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REVIEW ARTICLE

Pharmacological actions of curcumin in liver diseases or damage

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Abstract

Since 1900 BC, several therapeutic activities have been attributed to the rhizomes of the plant *Curcuma longa* for a variety of diseases, including liver disorders. Curcumin, the main active compound obtained from this plant, was first isolated two centuries ago and its structure as diferuloylmethane was determined in 1910. Curcumin has shown anti-inflammatory, anti-oxidant, antifungal, antibacterial and anticancer activities. The pharmacological properties of curcumin were reviewed recently and focused mainly on its anticancer properties. However, its beneficial activity on liver diseases (known centuries ago, and demonstrated recently utilizing animal models) has not being reviewed in depth until now. The curcumin ability to inhibit several factors like nuclear factor-κB, which modulates several pro-inflammatory and profibrotic cytokines as well as its anti-oxidant properties, provide a rational molecular basis to use it in hepatic disorders. Curcumin attenuates liver injury induced by ethanol, thioacetamide, iron overdose, cholestasis and acute, subchronic and chronic carbon tetrachloride (CCl_4) intoxication; moreover, it reverses CCl_4 cirrhosis to some extent. Unfortunately, the number of studies of curcumin on liver diseases is still very low and investigations in this area must be encouraged because hepatic disorders constitute one of the main causes of worldwide mortality.

Traditional agents derived from ancient Hindu medicine, such as curcumin, have been shown to have biological activity at physiologically relevant concentrations in preclinical studies. Dozens of medicinal properties of curcumin have been described that have been reviewed recently (1–3). The most studied beneficial properties of curcumin are perhaps its anti-oxidant, anticarcinogenic, anti-inflammatory and immunomodulatory activities, among others. However, the pharmacological hepatoprotective properties of curcumin known to Indians in the traditional medicine hundreds of years ago, and tested recently on the basis of modern scientific methods, have not been reviewed in depth. Therefore, this review summarized the most important and general actions of curcumin but focused on its effects on liver diseases.

Curcumin botany

Curcumin, a polyphenol (diferuloylmethane), is the main active compound found in the perennial plant *Curcuma longa* (commonly known as turmeric). The *Curcuma* genus belongs to the division of Magnoliophyta, class of Liliopsyda, subclass of Zingiberidae, order Zingiberales and family Zingiberaceae. Turmeric is a short-stemmed plant that grows around 1 m in height; it has curved leaves and oblong, ovate or cylindrical rhizomes (Fig. 1). *Curcuma longa* grows naturally throughout the Indian subcontinent and in Southeast Asia and other tropical countries.

Curcumin, demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin are the four principal curcuminoids obtained from the coloured extracts of dried roots from turmeric (Fig. 2). Whether all four analogues exhibit equal activity is not clear. Although in most systems curcumin was found to be the most potent (4, 5), in some systems bisdemethoxycurcumin was found to exhibit higher activity (6, 7). There are also suggestions that the mixture of all three is more potent than either one alone (8, 9).

India is the main producer of turmeric and consumes about 90% of it and exports the remainder (10). *Curcuma longa* and its constituents have been used in Asian cookery and traditional medicine for thousands of years: at present, they are used by the food industry as additives, like curry in England, flavourings, preservatives and colouring agents, in soft drinks, mustard and margarine.

Safety

One of the most prominent features of curcumin is its extremely good tolerance and its very low toxicity and

side effects. However, although turmeric and curcumin are natural products used in the diet, the doses used in clinical trials exceed those consumed in the diet; therefore, systematic toxicity studies are needed. Curcumin is Generally Recognized As Safe by the Food and Drug Administration, and this compound has been granted an Acceptable Daily Intake level of up to 3 mg/kg by the Joint FAO and WHO Expert Committee on Food Additives, 1996 (11). No studies in either animals (12, 13) or humans (14) have found any toxicity associated with the consumption of curcumin even at very high doses.

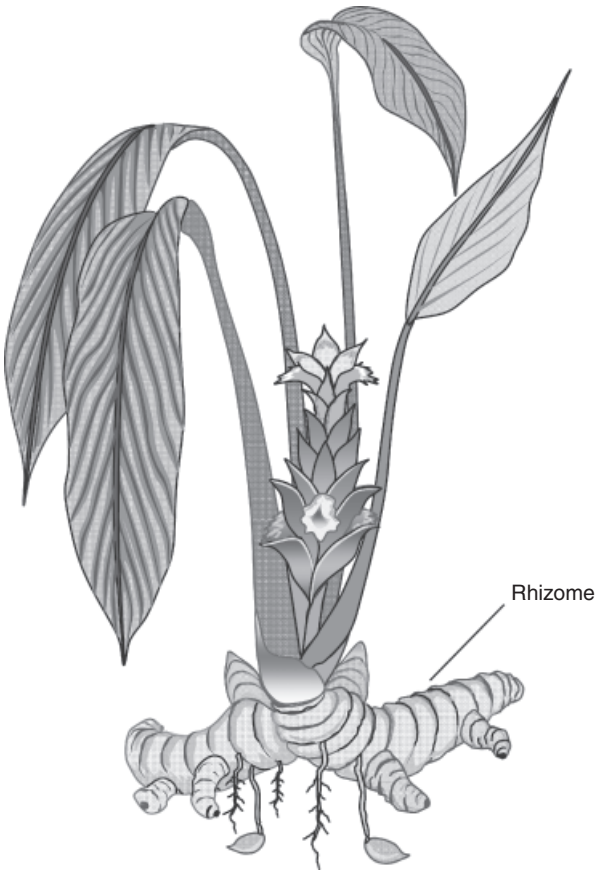

Fig. 1. *Curcuma longa* plant.

