Transcriptional factors of FAT/CD36, PTP1B, SREBP-1c and HNF4A are involved in dyslipidemia following Cyclosporine A treatment in the liver of rats: The rescue effect of curcumin

Abstract

Cyclosporine A (CsA) is a potent immunosuppressive drug which has been reported to cause various disorders including hepatotoxicity. However, the precise molecular mediators participated in CsA-induced liver injury remains poorly understood. This study aimed to characterize the transcription factors involved in lipid metabolism in the context of hepatic injury induced by cyclosporine A (CsA), both independently and in conjunction with curcumin. A total of twenty -eight male adult Wistar rats were assigned into four groups including control (Con), sham, cyclosporine A (CsA) and cyclosporineA +curcumin (CsA+cur). The rats were administered CsA at a dosage of 30 mg/kg and curcumin at 40 mg/kg via a gastric tube for a duration of 28 days. RT-PCR and also Masson trichrome staining were employed to measure related changes. Finally, CsA exposure caused a significant increase in protein tyrosine phosphatase 1B (PTP1B), Fatty acid translocase CD36 (FAT/CD36), sterol regulatory element-binding protein-1c (SREBP-1c) and a significant decrease in hepatocyte nuclear factor 4 Alpha (HNF4A) genes expressions compared to the control and sham group. The CsA treatment also significantly elevated plasma lipids (LDL, cholesterol, triglyceride) and liver enzymes (alanine aminotransferase (ALT) aspartate aminotransferase (AST), alkaline phosphatase (ALP)), compared to the control and sham group. Fibrotic changes were detected in CsA group by

Masson trichrome staining. Curcumin consumption resulted in a considerable improvement in histological disorders and molecular mediators involved in liver injury following CsA treatment. Taken together, these findings proposed that CsA can cause deleterious effect to liver tissue via lipid homeostasis disorders mediated by FAT/CD36, PTP1B and HNF4A gene expression alterations. It also reveals that these negative effects of CsA can be mitigated by using curcumin as an antioxidant and anti-inflammatory supplement.

Keywords: Cyclosporine A; liver; curcumin; fibrosis; FAT/CD36

Abbreviations: CsA, cyclosporine A; PTP1B, protein tyrosine phosphatase 1B; SEM, standard error of the mean; FAT/CD36, Fatty acid translocase CD36; SREBP-1c, sterol regulatory element-binding protein-1c; HNF4A, significant decrease in hepatocyte nuclear factor 4 Alpha; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; IL-2, interleukin-2; DILI, drug-induced liver injury; ROS, reactive oxygen specious; TC, total cholesterol; TGs, triglycerides; EDTA, Ethylenediaminetetraacetic acid; ANOVA, one-way analysis of variance; FATP 2/5, fatty acid transporter proteins 2 and 5.

1. Introduction

The survival of transplanted organs is one of the most outstanding concerns in today's medical world. So long-term immunosuppressive drugs are consistently being taken by organ transplant patients (1). In 1980 Cyclosporine A (CsA) - the most widely used immunosuppressant drug- entered the medical field. This hydrophobic ring is obtained from the *Tolypocladium fungus*. CsA specifically inhibits the activity of T-helper cells. consequently weakening the proliferative

effects of interleukin-2 (IL-2) (2). CsA, which has low toxicity on bone marrow cells, exhibits a significant inhibitory effect in preventing graft tissue rejection and suppressing autoimmune diseases. Although this drug is widely used on medical practice, especially to prevent the rejection of transplanted tissues, it has unwanted side effects that occasionally cause critical problems such as nephrotoxicity, hepatotoxicity, hypertension, and cardiotoxicity (3). Functional and morphological changes in liver tissue have been shown in research on humans or laboratory animal models linked to the consumption of CsA. These functional changes in the liver include increases in transaminase and alkaline phosphatase enzymes (4). Additionally, blood bilirubin and the production of bile salts increases. Most importantly a disturbance in the release of lipids from the liver correlated to this drug has been established in previous studies (1). Morphological changes include the activation of Kupffer cells, loss of the trabecular system, steatosis, necrosis, and hepatitis (1). Also, several studies on cells and animals have shown that CsA leads to hyperlipidemia, thereby increasing the risk of atherosclerosis and hepatic steatosis (5). Steatosis refers to the accumulation of lipids, primarily triglycerides, phospholipids, and cholesterol esters, within hepatocytes. In these conditions the continuation of damaging stimulus results in drug-induced liver injury (DILI), and hepatitis (6). Recently it has been shown that CsA causes hepatotoxicity by increasing the metabolic activities of the liver, mitochondrial damage, increasing reactive oxygen specious (ROS), and increasing oxidative stress (1). However, molecular mechanisms of CsA 's liver toxicity are still unclear.

