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Curcumin Modulates the Level of IL-17 and IL-10 Cytokines in Two Models of Experimental Liver Injury in Male Rats

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Abstract

Introduction: Curcumin, a natural antioxidant, has anti-inflammatory and protective effect on a large number of diseases like cancers and hepatic disorders in oxidative stress conditions by collecting free oxygen radicals and increasing intracellular glutathione. The aim of this study was to determine the effects of curcumin on the level of IL-17 and IL-10 cytokines in intrahepatic and extrahepatic liver injuries

Methods: A total of 72 male Wistar rats were randomly divided into two (A, B) categories, each of which was divided into 4 groups. A: One group as a control-sham group received distilled water as an acetaminophen vehicle and the other three groups received acetaminophen (500mg/kg IP). The third group received curcumin, and the fourth group was administrated curcumin vehicle. B: one group underwent Bile Duct Ligation (BDL), and another group received curcumin by gavage for seven days. The third group received distilled water as a curcumin vehicle and the fourth group was considered the sham group. Animals were sacrificed 48 hours after administration of acetaminophen under anesthesia with ketamine + xylazine. After that, liver tissue samples were taken for laboratory tests. Cytokines were measured by ELISA method.

Results: Levels of IL-17 and IL-10 in the liver tissue in groups A and BDL increased significantly, and in the Curcumin (CMN) group, decreased significantly in both in- and out-liver injury. Also, the body weight in the curcumin-treated groups showed a significant increase both in intrahepatic and extrahepatic injuries.

Conclusion: Our data suggest that curcumin undermines inflammation and damage to the inside and outside of the liver, but these findings need to be further investigated.

developed in the left eye. Two patients had no family history suspicious for keratoconus.

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Introduction

Intensification of liver cell inflammation and injury during cholestasis caused by accumulation of toxic bile acids are among major factors causing liver injury and fibrosis (1-3). Several diseases such as gallstone, bile duct ligation, pancreatic tumors, and drug toxicity sustain cholestasis and ultimately lead to liver disorders, fibrosis, cirrhosis, and death (4-6). Ursodeoxycholic acid (UDCA) and its analogues can inhibit the inflammation by eliminating toxic bile acids. This is the only approved drug that is widely used in clinical trials to treat patients with cholestasis, such as those with primary biliary cirrhosis (PBC) (7, 8). However, a large number of patients with PBC and cholestasis don't fully respond to UDCA (9,10). Farnesoid X receptor agonist exerts its anti-cholestasis effects by changing the metabolism of bile acids in in-vitro models (11-14). Therefore, to treat cholestasis, it is important to search for drugs potentially effective in improving the inflammation and reducing toxic bile acids. Curcumin is the yellow phenolic compound with the scientific name of diferuloylmethane 7, which is considered as the active component of turmeric and has powerful antioxidant and anti-inflammatory effects (15). Turmeric is comprised of three main combinations, namely, demethoxy curcumin, bis demethoxy curcumin, and curcumin, the latter of which is the effective substance of rhizome of turmeric plant called dafloyl-methane, which contributes to protecting brain and body organs, preventing the growth of cancer cell,s and treating Alzheimer's (16). The witnessed pharmacological effects of curcumin indicate the close association of these impacts with metabolic disorders, hepatic and digestive disorders, diabetes, and respiratory diseases (17). In terms of its structure, curcumin is a polyphenol, enjoys antioxidant capacity, has protective effect on lipid peroxidation in rat liver in oxidative stress conditions, functions as the collector of free oxygen radicals, and increases intracellular glutathione (18). Moreover, it increases glutathione-s-transferase (19). Furthermore, its anti-tumor, anti-inflammatory, and disinfectant effects have been shown.