Molecular targets

Curcumin possesses a diverse range of molecular targets; among them are transcription and growth factors, cytokines, enzymes and genes regulating cell proliferation and apoptosis. It binds to and inhibits the activity of enzymes, growth factor receptors, metals, albumin and other molecules. Curcumin binds *p*-glycoprotein (15, 16), multidrug resistance proteins 1 and 2 (MRP1 and MRP2) (17), glutathione (18), protein kinase C (PKC), ATPase (19, 20) and α-1-acid glycoprotein (21). It also blocks fibril formation *in vitro* and *in vivo* by directly binding small β-amyloid species (22). Curcumin binds CD13/aminopeptidase N and prevents angiogenesis and tumour invasion (23). It inhibits the activity of lipoxygenase by binding to it (24) or binding to phosphatidylcholine micelles and thereby inhibiting lipoxygenase 1 (25).

Various transcription factors are strongly inhibited by curcumin, including nuclear factor-κB (NF-κB), signal transducer and activator of transcription proteins, activated protein-1, β-catenin and peroxisome proliferator-activated receptor-γ (26). These transcription factors regulate the expression of genes that contribute to cell survival, inflammation, tumorigenesis, angiogenesis, cell proliferation and invasion. Curcumin also downregulates the activity of multiple kinases [for a review, please see Goel *et al.* (2, 3)].

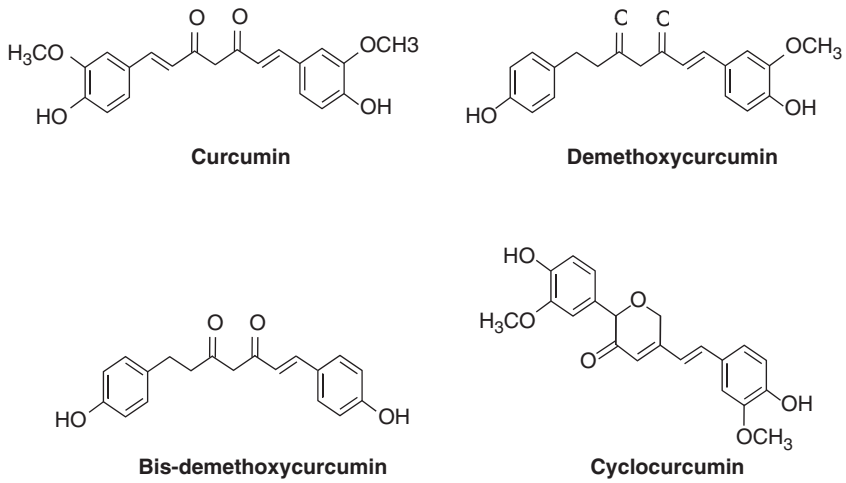

Fig. 2. The major curcuminoids present in the rhizome of the plant *Curcuma longa*.

Curcumin and liver damage

To date, there is no cure for cirrhosis, which is a leading worldwide cause of death (27). Some beneficial drugs for liver diseases have been studied but we are far from finding effective treatments. In this scenario, curcumin appears as a drug with possibilities to cure/ameliorate hepatic disorders.

Effects on iron-induced hepatic toxicity in rats

Iron is an essential constituent of the body and can be found in a functional form in cytochromes, haemoglobin, myoglobin and enzymes with iron–sulphur complexes and other iron-dependent enzymes. However, an excess of iron in the body is associated with toxic effects, leading to diseases (28). Because the body lacks any effective means to eliminate excessive iron, the toxic consequence of iron accumulation is determined by the rate, magnitude and distribution of iron in various compartments. Liver damage is the most common toxic effect observed with iron overloading. Excessive deposition of iron in hepatocytes produces fibrosis and cirrhosis (29, 30). Dietary curcumin lowers lipid peroxidation induced by Fe^{2+} intoxication to rats by enhancing the activities of anti-oxidant enzymes (31). Moreover, it has been demonstrated that curcumin and other spice ingredients can inhibit the oxidation of Fe^{2+} by H_2O_2 in the Fenton reaction (32), which generates $^{\bullet}OH$ radicals, involved in the initiation of lipid peroxidation (33). Based on these observations, Reddy and Lokesh (34) assessed whether the injury caused to hepatic parenchyma by iron intoxication could be normalized by curcumin. They found that administration of 30 mg/kg, p.o., of curcumin daily for 10 days to Wistar rats significantly reduced the lipid peroxidation degree induced by iron [30 mg/kg, intraperitoneal (i.p.)]. Serum enzyme activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are very important adjuncts to diagnosis and to measure liver injury; iron administration significantly elevated the hepatic lipid peroxides and serum AST, ALT and lactate dehydrogenase. Importantly, curcumin significantly prevented the serum levels of AST and ALT in iron-treated rats, indicating that this spice principle reduced the severity of iron toxicity by reducing the lipid peroxidation (34). Other targets of curcumin, like NF-κB inactivation (35), cannot be discarded to contribute to the beneficial effects of curcumin on iron toxicity. Recently, it was found that curcumin reduces the toxic effects of iron loading in rat liver epithelial cells (36). In summary, there is evidence that curcumin possesses beneficial properties on iron toxicity; however, more studies are needed to confirm this effect. New studies should include histopathological analysis and more in depth investigations of the action mechanism of curcumin in the light of their many and new described properties (1–3).