Several genes affect fat metabolism in the liver, among which PTB1-B SREBP-1c, FAT/CD36, and HNF4α genes can be mentioned. The sterol regulatory element-binding protein 1c (SREBP-1c) as a transcription factor is a pivotal regulator of lipid metabolism, influencing the expression of proteins associated with this process. An increase in this factor's activity escalates lipogenesis,

causes hyperlipidemia, and can initiate liver steatosis (7, 8). Several pharmacological agents can modify the expression of SREBP-1c, for instance, ethanol increases lipid synthesis in the liver by upregulating SREBP-1c, which is one of the mechanisms through which ethanol induces hepatic steatosis (9). Another essential factor in fat metabolism is (CD36/Fatty acid translocase) FAT/CD36, which plays a key role in the uptake of fatty acids by the liver. Hepatic uptake of fatty acids (long-chain fatty acids) is enabled by fatty acid transporter proteins 2 and 5 (FATP 2/5), caveolins, FA translocase (FAT)/CD36, and plasma membrane fatty acid-binding protein (10). Among these factors, FAT/CD36 plays a more important role in this process (11). Recent studies in lipid metabolism have revealed that the protein tyrosine phosphatase 1B (PTB1-B) is a newly identified activator of hepatic lipogenesis. This cytoplasmic enzyme regulates the activity of enzymes by dephosphorylating the tyrosine amino acid. Hyperlipidemia and liver steatosis are caused by increased PTB1-B activity, and inhibition of this enzyme has led to the downregulation of lipogenesis-related genes, such as SREBPs (12). Overall, PTP1B plays a significant role in hepatic lipogenesis and may serve as a novel therapeutic target for improving hepatic steatosis (13). Another cause of hyperlipidemia and hepatic steatosis is the reduction of β-oxidation of fatty acids under the influence of several factors, including a high-fat or High fructose diet, and the use of certain drugs. Hepatic Nuclear Factor 4α (HNF4α) is an intranuclear receptor that plays a pivotal role in regulating the metabolism of fats (β-oxidation of fatty acids), glucose, bile acids, and drugs. HNF4α expression is decreased in diabetes, obesity, non-alcoholic fatty liver, and following the consumption of a high-fat diet, probably as a result of increasing free fatty acids and cholesterol (14). However, whether these aforementioned molecules are participated in liver injury after CsA treatment remains undefined.

Curcumin is the main and active compound of turmeric, whose effects have been proven against liver damage caused by alcohol or non-alcoholic. This yellow phenolic pigment, exhibits a broad spectrum of biological and pharmacological activities, in addition to being a strong antioxidant and scavenger of free radicals, is able to prevent the production of all kinds of oxidant free radicals (15). In the study conducted by Rahmani et al., improvements in various disease characteristics were observed following short-term supplementation with curcumin in patients with non-alcoholic fatty liver disease (15). However, it remains unclear whether curcumin mitigates liver injury following treatment with CsA, and this issue requires further clarification. The first aim of this study is to evaluate the molecular mediators of CsA in liver damage (steatosis and hepatitis) namely PTB1-B (SREBP-1c, FAT/CD36, HNF4α. Secondly, we also aimed to investigate whether curcumin reduces liver injury resulting from the administration of CsA.

2. Materials and methods

In the current study, the animal care and experimental procedures received approval from the Ethics Committee, a section of Research Deputy of the Urmia University of Medical Sciences (IR.UMSU.REC.1398.005). Twenty-eight male adult Wistar rats, with an average weight of 220 ± 10 g (aged 6 to 8 weeks), were randomly assigned to four distinct groups.