Curcumin is used for the treatment of several diseases, including rheumatism, body pain, intestinal parasites, diarrhea, turn fever, liver disorders, stomach discomfort, urinary tract infections, dyspepsia, inflammation, white patches in the body, menstrual problems, colonization of the intestines, and skin diseases

Curcumin is a vasodilator that helps the treatment of jaundice. Choking curcumin for tooth pain relief is effective. It also causes wounds to dry and relieves their swelling. It is useful for joint pain and swelling (20). Some studies have shown the anti-cirrhotic effects of curcumin. It is claimed that the hepatoprotective effects of this plant is due to its antioxidant properties (21). Curcumin affects inflammatory reactions through its prostaglandin inhibiting effect and has been known to be effective in treatment of inflammatory diseases, diabetes, tumors, cardiovascular, respiratory, nervous system, skin, liver, and bone diseases, and menopausal symptoms (22). Curcumin is also effective in the treatment of depression (22, 23). Furthermore, curcumin is a well-known antioxidant and one of the most powerful free-radical-cleansing agents, which can prevent the production of different types of oxygen free radicals (24). Also, inflammation is caused by prostaglandins where cyclooxygenase-2 plays a key role. Therefore, the aqueous extract of curcumin improves wound healing in healthy rats by suppressing the activity of cyclooxygenase-2 (25).

Cytokines have pro inflammatory or anti-inflammatory activity or suppress the immune system (26). In fact, cytokines play an important role in liver injuries; thus, measuring their levels can be an important indicator of response to treatment with drugs (27). Overall, produced in rats and humans by a wide range of cells including macrophages, monocytes, B cells, TCD4+ cells, and TCD8 cells, IL-10 is an interleukin regulating the immune system (28-30). It has been reported that in infections, IL-10 inhibits functions of Th1, NK cells, macrophages, as well as whatever required for efficient removal of pathogens. However, it is involved in damages to tissues and can, as a cytokine, both preventremoval of pathogens and modify damage incurred to immunity (31). Additionally, it has been indicated that in addition to time, relative production amounts of pre-inflammatory and anti-inflammatory cytokines are of utmost significance to efficient and effective improvement of damage (32).

It has been also shown that IL-10 can directly inhibit the production of cytokines by T cells in vitro (33), and that the same cytokines block production of pro-inflammatory cytokines, contribute to stimuli, expresse MHC II, and generate chemokines (34).

Generated by a wide range of cells including intrinsic immune cells and epithelial cells, IL-17 induces production of pro-inflammatory cytokines. It is mainly produced by Th17 cells, lymphocytes and neutrophils playing a role as well. Th17 cells are among the main components of pro-inflammatory lymphpcytes, and plays a crucial part in host defense against extracellular pathogens and autoimmune diseases (35, 36). The studies carried out recently have revealed the close association between virus infection in the liver, activation of Th17 cells, as well as liver disorders inflicted by immune responses. It has been also shown that the expression degree of IL-17 is related to liver diseases and fibrosis levels (37). Cytokines control a wide range of pathological and biological processes and it has been shown that IL-1 levels increase in some types of inflammation that trigger the production of TNFα and that the disease severity has been decreased by blocking these cytokines (38). Since cholestasis-induced liver injuries are caused by increased inflammation and oxidant agents and considering that curcumin has anti-inflammatory and antioxidant effects, it may exert its protective effect on the liver by inhibiting inflammation. Therefore, whether the effect of curcumin on reducing cholestasis-induced injury is applied through reducing modulations of cytokines levels has been tested in the current study. The aim of this study was to investigate the protective effects of curcumin on intra- and extra-hepatocyte injuries.

Materials and methods:

In this study, 72 male Wistar rats weighing 250-300g were prepared. Animals were purchased from of Kerman University of Medical Sciences and were kept at normal conditions, 12 hours of darkness and 12 hours of lighting and a standard diet at 25°C.

Experimental groups

The animals were divided randomly into two, A and B, categories, each of which includes four groups of nine rats (n = 9).

A. Intrahepatic injury

1. Control-Sham group (C-Sh): Animals that receive 0. 5ml of acetaminophen vehicle (distilled water).

2. Acetaminophen group (A): Animals that receive a single dose of 500 mg/kg IP injection of acetaminophen (39).

3. Curcumin group (CMN): Animals that were treated with 300 mg/kg curcumin.

4. Curcumin Vehicle Group (Distilled water): Rats that receive IP injection of distilled water 24 hours after taking acetaminophen (40).

Intrahepatic injury induction method

Animals were kept without food 16 hours before injury, and water was placed freely at their disposal. To induce intrahepatic injury, 500 mg/kg acetaminophen was injected intraperitoneally (41).

B. Extrahepatic cholestasis

1. Sham group: Healthy animals on which all bile duct ligation (BDL) preparations are carried out but whose bile ducts are not blocked.