Ethanol administration resulted in biochemical, histopathological changes in the liver, kidney and brain that were reverted when curcumin and *N*-acetylcysteine were given to rats intoxicated with ethanol (37).

Evidence shows that alcohol-induced changes can cause a significant decrease in arachidonic acid in human plasma, erythrocytes, platelets and liver tissue (38–40). Arachidonic acid is released from phospholipids inserted into the plasma membrane by the action of Ca^{2+}-dependent phospholipase 2 (PLA_2) (41). There are two routes for arachidonic acid metabolism. One is the cycloxygenase (COX) pathway that produces prostaglandins (PGs), thromboxanes (TXs) and prostacyclins. The other pathway produces hydroxyeicosatetraenoic acids, and leukotrienes that are well-known mediators of the acute vascular changes that accompany the process of inflammation (42).

Researchers have demonstrated that chronic exposure to ethanol increases, progressively, PLA_2 activity (43, 44). In turn, PLA_2 activity increases the formation of arachidonic acid release from phospholipids, which is then converted to physiologically relevant eicosanoids (45). Interestingly, curcumin has been shown to inhibit the production of arachidonic acid in the liver, kidney and brain (46). Moreover, curcumin inhibits PLA_2 activity (47).

Oral alcohol administration to rats increases the levels of COX mRNA, which in turn enhances the production of PGs (48). Rajakrishnan *et al.* (46) found a significant increase in the levels of PGE_1, PGE_2, $PGF_{2\alpha}$ and PGD_2 in the liver, kidney and brain in alcohol-fed rats, while cotreatment with curcumin decreased the level of PGs significantly.

It has been reported that curcumin decreases the PG formation of PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2 and 6-keto-PGF_1 in azoxymethane-induced colon carcinogenesis (49). The mechanism by which curcumin decreases the production of PGs is through inhibition of PLA_2, lipoxygenase and COX activities (50). In addition, curcumin administration to rats intoxicated chronically with ethanol resulted in a decrease in PGs. This decrease in PGs suggests that increased arachidonic acid may be utilized for the synthesis of phospholipids, which can be useful for plasma membrane synthesis (46). In fact, Rajakrishnan *et al.* (46) administered curcumin after 1 month of ethanol administration; because ethanol may have resulted in membrane hydrolysis and dysfunction, administration of curcumin may result in the resynthesis of plasma membrane, as evident from decreased PG synthesis, an increased arachidonic acid level and an increased phospholipid concentration. Therefore, evidence strongly suggests that curcumin helps in maintaining the membrane structure, integrity and function, protecting the liver, brain and kidney from alcohol toxicity.

Attenuation of thioacetamide-induced hepatitis by curcumin

The liver has a remarkable ability to metabolize and aid in the excretion of xenobiotics. However, this organ is

susceptible to damage from several drugs and toxins (51). Hepatic damage following the intake of such agents can be reversible and subclinical, but may also cause fulminant hepatic failure (FHF) and death (51, 52). Experimental models of toxic liver injury are utilized to evaluate the biochemical processes involved in many forms of liver disease and to evaluate the possible pharmacological effects of candidate hepatoprotectants. The administration of thioacetamide (TAA) to rats causes FHF or cirrhosis, depending on the dose used and the duration of administration (53, 54). Animals treated with TAA exhibit, in their livers, enhanced formation of reactive oxygen species (ROS) and lipid peroxides (55), stimulation of NF-κB and the resultant production of pro-inflammatory molecules (56, 57), mechanisms that have an established role in FHF (52).

One of the most known mechanisms underlying curcumin's beneficial effects on several illnesses is its pleiotropic anti-oxidant activity (58). It prevents formation and scavenges ROS (59, 60) and reactive nitrogen species (61, 62). Furthermore, curcumin was shown to induce several enzymatic anti-oxidants, like glutathione transferase (63), haeme-oxygenase-1 (64) and catalase (63). Curcumin inhibits the activation of NF-κB by ROS, PKC and pro-inflammatory cytokines. NF-κB-mediated expression of inducible nitric oxide synthase (iNOS) is also inhibited by curcumin (35, 65).

The information presented above prompted Shapiro *et al.* (66) to evaluate whether curcumin's ability to inhibit iNOS and NF-κB, and to scavenge free radicals would protect from TAA-induced acute liver damage in rats. In their work, FHF was induced by two i.p. injections of 300 mg/kg of TAA at 24-h intervals. Two doses of curcumin were evaluated, a low dose (200 mg/kg per day, i.p.) or a high dose (400 mg/kg per day), initiated 48 h before the first TAA injection. Appropriate controls were performed. Survival was higher in the curcumin-treated groups compared with the TAA-only-treated animals. Liver necrosis and blood ammonia were reduced by curcumin, and the effect was better in the high-dose group. Lipid peroxidation, NF-κB activation and iNOS expression were increased by TAA and reduced by curcumin. The findings of the Shapiro *et al.* (66) study confirm a role for ROS, nitric oxide and NF-κB activation in the pathogenesis of TAA-induced liver damage and indicate that a blockade of their formation or activation by curcumin administration has a potent anti-inflammatory effect. Because curcumin is safe in humans (67), its use in patients with acute liver injury can be evaluated based on these results.