1. Control (Con) group: The rats were administered tap water as a vehicle once daily for a duration of 28 days.
2. Sham group: The rats were administered a dimethyl sulfoxide solution (5% DMSO) via gastric gavage once daily for a period of 28 days.

3. Cyclosporine A group: Cyclosporine A (CsA, Sandimmune®, New Jersey) was administered to the rats at a dosage of 30 mg/kg, diluted in dimethyl sulfoxide (DMSO), via gastric tube once daily in the morning at 8:00 AM for a duration of 28 days (16).
4. Cyclosporine A+curcumin (CsA+cur, Merck, India) group. The rats were administered CsA at a dosage of 30 mg/kg at 8:00 AM and curcumin at 40 mg/kg at 10:00 AM, both via oral administration. (17) diluted in DMSO once daily for 28 days.

At the end of 4 weeks, the rats were anesthetized using a combination of with ketamine (60 mg/kg) and xylazine (6 mg/kg), then the blood samples were obtained from the heart of animals in tube containing ethylenediaminetetraacetic acid (EDTA) then centrifuged at 4000 × g for 20 min and obtained plasma stored at −80 °C for later analysis. The liver tissue was excised, carefully separated from surrounding tissues, adipose deposits, and blood clots, and subsequently washed in ice-cold physiological saline. A portion of the liver was frozen using liquid nitrogen and stored at -80°C for gene expression analysis, while another portion was fixed in a 10% buffered formalin solution for histopathological examination.

2.1.Biochemistry analysis

Plasma levels of total cholesterol (TC) and triglycerides (TGs) were quantified using colorimetric and enzymatic methods. The concentrations of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured directly utilizing kits from Biosystem, Barcelona, Spain. The plasma levels of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), were measured through a colorimetric assay in accordance with the manufacturer's instructions (Lab test Diagnostika SNord GmbH, Nordhorn, Germany). Serum albumin levels were determined using an automatic analyzer (Architect c8000 Clinical Chemistry System, Abbott, IL, USA).

2.2.Quantitative real-time PCR

To determine PTP1B, FAT/CD36, SREBP-1c and HNF4A genes expressions in the liver tissue, the samples were homogenized and total RNA was extracted in accordance with the kit instructions. (GeneAll, Cat No. 305-101). The quantity and purity of the extracted RNA were evaluated using a Nanodrop spectrophotometer (Thermo Scientific, USA) to ensure suitability for subsequent molecular techniques. Following this, complementary DNA (cDNA) was synthesized according to the manufacturer's instructions using the Pocket Script RT perMix (BioNeer, Alameda, CA, USA). The synthesized cDNAs served as templates for real-time PCR. The relative expression of mRNA was then analyzed using the accurate 2-(ΔΔCt) method, with results reported as fold changes relative to a housekeeping gene. The primers for the target genes are detailed in Table 1, with sequences obtained from GenBank (http://blast.ncbi.nlm.gov/Blast.cgi). The corresponding primers were validated on the NCBI website using Gene Runner software (Syngene, Cambridge, UK), and the specificity of the novel primer sets was confirmed using Oligo 7 software.

Table 1. Sequences of primers used to evaluate expression of GAPDH, PTP1B, HNF4α, and CD36.

primers	**sequence**
PTP1B(forward)	TTCAAAGTCCGAGAGTCAGG
PTP1B(reverse)	CGGGTCTTTCCTCTTGTCCA
HNF4α(forward)	TGCGACTCTCTAAAACCCTC
HNF4α(reverse)	CTTCAGATGGGGATGTGTCA
CD36(forward)	GACTTGTACTCTCTCCTCGG
CD36(reverse)	AGTAATGAGCCCACAGTTCC
SREBP-1c (forward)	GCGCCTTGACAGGTGAAGTC

SREBP-1c (reverse)	GCCAGGGAAGTCACTGTCTTG
GAPDH (forward)	AGA CAG CCG CAT CTT CTT GT
GAPDH (reverse)	CTT GCC GTG GGT AGA GTC AT