2. BDL group: Animals that undergo BDL but not receive any drug.

3. Curcumin group (CMN): Includes rats, the bile ducts of which are ligated and are treated with 300 mg/kg with volume of 1.2 ml/kg IP injection of curcumin 28 days after BDL (42).

4. Curcumin Vehicle Group (distilled water): Includes rats the bile ducts of which are ligated and are treated with 1.2 ml/kg IP injection of curcumin 28 days after BDL.

Extrahepatic cholestasis technique (BDL)

Animals were completely anesthetized by IP injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) and the bile duct was blocked with two nodes at the distance of a few mm after laparotomy. The distance between the two nodes was then cut with scissors. Then the abdominal wall were sutured in the two layers of fascia and skin using plain absorbable thread and non-absorbable silk thread (43)

Drug prescription

300 mg/kg curcumin was given to the experimental animals by gavage 28 days after bile duct ligation for seven days (44) (Being in the form of pure powder provided by Sigma Company, the curcumin used in the present study was solved in distilled water (so as to penetrate through needle tip). It was then injected in rats intra-peritoneally) (45). After drug administration on the seventh day, the rats were anesthetized using ketamine (50 mg/kg) and xylazine 10 mg/kg) and their liver samples were taken for laboratory analysis (42).

Administration method

300 mg/kg curcumin was intraperitoneally injected to the experimental animals 48 hours after the administration of acetaminophen, which induces the liver injury. Animals were killed 48 hours after administration of acetaminophen after anesthesia with ketamine (50 mg/kg) and xylazine (10 mg/kg), and their liver tissue was frozen at a temperature of -70 °C to measure the levels of cytokines (39).

Homogenization method used for measuring cytokines levels

To carry out the homogenization process, 500 mg of each liver tissue was mixed with 2 ml of buffer (pH = 7.2) containing 50 mmol Tris, 0.5 Triton 100-x, 150 mmol Nacl, and protease inhibitor cocktail) using the homogenizer (Roche, Germany). The homogenized solution was then refrigerated centrifuge at 4000 g and 4-°C for 15 minutes, and was finally used to measure the level of cytokines.

Measuring technique of cytokin level

IL-17 and IL-10 cytokine levels of tissue samples, which are prepared according to the ELISA kit instructions purchased from Eastbiopharm, a German Company, are measured.

Statistical Analysis

Data analysis was carried out using SPSS v. 20. The results were expressed as mean ± SE, the data significance was shown using one-way ANOVA and Tukey test and $P < 0.05$ was considered statistically significant.

Results

BDL symptoms such as jaundice, dark urine, itching, and light-colored stools were seen in the tested animals. Table 1 shows the body weight at the beginning and end of the experiment phase as well as weight changes in all groups. The results show that compared with the control group, weight gain was not observed in the acetaminophen treated group and BDL animals and even showed significant weight loss compared to the beginning of the experiment ($p < 0.001$). Also, 300 mg/kg curcumin significantly compensated for the weight loss caused by intrahepatic and extrahepatic injury ($P < 0.05$ and $P < 0.01$ respectively).

Table 1. The weights of the male rats at the beginning and end of the test and the weight variation during the test. There are 9 animals (n = 9) in each group. The results are shown in the form of mean ± SE.

Groups \ index	Body weight at the beginning of the experiment (g)	Body weight at the end of the experiment (g)	Body weight changes (g)
Control-Sham (C-SH)	259.66 ± 2. 99	290.33 ± 2.53	30.66 ± 3.27
Acetaminophen group (A)	279.33 ± 4. 13	255.77 ± 2.21	-23.55 ± 4.17 ***
Curcumin group (CMN)	271.77 ± 4. 39	288.55 ± 2.93	16.77 ± 3.91 **
Curcumin Vehicle group (VCMN)	269.44 ± 3. 65	263.44 ± 3.63	-6.00 ± 2.60
Sham group	274.88 ± 4. 86	299.33 ± 3.93	24.44 ± 4.08
BDL group	279.22 ± 3. 86	263.11 ± 3.64	-16.11 ± 2.70 ***
Curcumin group (CMN)	275 ± 4.27	282.66 ± 4.15	7.66 ± 2.96 *
Curcumin Vehicle group (VCMN)	272.88 ± 4.84	265.32 ± 4.54	-7.55 ± 2.29
Control-Sham group (C-SH)	259.66 ± 2.99	290.33 ± 2.53	30.66 ± 3.27

* $P < 0.05$ denotes the significant difference compared with the BDL group.