Attenuation of thioacetamide-induced cirrhosis by curcumin

Although progress has been made in the management of liver cirrhosis and its complications, preventive strategies should be beneficial in reducing the burden of this disease. Based on these facts, Bruck *et al.* (68) studied the preventive effect of curcumin on TAA-induced cirrhosis. TAA, although not toxic in itself, is converted into potent toxic substances by cytochromes (69). Hepatic damage is produced by the action of these reactive compounds (70) and possibly by activating NF-κB (66, 71). The type of liver injury induced by TAA depends on the dosage and duration of its administration: high doses can produce FHF (72), whereas lower doses administered periodically can lead to cirrhosis (54). In the study of Bruck *et al.* (68), rats that received biweekly, i.p. injections of TAA for 12 weeks developed liver cirrhosis, manifested by a gross macroscopic appearance, liver histopathology, hepatic hydroxyproline content and increased spleen weight; the liver showed hepatic stellate cells (HSC) activation determined as increased expression of α-smooth muscle actin and type I collagen gene expression. Curcumin (300 mg/kg/day/12 weeks) co-administration to TAA-treated rats resulted in a significant improvement of all alterations observed in the group receiving TAA alone. The protective effect of curcumin against liver fibrosis may be associated with its ability to inhibit NF-κB in Kupffer cells and infiltrating macrophages (35), therefore preventing liver inflammation, necrosis and apoptosis.

In order to further explore the potential antifibrotic actions of curcumin, Bruck *et al.* (68) studied whether curcumin would have a fibrolytic effect on established TAA cirrhosis. Unfortunately, administration of curcumin for 4 or 6 weeks to rats that already received TAA for 12 weeks did not reduce hepatic hydroxyproline levels or spleen weights, suggesting that curcumin exerts no beneficial effects on pre-established TAA-induced cirrhosis. This is in agreement with the observations in the same study that curcumin showed no *in vitro* effect on HSC. The failure of curcumin to inhibit HSC profibrogenic activity observed by Bruck *et al.* (68) is not in agreement with a previous study (72); the use of different HSC cell lines may explain this discrepancy. The most relevant result of this study is that curcumin prevented the development of hepatic cirrhosis induced by TAA. Because curcumin is safe for consumption by humans, it may have a beneficial role in chronic liver diseases characterized by ongoing necroinflammation.

Effects on cholestasis and biliary cirrhosis

Cirrhosis produced by bile duct ligation (BDL) in the rat is a useful model to evaluate possible beneficial drugs (73). Cirrhosis is accompanied by a perturbation in liver homeostasis with the release of cytokines and signalling molecules (74). One of the most important profibrogenic cytokines, transforming growth factor- β (TGF-β), is central to the development of fibrosis through its stimulating properties on ECM production and inhibitory effect on its removal (75). TGF-β plays an important role in initiating and promoting the activation of HSC to myofibroblasts during hepatic fibrogenesis. The TGF-β expression is elevated in experimental and human liver

diseases ranging from hepatitis and cholestasis to cirrhosis (76, 77).

Curcumin blocks the profibrotic actions of TGF-β and has been reported to prevent fibrosis in a TGF-β-driven model of fibrotic lung (78) and kidney (79) disease. Recently, we showed that curcumin protects against inflammation and oxidative stress in carbon tetrachloride (CCl_4)-induced acute hepatic injury (80). It was also found that curcumin prevents TAA-induced cirrhosis (68), but the role of TGF-β was not studied. Therefore, Reyes-Gordillo *et al.* (80) studied the effect of curcumin on BDL-induced cirrhosis and possible mechanisms like TGF-β downregulation and oxidative stress participation. Rats were bile duct obstructed for 4 weeks and curcumin was administered at a dose of 100 mg/kg, p.o., daily. Serum ALT activity increased about two-fold after BDL; as expected in a model of cholestasis, γ-GTP and bilirubins were highly increased by BDL (22.5- and seven-fold respectively). Curcumin treatment partially, but significantly, prevented the increase of these serum markers of liver damage. Haematoxylin and eosin staining of liver sections showed important areas of necrosis in the BDL group that were absent in the BDL+curcumin group, confirming the ALT observations. Liver glutathione levels were measured as an indicator of oxidative stress at the hydrophilic level. GSH, GSH/GSSG ratio and total (GSH+GSSH) glutathione decreased significantly in the BDL group; in contrast, curcumin administration increased glutathione levels in BDL and in rats administered curcumin alone. The main source of energy in the liver, glycogen, was depleted by BDL, while curcumin maintained the normal levels. Fibrosis, measured by the liver content of hydroxyproline and visualized by trichromic staining, increased five-fold by BDL; this effect was prevented partially but significantly by curcumin. Numerous studies have shown that TGF-β treatment upregulates the expression of various profibrotic genes (75). The results of Reyes-Gordillo *et al.* (80) show increases in TGF-β mRNA in livers from BDL rats; importantly, curcumin treatment markedly inhibited this increase. TGF-β protein was also increased by BDL, but significantly inhibited by curcumin.