2.3.Histopathological examination

To assess liver tissue fibrosis, 5 μm sections of kidney tissue were stained using Masson's Trichrome staining in accordance with the manufacturer's guidelines (Asiapajohesh, Amol, Iran). The severity of liver fibrosis was evaluated through a semiquantitative method as previously described (18). The scoring system ranged from 0 (indicating normal liver) to 8 (indicating total fibrosis). The criteria for scoring liver fibrosis were defined as follows: grade 0 = normal liver; grade 1 = minimal fibrosis characterized by slight thickening of liver tissue; grades 2 and 3 = moderate thickening of liver tissue without significant structural damage; grades 4 and 5 = increased fibrosis accompanied by complete structural damage and the formation of fibrotic bands or small fibrotic masses; grades 6 and 7 = severe structural disturbances with extensive fibrotic areas in the liver tissue; and grade 8 = total obliteration due to fibrosis (18).

2.4.Statistical analysis

First, data normality was checked by the Kolmogorov-Smirnov test. To identify differences between the groups, one-way analysis of variance (ANOVA) and Tukey's post hoc test were

employed. Results are presented as the mean ± standard error of the mean (SEM). P-values less than 0.05 were considered statistically significant.

3. Results

3.1.Biochemical findings

Table 2 reveals plasma lipid profile in diff erent groups of this study. plasma levels of cholesterol, triglycerides, and low-density lipoprotein (LDL) were markedly elevated in rats treated with CsA ($p < 0.05$) compared control and sham groups. Curcumin administration along with CsA, reduced the cholesterol, triglyceride and LDL level remarkably in the plasma compared to CsA groups ($p < 0.05$). In addition, CsA treatment decreased plasma HDL level and curcumin administration increased it in plasma of CsA treated rats ($p < 0.05$).

As it was shown in Table 3, CsA consumption significantly ($p < 0.05$) increased the levels of AST, ALT and ALP in rat plasma compared to the control group. In contrast, the concurrent administration of curcumin with CsA treatment significantly decreased these enzyme levels ($p < 0.05$). No significant differences were observed in plasma albumin levels among the different groups.

Table 2. Eff ect of CsA with or without curcumin treatment on changes of lipid profile after 28 days. Values are mean ±SEM for 7 rats per group. $p < 0.05$ * Denotes significant diff erence compared to the control and sham groups. † Denotes significant diff erence compared to the CsA group. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin.

	Con	Sham	CsA	CsA +Cur
Cholesterol	64±5.7	63.5±6.1	85.4±5.9*	67.6±13.5†
HDL	34.2±1.03	36.9±2.1	30.2±2.7*	39.8±2.02†
LDL	12±0.82	11±0.9	20.7±3.5*	14.02±2.5†
Triglycerides	111.2±20.2	109±7.2	115.6±4.8*	88±.3.1†

Table 3. Effect of CsA with or without curcumin treatment on liver enzymes and serum albumin after 28 days. Values are mean ±SEM for 7 rats per group. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin. $p < 0.05$ * Denotes significant difference compared to the control and sham groups. † Denotes significant difference compared to the CsA group.

	Con	Sham	CsA	CsA+Cur
ALT	56.48±10.3	57.41±5.21	126.4±5.29*	45.68±3.6†
AST	79.1±5.1	82.11±7.2	215±21.18*	82.5±5.9†
ALP	50.28±1.6	49.31±2.1	60.68±2.38*	39±4.01†
Albumin	3.42±0.13	3.22±0.17	3.06±0.29	3.02±0.4

3.2.FAT/CD36, PTP1B, SREBP-1c and HNF4A gene expressions in the liver tissue

Fig 1, 2, 3, 4 illustrate the impact of CsA administration, both with and without curcumin, on gene expression levels of FAT/CD36, PTP1B, SREBP-1c and HNF4A as mediators of steatosis in the liver of male rats. According to our data, in the present study we reported that CsA consumption significantly increased FAT/CD36, PTP1B and SREBP-1c gene expressions in liver tissue compared to the control and sham groups ($p < 0.05$). While, curcumin usage significantly ($p < 0.05$) declined the mentioned gene expressions in the liver samples of the CsA group. CsA exposure markedly diminished the expression of HNF4A mRNA in the liver samples of male rats compared to the control and sham groups ($p < 0.05$). In the CsA+cur group, there was a significant increase compared to the CsA group ($p<0.05$).