** $P < 0.01$ denotes the significant difference compared with the Acetaminophen group

*** $P < 0.001$ denotes the significant difference compared with the Control group

Effect of curcumin on level of IL-17, IL-10 in intrahepatic injury groups

Figure 1 shows that IL-17 levels in the Acetaminophen group (1.160 ± 0.046 pg/ml) significantly increased compared with the Control-Sham group (0.4850 ± 0.015 pg/ml) (P < 0.001) and the same level was significantly reduced in the Curcumin group (0.807 ± 0.092 pg/ml) compared with the Acetaminophen group (1.160 ± 0.046 pg/ml) (P < 0.01). However, there is no significant difference between the Curcumin Vehicle group (1.217 ± 0.047 pg/ml) and the Acetaminophen group (1.160 ± 0.046 pg/ml). Figure 2 shows that IL-10 level in the Acetaminophen Group (0.880 ± 0.054 pg/ml) significantly increased compared with the Control-Sham Group (0.267 ± 0.010 pg/ml) (P < 0.001) and the same level significantly decreased in Curcumin group (0.55 ± 0.121 pg/ml) compared with Acetaminophen group (0.880 ± 0.054 pg/ml) (P < 0.01). However, there was no significant difference between the Curcumin Vehicle group (0.948 ± 0.06 pg/ml) and the Acetaminophen group (0.880 ± 0.054 pg/ml).

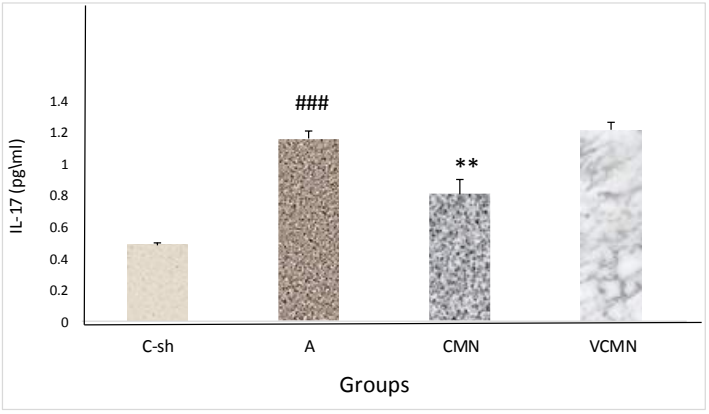

Figure 1. The effect of curcumin on IL-17 in intrahepatic injury. Data are presented as mean ± SEM. (n = 9)

** P < 0.01 denotes the significant difference between the curcumin-treated group at a dose of 300 mg/kg and Group A
P < 0.001 denotes the significant difference between Group A and the C-SH group
C-Sh: the Sham-Control group A: Acetaminophen, CMN: 300 mg/kg curcumin, VCMN: Curcumin Vehicle (distilled water)

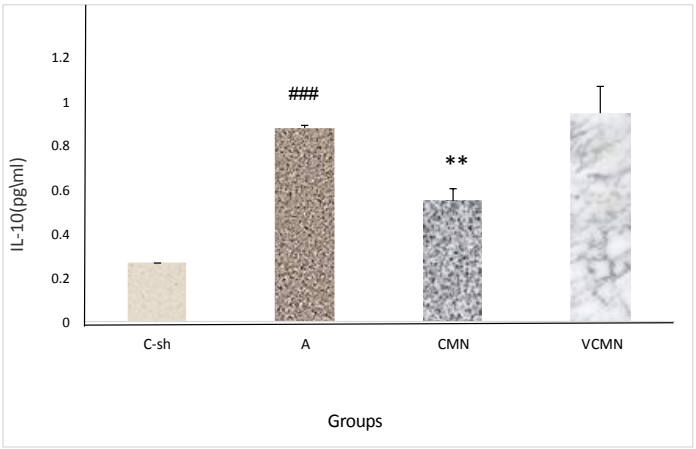

Figure 2. The effect of curcumin on IL-10 in intrahepatic injury. Data are presented as mean ± SEM (n = 9).