It can be concluded that curcumin attenuates experimental fibrosis of various aetiologies including biliary cirrhosis and that some of the mechanisms include NF-κB and TGF-β downregulation and its anti-oxidant properties.

Effects on acute and subacute carbon tetrachloride toxicity

Acute toxicity to the liver induces necrosis, inflammation and oxidative stress of hepatocytes (73). Recently, it has been suggested that hepatocellular injury is because of the inflammatory cells that have been attacked by the stressed hepatocytes (74). The inflammatory response is mediated by cytokines, mainly interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α); these cytokines may induce an acute-phase response or modulate the effects of IL-6 (81). The relevance of these cytokines in liver damage is demonstrated by evidence that circulating levels of IL-1β and TNF-α are elevated in rats that develop hepatic injury (74). It is also known that inflammatory events are regulated by NF-κB activation by the release of IL-1β and TNF-α (82). NF-κB is an important regulator of the expression of genes encoding cytokines (71) including IL-1β and TNF-α. Therefore, a vicious cycle is established in the damaged liver: IL-1β and TNF-α induce NF-κB activity and these factors increase the production of additional IL-1β and TNF-α. This cycle destroys the hepatic parenchyma and impairs liver function. Therefore, NF-κB is a suitable target to treat liver diseases (71). Studies involving systemic curcumin administration have demonstrated its beneficial effects by modulating NF-κB activity (35); in addition, the ability of curcumin to scavenge a variety of ROX (83) makes this compound a suitable tool to be studied in CCl_4-induced liver damage.

Park *et al.* (84) studied the protective effect of curcumin in acute and subacute rat liver injury induced by CCl_4. In acutely liver-damaged rats (0.2 ml/kg, i.p.), AST and ALT serum activities were increased to 340 and 397% as compared with control animals. Treatment with curcumin (100 or 200 mg/kg) significantly prevented the increase of these enzymes. In the subchronic liver damage, CCl_4 was administered by gavage (1 ml/kg, mixed with an equal volume of corn oil) twice a week for 4 weeks; curcumin (50 or 100 mg/kg, daily) was administered along with CCl_4. Serum activities of alkaline phosphatase, AST and ALT increased by subacute CCl_4 intoxication, 1041, 840 and 1653%, respectively, while serum albumin levels decreased 22%. Curcumin (100 mg/kg) prevented partially, but significantly, these alterations. A 50 mg/kg dose of curcumin showed modest and no significant effects on these parameters.

The liver hydroxyproline content increased to 277% in subacutely intoxicated rats as compared with controls; daily administration of 100 mg/kg of curcumin maintained the liver hydroxyproline content within normal levels. The malondialdehyde in the livers of CCl_4-treated animals for 4 weeks increased significantly, but again, curcumin maintained normal lipid peroxidation degree.

In summary, the study of Park *et al.* (84) demonstrated that curcumin can effectively inhibit the hepatic damage produced by either acute or subacute CCl_4 treatment as monitored by serum biochemical parameters, fibrosis and lipid peroxides in the liver.

The ability of oral curcumin (200 mg/kg) to prevent acute CCl_4 (4 g/kg, p.o.) intoxication was studied recently (85). Acute CCl_4 administration produced liver injury measured by serum ALT, γ-GTP and bilirubins, and by histopathological analysis. The damage was associated with a decreased hepatic GSH/GSSG ratio, indicating oxidative stress. Furthermore, NF-κB was activated and pro-inflammatory cytokines upregulated by this factor, IL-1β, TNF-α and IL-6, increased several fold. Curcumin

treatment prevented all the alterations induced by acute CCl_4 intoxication (85). It seems likely that the anti-oxidant properties of curcumin (83) and its ability to inactivate NF-κB (35), and thus pro-inflammatory cytokine production (86), are the most important mechanisms of action of curcumin to prevent acute CCl_4-induced liver injury.

Reversion of carbon tetrachloride cirrhosis

As shown previously, the ability of curcumin to prevent acute and chronic liver damage has been demonstrated in different models (CCl_4, TAA and BDL). However, the capacity of this compound to reverse well-established cirrhosis was not demonstrated. This is a very interesting point because usually patients are treated when the disease is already present.

Advanced cirrhosis is generally considered to be an irreversible process even after removal of the causative agent. The disease seems to be characterized by the inability of the damaged liver to remodel the fibrotic parenchyma (75). Discontinuation of CCl_4 after 2 months of chronic (three times per week) treatment is usually followed by rapid fibrosis remission (87). Therefore, based on previous observations (88), we decided to perform the evaluation of curcumin to reverse CCl_4 well-established cirrhosis by using a prolonged CCl_4 treatment (3 months vs. the 2 months normally used in experimental protocols); after 3 months of chronic CCl_4 administration, the toxin was discontinued and curcumin (100 mg/kg, p.o., daily) was administered for 2 months; a group receiving vehicle was studied to monitor spontaneous resolution of liver damage (80). ALT activity increased significantly after 3 months of chronic CCl_4 administration. Importantly, discontinuation of CCl_4 produced a further increase in plasma ALT; curcumin treatment for 2 months of rats pretreated with CCl_4 restored the levels of ALT enzyme activity to normal values. Because it is well known that oxidative stress participates in the damage caused by CCl_4, glutathione was evaluated: an important decrement in GSH, GSH/GSSG ratio and total glutathione produced by CCl_4 administration for 3 months was observed. Interestingly, discontinuation of the toxin increased oxidative stress at the hydrophilic level as the GSH, GSH/GSSG ratio and GSH+GSSG were lower than those in rats treated with CCl_4 for 3 months. Very importantly, administration of curcumin for 2 months resulted in normalization of glutathione levels.