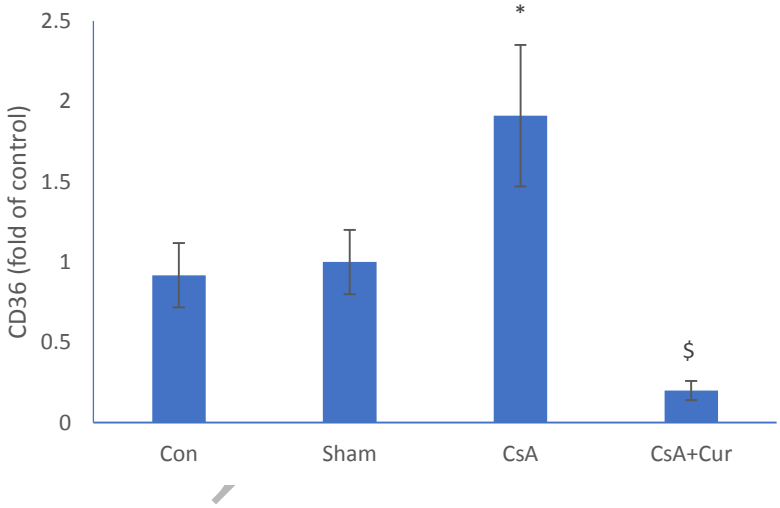

Fig1. Eff ect of CsA with or without curcumin treatment on the expression of FAT/CD36 gene in liver tissue after 28 days. The relative amounts of mRNA were examined using quantitative realtime PCR analysis. Values are mean ±SEM for 7 rats per group. * $p < 0.05$ Denotes significant diff erence compared to the control and sham groups. $ $p < 0.05$ Denotes significant diff erence compared to the CsA group. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin.

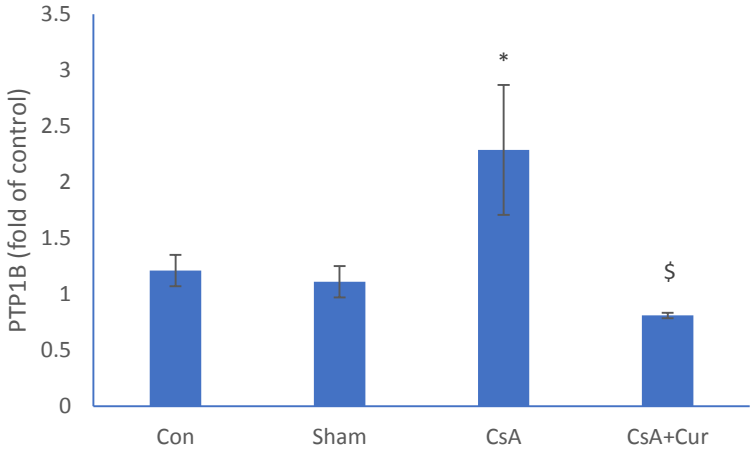

Fig 2. Effect of CsA with or without curcumin treatment on the expression of PTP1B gene in liver tissue after 28 days. The relative amounts of mRNA were examined using quantitative realtime PCR analysis. Values are mean ±SEM for 7 rats per group. * $p < 0.05$ Denotes significant difference compared to the control and sham groups. $ $p < 0.05$ Denotes significant difference compared to the CsA group. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin.