** P < 0.01 denotes the significant difference between the curcumin-treated group at a dose of 300 mg/kg and Group A

P < 0.001 denotes the significant difference between Group A and the C-SH group

C-Sh: Sham-Control group A: Acetaminophen, CMN: 300 mg/kg curcumin, VCMN: Curcumin Vehicle (distilled water)

Effect of curcumin on the levels of IL-17 and IL-10 in extrahepatic cholestasis groups

Figure 3 shows that IL-17 level in the BDL group (0.839 ± 0.050 pg/ml) significantly increased compared with the Control-Sham group (0.267 ± 0.010 pg/ml) ($P < 0.05$) and the same level significantly decreased in the Curcumin group (CMN = 0.768 ± 0.028) compared with the BDL group (0.839 ± 0.050 pg/ml) ($P < 0.05$). However, there was no significant difference between the Curcumin Vehicle group (0.913 ± 0.059 pg/ml) and the BDL Group (BDL = 0.839 ± 0.050). Figure 4 shows that the IL-10 level in the BDL group (0.713 ± 0.050 pg/ml) significantly increased compared with the Sham group (0.252±0.012 pg/ml) ($P < 0.05$) and the same level significantly decreased in the Curcumin group (0.628 ± 0.306 pg/ml) compared with the BDL group (0.713 ± 0.050 pg/ml) ($P < 0.05$). However, there was no significant difference between the Curcumin Vehicle group (0.801 ± 0.032 pg/ml) and the BDL group (0. 713 ± 0.050 pg/ml).

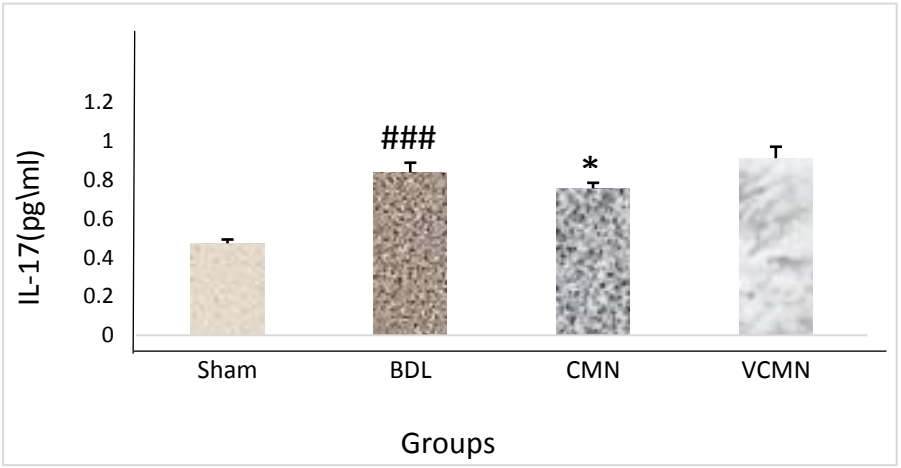

Figure 3. The effect of different doses of curcumin on IL-17 in extrahepatic cholestasis. Data are presented as mean ± SEM. (n = 9)
* P < 0. 5 denotes the significant difference between the Curcumin group (CMN) and the BDL group
P < 0.005 denotes the significant difference between the BDL group and the Sham group
BDL: Bile Duct Ligation, VCMN: Curcumin Vehicle (distilled water), CMN: 300 mg/kg curcumin