Hepatic fibrosis occurs as a result of an imbalance between fibrogenesis and fibrolysis and is a major factor contributing to liver failure in cirrhotic patients. Administration of CCl_4 for 3 months led to a five-fold increase in the collagen content; curcumin administration to these rats for 2 months produced a partial but significant reversion of CCl_4-induced fibrosis. Liver glycogen content was found to be decreased because of CCl_4 administration and discontinuation of CCl_4 led to a further decrease of glycogen; importantly, curcumin-restored control glycogen levels (80).

It has been considered that the beneficial effects of curcumin are mediated, in part, by its anti-oxidant defence ability and the scavenging of free radicals; furthermore, curcumin is 10 times more active as an anti-oxidant than vitamin E (89). CCl_4 decreases the anti-oxidant capacity of the liver (73, 90) even after CCl_4 discontinuation for 2 months (80); thus, the anti-oxidant activity of curcumin may account for its beneficial properties in the reversion of cirrhosis. Curcumin also demonstrated its ability to restore glycogen levels previously depleted by hepatic injury induced by CCl_4 administration; Pari and Murugan (91) reported the ability of curcumin to preserve blood glucose levels. They attributed this action to the ability of curcumin to restore the altered activities of the enzymes 6-phosphate dehydrogenase and glucose-6-phosphatase that participate in gluconeogenesis and glucogenolysis. The same mechanism may be responsible for the recuperation of glycogen in the liver.

Liver recovery from fibrosis involves the degradation of fibrous bands. Metalloproteinase enzymes (MMPs), especially pro-MMP-2 and proMMP-9, as well as their active forms, are responsible for the degradation of matrix proteins like gelatin, collagen IV, collagen V, fibronectin and elastin (92, 93). It has been reported that curcumin upregulates the expression and activity of matrix pro-MMP-2 and proMMP-9 in human bronchial epithelial cells, and during the prevention and healing of indomethacin-induced gastric ulcers (94, 95). Thus, the reversion of liver fibrosis observed by Reyes-Gordillo *et al.* (80) may be explained through modulation of MMPs; in addition, downregulation of NF-κB and TGF-β and other cytokines may provide likely antifibrotic and fibrolytic mechanisms (71, 85). Furthermore, Kang *et al.* (96) reported that curcumin inhibits collagen synthesis and HSC activation *in vivo* and *in vitro*, supporting the ability of curcumin to reverse CCl_4 fibrosis (85).

Conclusions

Curcumin has shown beneficial properties in diverse experimental models of liver damage. It prevents liver damage induced by aflatoxins, iron overdose, erythromycin estolate, ethanol, TAA acute and chronic intoxication, cholestasis (BDL) and acute, subacute and chronic CCl_4 intoxication; moreover, it reverses CCl_4 cirrhosis to some extent. Curcumin may act at several molecular targets, but two have been utilized the most to explain its pharmacological properties: one is its anti-oxidant effect and the other is its ability to inhibit NF-κB factors. Oxidative stress plays a causative role in most liver disorders and models of hepatic injury, while NF-κB is responsible for the transcription of the fundamental mediators of inflammation and liver damage.

In the case of acute CCl_4 intoxication, for example, oxidative stress and expression of pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 (all of them upregulated by NF-κB) and activation of NF-κB were associated with increases in several markers of liver damage and distortion in the hepatic microscopical structure; curcumin pretreatment prevented oxidative stress, activation of NF-κB and liver damage. Similarly, in biliary cirrhosis, curcumin showed antifibrogenic properties associated with a downregulation of the most profibrotic cytokine, TGF-β. Reversion of CCl_4-induced cirrhosis by curcumin is, perhaps, the most exciting discovery due to its possible implications in human chronic liver disease. However, there is only one report supporting the ability of the compound to reverse the disease. Therefore, more basic and even clinical (due to the low toxicity of curcumin) studies are urgently needed before this drug can be recommended for the treatment of human acute and chronic liver disorders.

