intraperitoneal injection of CCl4 [17, 18]. The present study observed that TGF-β1 gene expression and hydroxyproline levels in the 6-week liver fibrosis group were significantly higher than in the 4-week liver fibrosis group. It was a week. This difference may be due to the longer duration of exposure to carbon tetrachloride (CCl_4) in the 6-week liver fibrosis group. As mentioned in previous studies, increasing the dose and duration of CCl_4 exposure aggravated the liver damage and accelerated the progression of fibrosis. Studies have shown that carbon tetrachloride significantly increases the level of fibrotic genes such as TGF-β1 by causing oxidative damage and chronic inflammation in the liver. It causes the accumulation of collagen and fibrosis [17, 19].

Liver fibrosis is characterized by progressive accumulation of extracellular matrix (ECM), which destroys the physiological architecture of the liver [20]. TGF-β1 is found in the liver at all stages of the development of pathological conditions. Early on, it induces apoptosis of hepatocytes and differentiation of stellate cells into myofibroblasts after chronic injury, leading to the inflammatory process and liver fibrosis. also acts to promote the proliferation of stellate cells and maintain the myofibroblastic phenotype, leading to cirrhosis [21]. TGF-β1 is the key cytokine driving liver fibrogenesis, and inhibiting its synthesis is one of the primary targets in creating antifibrotic drugs [16, 22].

Curcumin has potential utility in the prevention and treatment of various types of liver

diseases, including types of liver diseases, including non-alcoholic and alcoholic fatty liver, fibrosis, cirrhosis, and liver cancer [10].

We have demonstrated that curcumin treatment significantly reduced the expression levels of TGF-β1 in liver tissue (Figure 3). The reduction in of expressionTGF-β1 was associated with a decrease in the degree of liver fibrosis. Curcumin's pronounced mitigation of CCl_4-induced liver inflammation and fibrosis likely occurred through the inhibition of the TGF-β1/Smad signaling pathway [23, 24]. In addition, the level of hydroxyproline, which is an indicator of the amount of collagen in the liver tissue, was significantly reduced in the groups treated with curcumin. Our results are consistent with previous findings that showed Curcumin not only inhibits the proliferation of hepatic stellate cells (HSCs) and their transformation into an activated phenotype; but also promotes HSC apoptosis. Inducing apoptosis in HSCs reduces the secretion of type I collagen and decreases TGF-β1 production [25, 26]. Overall, curcumin may safely improve liver fibrosis by several different mechanisms.

CONCLUSION

our findings demonstrate that the administration of curcumin effectively protected mice livers from CCl4-induced liver injury and fibrogenesis possibly by reducing TGF-β1 levels. Further research is needed to explain these mechanisms in more detail.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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