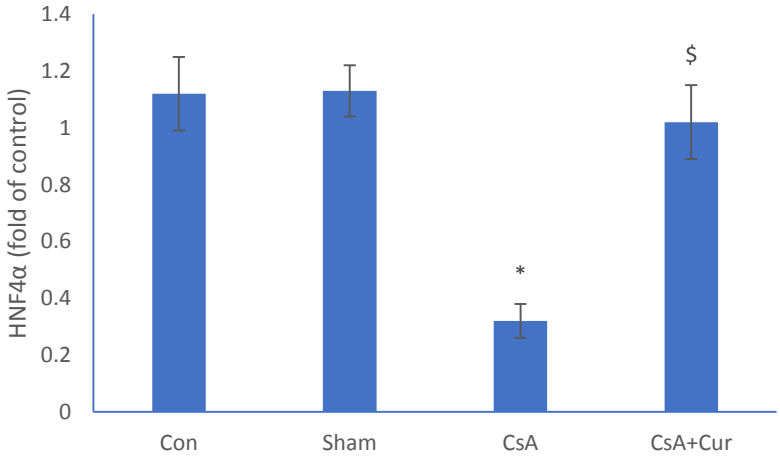

Fig 3. Effect of CsA with or without curcumin treatment on the expression of HNF4α gene in liver tissue after 28 days. The relative amounts of mRNA were examined using quantitative realtime PCR analysis. Values are mean ±SEM for 7 rats per group. * $p < 0.05$ Denotes significant difference compared to the control and sham groups. $ $p < 0.05$ Denotes significant difference compared to the CsA group. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin.

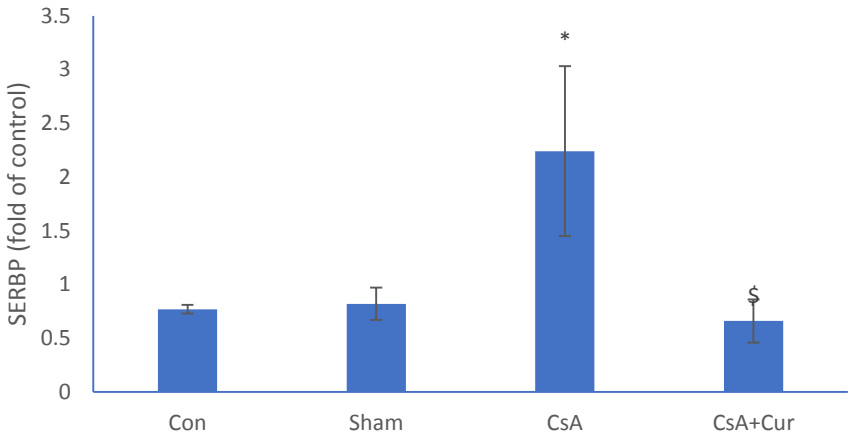

Fig 4. Eff ect of CsA with or without curcumin treatment on the expression of SERBP gene in liver tissue after 28 days. The relative amounts of mRNA were examined using quantitative realtime PCR analysis. Values are mean ±SEM for 7 rats per group. * $p < 0.05$ Denotes significant diff erence compared to the control and sham groups. $ $p < 0.05$ Denotes significant diff erence compared to the CsA group. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin.

3.3.Histopathological alterations in the liver tissue

Masson's Trichrome staining was conducted to evaluate the effects of CsA consumption and curcumin treatment on liver tissue fibrosis. As shown in Figure 5, there were no lesion scores observed in the liver tissue of control and sham rats (grade 0). In contrast, the microscopic lesion score for the liver tissue from the CsA-treated group ranged from 4 to 5, indicating severe liver tissue damage. Furthermore, the administration of curcumin alongside CsA reduced fibrosis in the liver tissue (grade 1).