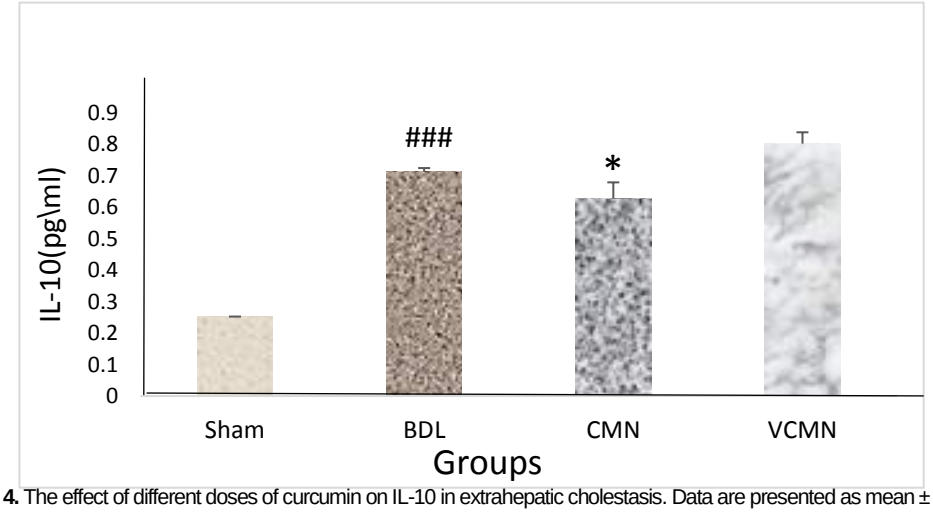

Figure 4. The effect of different doses of curcumin on IL-10 in extrahepatic cholestasis. Data are presented as mean ± SEM. (n = 9)

* P < 0.05 denotes the significant difference between the curcumin-treated group (CMN) and the BDL Group
P < 0.001 denotes the significant difference between the BDL group and the Sham group
BDL: Bile Duct Litigation, VCMN: Curcumin Vehicle (distilled water), CMN: 300 mg/kg curcumin

Discussion

This experimental study examines the effect of curcumin on liver injuries induced following intrahepatic injury (using acetaminophen) and extrahepatic injury (bile duct ligation). Weight changes in the cholestasis groups showed lack of proper weight gain and even weight loss (46), and BDL causes an accumulation of toxic bile acids in the liver (47). In other words, the liver tissue injury affects metabolic reactions in presence of bile acids. On the other hand, bile secretion disorder causes insufficient absorption of fat, leading to weight loss, lack of energy, fat-soluble vitamin deficiency, and lack of essential fatty acids for the body (48). Curcumin treatment can prevent this weight loss to a great extent, which can be attributed to the antioxidant effect of curcumin, because many studies have shown that hepatic oxidative injury is directly caused by reducing the antioxidant capacity of the liver and increasing hepatic lipid peroxidation (49). Fibrosis is the main disorder in chronic liver diseases; it is a leading cause of death in many people with cholestatic liver disease (50). In an experimental BDL model, the oxidative stress and defects in antioxidant defense system play a key role in hepatocellular injury and the liver fibrosis process (51). Many studies suggest that antioxidant supplements significantly inhibit hepatic fibrosis and necrosis (52), thereby preventing hepatic injury and failure during cholestasis (53). Reduced liver complications achieved by reducing tissue levels of cytokines in rats with intrahepatic and extrahepatic cholestasis that were treated with 300 mg/kg of curcumin in our study is consistent with results of other studies (54). The tissue inflammation is initiated under the cholestatic conditions as a result of accumulation of toxic bile salts in the liver in the form of accumulation of leukocytes (cellular components of cytokines) and activation of Kupffer cells (50, 55). Drugs such as UDCA and INT747, which increase the removal of toxic bile acids, are useful for the treatment of cholestasis (7-14). The presence of toxic bile acids during the cholestasis is the leading cause of liver injuries (1, 2). This study showed that IL-17, IL-10 levels increased in liver tissue in rats as a result of intrahepatic and extrahepatic cholestasis, and the levels of these cytokines significantly decreased during intrahepatic cholestasis when 300 mg/kg of curcumin was administered, which suggests that curcumin leads to a reduction in the inflammation caused by liver injuries and intrahepatic cholestasis recovery. Since bile is the primary excretion path for bilirubin, bilirubin is excreted into the intestine with a reduced flow of bile during cholestasis. On the other hand, chronic biliary retention leads to the expansion of the bile ducts and their proliferation; as a result, bilirubin is released into the blood probably due to the rupture of dilated bile ducts and the direct discharge of bile into the lymph leaving the liver. Therefore, it can be concluded that curcumin prevents inflammation and the development of cholestasis probably by reducing the IL-10 and IL-17 levels and thus increases bile flow, thereby preventing the entry of bile into the blood (56). Considering that the liver is able to regulate its growth and size in response to injury (57), , it may be said that curcumin may accelerate the process of recovery and the improvement of lever tissue by participating in numerous biochemical reactions. IL-10 is mainly produced by activated macrophages and regulatory T cells. Because IL-10 is produced by macrophages and also inhibits macrophage functions, it is a good example of negative feedback regulation (58). Thus, with increased cholestasis-induced inflammation (54), the number of activated macrophages increases and the levels of