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References

1. Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid Redox Signal* 2008; **10**: 511–45.
2. Goel A, Jhurani S, Aggarwal BB. Multi-targeted therapy by curcumin: how spicy is it? *Mol Nutr Food Res* 2008; **52**: 1010–3.
3. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. *Biochem Pharmacol* 2008; **75**: 787–809.
4. Sreejayan N, Rao MN. Free radical scavenging activity of curcuminoids. *Arzneimittelforschung* 1996; **46**: 169–71.
5. Ahsan H, Parveen N, Khan NU, Hadi SM. Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivative demethoxycurcumin and bisdemethoxycurcumin. *Chem Biol Interact* 1998; **121**: 161–75.
6. Syu WJ, Shen CC, Don MJ, *et al*. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. *J Nat Prod* 1998; **61**: 1531–4.
7. Aggarwal BB, Sundaram C, Malani M, Ichikawa H. Curcumin: the Indian solid gold. *Adv Exp Med Biol* 2007; **595**: 1–75.
8. Sreejayan N, Rao MN. Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol* 1997; **49**: 105–7.
9. Huang MT, Lou YR, Xie JG, *et al*. Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz[a]anthracene-induced mammary tumors and lymphomas/leukemias in Sencar mice. *Carcinogenesis* 1998; **19**: 1697–700.
10. Ploto A. *Turmeric: Post-Production Management for Improved Market Access for Herbs and Spices – Turmeric*. Rome: Food and Agriculture Organization of the United Nations (FAO), 2003.
11. NCI. Clinical development plan: curcumin. *J Cell Biochem* 1996; **26S**: 72–85.
12. Shankar TN, Shantha NV, Ramesh HP, *et al*. Toxicity studies on turmeric (*Curcuma longa*): acute toxicity studies in rats, guinea pigs & monkeys. *Indian J Exp Biol* 1980; **18**: 73–5.
13. Qureshi S, Shah AH, Ageel AM. Toxicity studies on *Alpinia galangal* and *Curcuma longa*. *Planta Med* 1992; **58**: 124–7.
14. Lao CD, Ruffin MT, Normolle D, *et al*. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med* 2006; **6**: 10–3.
15. Romiti N, Tongiani R, Cervelli F, Chieli E. Effects of curcumin on P-glycoprotein in primary cultures of rat hepatocytes. *Life Sci* 1998; **62**: 2349–58.
16. Anuchapreda S, Leechanachai P, Smith MM, *et al*. Modulation of P-glycoprotein expression and function by curcumin in multidrug-resistant human KB cells. *Biochem Pharmacol* 2002; **64**: 573–82.
17. Wortelboer HM, Usta M, van der Velde AE, *et al*. Interplay between MRP inhibition and metabolism of MRP inhibitors: the case of curcumin. *Chem Res Toxicol* 2003; **16**: 1642–51.
18. Iersel ML, Ploemen JP, Struik I, *et al*. Inhibition of glutathione-S-transferase activity in human melanoma cells by alpha, beta-unsaturated carbonyl derivatives. Effects of acrolein, cinnamaldehyde, citral, crotonaldehyde, curcumin ethacrynic acid, and trans-2-hexenal. *Chem Biol Interact* 1996; **102**: 117–32.
19. Logan-Smith MJ, Lockyer PJ, East JM, Lee AG. Curcumin, a molecule that inhibits the Ca^{2+}-ATPase of sarcoplasmic reticulum but increases the rate of accumulation of Ca^{2+}. *J Biol Chem* 2001; **276**: 46905–11.
20. Bilmen JG, Khan SZ, Javed MH. Inhibition of the SERCA Ca^{2+} pumps by curcumin. Curcumin putatively stabilizes the interaction between the nucleotide-binding and phosphorylation domains in the absence of ATP. *Eur J Biochem* 2001; **268**: 6318–27.
21. Zsila F, Bikadi Z, Simonyi M. Induced circular dichroism spectra reveal binding of the antiinflammatory curcumin to human alpha1-acid glycoprotein. *Bioorg Med Chem* 2004; **12**: 3239–45.
22. Yang F, Lim GP, Begum AN, *et al*. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J Biol Chem* 2005; **280**: 5892–901.
23. Shim JS, Kim JH, Cho HY, *et al*. Irreversible inhibition of CD13/aminopeptidase N by the antiangiogenic agent curcumin. *Chem Biol* 2003; **10**: 695–704.
24. Skrzypczak-Jankun E, Zhou K, McCabe NP, *et al*. Structure of curcumin in complex with lipoxygenase and its significance in cancer. *Int J Mol Med* 2003; **12**: 17–24.
25. Began G, Sudharshan E, Appu Rao AG. Inhibition of lipoxygenase 1 by phosphatidylcholine micelles-bound curcumin. *Lipids* 1998; **33**: 1223–8.