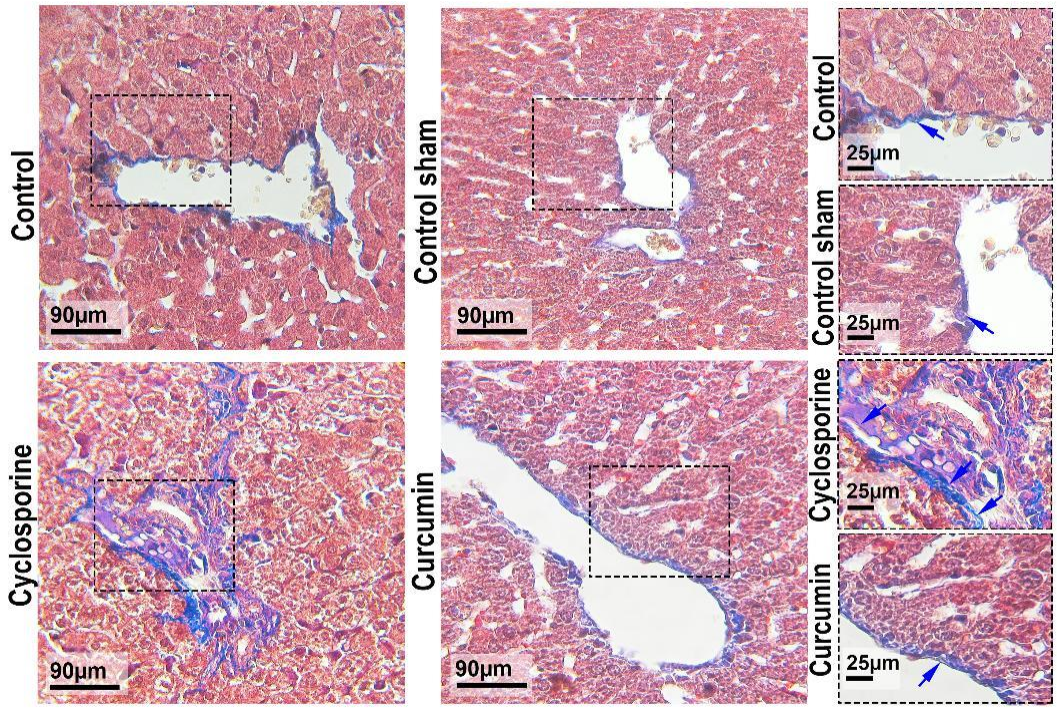

Fig 5. Photomicrographs of liver tissue staining by Masson Trichrome staining show that microscopic lesion score in the liver tissue 4 to 5, which was an indication of severe lesion of liver tissue. Moreover, curcumin administration along CsA had attenuate fibrosis induced by CsA on liver tissue (grade 1). magnification ×400. Con: Control, Sham, CsA: Cyclosporine A, CsA+cur: Cyclosporine A+curcumin

4. **Discussion**:

Considering the necessity of controlling the side effects of CsA based on the large target community that consumes this drug (recipients of transplanted tissue and autoimmune patients) (3), this article focuses on this drug's hepatotoxicity and its mechanism since there are few studies with this subjective.

The primary findings of the current study indicated that following prolonged exposure to CsA: (i) CsA administration led to a disturbance in lipid profile and liver enzymes as evidenced by an increase in plasma LDL, cholesterol, triglyceride, ALT, AST and ALP compared to the control group; (ii) CsA caused liver fibrosis compared to the control animals; (iii) additional molecular

analysis revealed that CsA enhanced protein tyrosine phosphatase 1B (PTP1B) , FAT/CD36, SREBP-1c and decreased hepatocyte nuclear factor 4 Alpha (HNF4A) genes expressions in the liver of male rats. Furthermore, significant improvements in fibrotic disorders and the molecular mediators associated with liver injury were observed in the animals treated with curcumin. These changes were likely mediated through the transcription factors FAT/CD36, PTP1B, and HNF4A.

In the current study, evaluating the level of lipids in plasma demonstrated that CsA group showed significantly higher plasma LDL, triglyceride and cholesterol levels as well as lower plasma level of HDL than the control group. Also, ALT, AST, and alkaline phosphatase (ALP) were higher in this group than in the control group and curcumin cotreatment improve this undesirable effect of CsA. These results indicates that CsA can cause damage to liver tissue. It was known that the effects of CsA on the liver come from increasing metabolic activities, mitochondrial damage, increasing ROS, and increasing oxidative stress (1).

Korolczuk et al. conducted a study in adult rat to assess the effect of CsA on oxidative stress and morphological changes in liver tissue. Accordingly, Korolczuk et al reported that CsA administration significantly impaired liver function as manifested by elevation of ALT and AST enzymes, blood bilirubin, and lipid peroxidation products as well as progressive inflammation and steatosis through oxidative stress insult (1, 19) which is in agreement with our results.