produced IL-10 increases as well. Also, reduction in the number of activated macrophages due to the inflammation recovery leads to a reduction in cytokine levels. Reports have shown that empirical removal of IL-10 and/or inhibition of its signaling can improve pathogen control, thereby reducing the severity of the disease (32, 59). In another study, it has been implied that if high concentrations of IL-10 during infections are regarded as a cause, the process can inhibit pathogen clearance. On the contrary, if this increase in concentration is the result of high damage power, the increased concentration leads to decrease in inflammation (60). The immune system's deviation towards production of cytokines such as IL-10 undermines the performance of T cell. In accordance with recent observations, the neutralizing effect of IL-10 improves chronic inflammation and infection. Therefore, temporarily neutralizing the function of IL-10 allows for the treatment of other chronic infections associated with high levels of IL-10, thereby preventing the occurrence of autoimmune diseases in the host due to interim reduction in activities of the host immune system (61, 62). In another study, it has been indicated that inflammation and infection are eliminated in rats not producing IL-10 or those in which IL-10 is blocked (63). Additionally, it has been reported that IL-10 plays a significant role in transition of infection towards chronic phase in rats (64). Therefore, it can be concluded that the reduced IL-10 level achieved by taking curcumin in the present study leads to a reduction in the inflammation rate and subsequent recovery.

IL-17 is a pro-inflammatory cytokine, which is mainly produced by TH-17 cells. It stimulates macrophages in the endothelial cells, fibroblasts, and epithelial cells and leads to the secretion of other pro-inflammatory cytokines such as TNF, IL-6, IL-1, chemokines, and metalloproteinases (65, 66). Therefore, IL-17 leads to the occurrence and severity of inflammation by inducing the production of these factors. Recently, increased expression of IL-17 has been reported in inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, asthma, and Crohn's disease (67-70). Therefore, it can be said that increased inflammation and intrahepatic and extrahepatic cholestasis was associated with the increased expression of this cytokine in our study, and the recovery observed in the curcumin-treated groups could be due to a significant decrease in the level of these cytokines in the liver tissue. It has been reported that the protective role of IL-17 has been attributed to its ability in absorbing neutrophils to sites of infection (66). On the other hand, it was also reported that IL-17 may not always have a protective role. Rutitzky et al. and Zelante et al. demonstrated that pathological inflammatory reactions can be reduced by injecting anti-IL-17 antibodies (71, 72). These observations confirm the fact that IL-17 may be harmful and cause tissue damage that is consistent with increased expression of this cytokine during the intrahepatic and extrahepatic cholestasis in our study, which suppressed the immune system and increased the inflammation rate. The present study showed that the levels of the inflammatory cytokine IL-17 significantly decrease in intrahepatic and extrahepatic cholestasis rat groups treated with curcumin. Therefore, it can be said that curcumin has an anti-inflammatory effect in the cholestasis process. These observations in intrahepatic and extrahepatic cholestasis rat groups treated with curcumin are similar to the effect of swertianlarin on BDL rats in the study conducted by Jin Chai et al. (73). Obstructive cholestasis is associated with systemic release of inflammatory cytokines (74). Thus, eliminating or

reducing inflammatory cytokines in cholestasis leads to a reduction in liver injuries and fibrosis (75). Therefore, with a significant reduction in IL-17 and IL-10 levels in intrahepatic and extrahepatic cholestasis treated with curcumin in our study, it can be concluded that curcumin will prevent the spread of cholestasis by inhibiting the inflammation, thereby reducing liver injuries and fibrosis. Although, in our study, liver injuries, inflammation, and cholestasis rates decreased in cholestasis rats treated with curcumin compared to the untreated rats, whether we can state that curcumin can play a protective role in other cholestases such as human cholestasis, demands further study in this field.

Conclusions

The present study indicated that levels of cytokines including IL-10 and IL-17 show a significant decrease in extra- and intra-hepatic damages treated by curcumin in such animals. Thus, it is safe to say that curcumin, as a hepatoprotective substance, may be effective in extra- and intra-hepatic damages against high consumption of acetaminophen and the empirical type of biliary obstruction (cholestasis).

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