In the present study, we demonstrated that CsA caused fibrotic changes in the liver tissue of male rats. This is the first study to evaluate fibrotic alterations following CsA consumption. This finding confirms the biochemical data that CsA induce liver injury and unwanted deleterious changes in this organ. Previous studies confirmed our results in which CsA caused structural changes in liver including hepatocyte necrosis, inflammation, steatosis, sinusoid expansion, and congestion (1, 20).

Despite the many evidences of liver damage following CsA consumption, the molecular mechanisms involved in this damage have not been fully identified. A notable increase in protein tyrosine phosphatase 1B (PTP1B), FAT/CD36, SREBP-1c, and a significant reduction in hepatocyte nuclear factor 4 Alpha (HNF4A) genes expressions were shown in the CsA group by real time-PCR in comparison to the control group. These data demonstrate the mentioned molecules as possible mediators involved in the side effects of cyclosporine. In 2018, Shirpoor et al designed a study to investigate the molecular mechanism (FAT/CD36, PTP1B, and HNF4A) involved in liver damage caused by alcohol consumption in rats. They showed gene expression of molecules involved in fat metabolism (FAT/CD36, PTP1B, and HNF4A) changes in animals treated with alcohol compared to the control rats. To be more specific, the level of FAT/CD36, and PTP1B genes increases, and the expression of HNF4A gene decrease (9). Also, an in vitro study reported by Jin et al In 2004 to investigate the molecular mechanisms used (liver-X-receptor, FAT/CD36) by CsA in macrophages in the development of atherosclerosis showed increased expression of liver-X-receptor, FAT/CD36 genes in CsA group incomparison to the control group (21). Other study conducted by Borlak et al., shows that CsA can induce diabetes by suppressing the expression of the HNF4a gene in mice (22). These articles confirm our study's results about CsA effects being mediated by these molecules.

Another finding of this study was that curcumin administration along with CsA, reduced liver structural and functional changes which were possibly mediated by FAT/CD36, PTP1B, SREBP-1c and HNF4A gene expressions.

Previous research has demonstrated that curcumin supplementation influences lipid profiles, oxidative stress, and function of the liver in various disorders (23, 24), which are in line with our study.

Curcumin whose effects have been proven against liver damage caused by alcohol or non-alcoholic has a strong antioxidant function (15, 25). Considering the positive effect of this substance on liver function compared to the CsA group (which is affected by the oxidative stress of this drug), our study proves the effect of curcumin against liver damage by antioxidant action.

All these information introduces curcumin as a substance that reduce the adverse effects of CsA on liver tissue.

This study could confirm the efficacy of curcumin as an antioxidant and anti-inflammatory agent in mitigating liver damage induced by CsA. Considering that this substance is a strong antioxidant and scavenger of free radicals, along with curcumin's protective effects against liver damage (22, 25), these results seem reasonable. To assese the effect of an antioxidant on the liver side effects of CsA, Shirpoor studied the effects of ginger, which showed that treatment with ginger improves all unwanted changes in the liver (9).

As a summary of all the things mentioned, this article suggests that CsA can cause damage to liver tissue through lipid homeostasis disorders mediated by FAT/CD36, PTP1B, and HNF4A gene expression alterations. It also indicates that these negative effects of CsA can be mitigated by using curcumin as an antioxidant and anti-inflammatory agent.

This article indicates that CsA can induce damage to liver tissue through disruptions in lipid homeostasis, which are mediated by alterations in the FAT/CD36, PTP1B, expressions and HNF4A genes. Additionally, it suggests that these adverse effects of CsA may be alleviated by curcumin, which acts as an antioxidant and anti-inflammatory supplement.

Ethical statement

All animal procedures were approved by the guidelines of the Ethics Committee of Urmia University of Medical Sciences (Ethical Code: IR.UMSU.REC.1398.005).

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None

Author contributions

Alireza Shirpoor: Conceptualization, Methodology. Mahrokh Samadi: Data curation, Shiva Gholizadeh-Ghaleh Aziz: Writing – original draft. Roya Naderi: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Conflicts of interest

All authors declare no competing interests.

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Data availability

Data will be available on request.

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