26. Shishodia S, Singh T, Chaturvedi MM. Modulation of transcription factors by curcumin. *Adv Exp Med Biol* 2007; **595**: 127–48.
27. Bosetti C, Levi F, Lucchini F, *et al*. Worldwide mortality from cirrhosis: an update to 2002. *J Hepatol* 2007; **46**: 827–39.
28. Briton RS, Bacon BR, Recknagel RO. Lipid peroxidation and associated hepatic organelle dysfunction in iron overload. *Chem Physiol Lipids* 1987; **45**: 207–39.
29. Weintraub LR, Gorel A, Grasso J, *et al*. Pathogenesis of hepatic fibrosis in experimental iron overload. *Br J Haematol* 1985; **59**: 321–3.
30. Deugnier Y, Brissot P, Loréal O. Iron and the liver: update 2008. *J Hepatol* 2008; **48**: S113–23.
31. Reddy ACP, Lokesh BR. Alterations in lipid peroxides in rat liver by dietary n-3 fatty acids: modulation of antioxidant enzymes by curcumin, eugenol, and vitamin E. *J Nutr Biochem* 1994; **5**: 181–8.
32. Reddy ACP, Lokesh BR. Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol Cell Biochem* 1994; **137**: 1–8.
33. Girotti AW, Thomas JP. Damaging effects of oxygen radicals on resealed erythrocyte ghosts. *J Biol Chem* 1984; **259**: 1744–52.
34. Reddy PACh, Lokesh BR. Effect of curcumin and eugenol on iron-induced hepatic toxicity in rats. *Toxicology* 1996; **107**: 39–45.
35. Singh S, Aggarwal BB. Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane). *J Biol Chem* 1995; **270**: 24995–5000.
36. Messner D, Sivam G, Kowdley KV. Curcumin reduces the toxic effects of iron loading in rat liver epithelial cells. *Liver Int* 2009; **29**: 63–73.
37. Rajakrishnan V, Viswanathan P, Rajashekaran KN, Menon VP. Neuroprotective role of curcumin from *Curcuma longa* on ethanol-induced brain damage. *Phytother Res* 1999; **13**: 571–4.
38. Clemens MR, Kessler W, Schied HW, *et al*. Plasma and red cell lipids in alcoholics with macrocytosis. *Clin Chim Acta* 1986; **156**: 321–8.
39. Horrobin DF. Essential fatty acids, prostaglandins and alcoholism: an overview. *Alcoholism: Clin Exp Res* 1987; **11**: 2–9.
40. Morimoto M, Hagbjork AL, Wan YJY, *et al*. Modulation of experimental alcohol-induced liver disease by cytochrome P450E1 inhibitors. *Hepatology* 1995; **21**: 1610–7.
41. Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipase A2. *Ann Rev Biochem* 2008; **77**: 495–520.
42. Harizi H, Corcuff JB, Gualde N. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. *Trends Mol Med* 2008; **14**: 461–9.
43. Zheng Z, Barki AI, Hungund BL. Effects of ethanol on the incorporation of free fatty acids into cerebral membrane phospholipids. *Neurochem Int* 1996; **28**: 551–5.
44. Hugund BL, Zheng Z, Lin L, Barkai AI. Ganglioside GMI reduces ethanol induced phospholipase A_2 activity in synaptosomal preparations from mice. *Neurochem Intern* 1994; **25**: 321–5.
45. Holtzmann MJ. Arachidonic acid metabolism. *Am Rev Resp Dis* 1991; **143**: 188–203.
46. Rajakrishnan V, Jayadeep A, Arun OS, *et al*. Changes in the prostaglandin levels in alcohol toxicity: effect of curcumin and N-acetylcysteine. *J Nutr Biochem* 2000; **11**: 509–14.
47. Aggarwal BB, Takada Y, Oommen OV. From chemoprevention to chemotherapy: common targets and common goals. *Exp Opin Investig Drugs* 2004; **13**: 1327–38.
48. Nanji AA, Zakim D, Rahematulla A, *et al*. Dietary saturated fatty acids down-regulate cycloxygenase-2 and tumor necrosis factor alpha and reverse fibrosis in alcohol-induced liver disease in the rat. *Hepatology* 1997; **26**: 1538–45.
49. Rao CV, Simi B, Reddy BS. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. *Carcinogenesis* 1993; **14**: 2219–25.
50. Huang MT, Lysz T, Ferraro T, *et al*. Inhibitory effects of curcumin on in vitro lipoxygenase and cycloxygenase activities in mouse epidermis. *Cancer Res* 1991; **51**: 813–9.
51. Lee WL. Drug-induced hepatotoxicity. *N Engl J Med* 2003; **349**: 474–85.
52. O'Grady J, Schalman SW, Williams R. Acute liver failure: redefining the syndrome. *Lancet* 1993; **324**: 273–5.
53. Bruck R, Aeed H, Avni Y, *et al*. Melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. *J Hepatol* 2004; **40**: 86–93.
54. Abul H, Mathew TC, Dashti HM, Al-Bader A. Level of superoxide dismutase, glutathione peroxidase and uric acid in thioacetamide-induced cirrhotic rats. *Anat Histol Embryol* 2002; **31**: 66–71.
55. Sun F, Hayami S, Origiri Y, *et al*. Evaluation of oxidative stress based on lipid hydroperoxide, vitamin C and vitamin E during apoptosis and necrosis caused by thioacetamide in rat liver. *Biochim Biophys Acta* 2000; **1500**: 181–5.
56. Lu SC, Huang ZZ, Yang H, Tsukamoto H. Effect of thioacetamide on the hepatic expression of gamma-glutamylcysteine synthetase subunits in the rat. *Toxicol Appl Pharmacol* 1999; **159**: 161–8.
57. Rahman TM, Hodgson HJ. The effect of early and late administration of inhibitors of inducible nitric oxide synthase in a thioacetamide-induced model of acute hepatic failure in the rat. *J Hepatol* 2003; **38**: 583–90.
58. Miquel J, Bernd A, Sempere JM, *et al*. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. *Arch Gerontol Geriatr* 2002; **34**: 37–46.
59. Das KC, Das CK. Curcumin (diferuloylmethane), a singlet oxygen ((1)O(2)) quencher. *Biochem Biophys Res Commun* 2002; **295**: 62–6.
60. Ak T, Gülçin I. Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact* 2008; **174**: 27–37.
61. Brouet I, Ohshima H. Curcumin, an anti-tumor promoter and anti-inflammatory agent, inhibits induction of nitric

oxide synthase in activated macrophages. *Biochem Biophys Res Comm* 1995; **206**: 533–40.
62. Kim JE, Kim AR, Chung HY, *et al*. In vitro peroxynitrite scavenging activity of diarylheptanoids from *Curcuma longa*. *Phytother Res* 2003; **17**: 481–4.
63. Iqbal M, Sharma SD, Okazaki Y, *et al*. Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity. *Pharmacol Toxicol* 2003; **92**: 33–8.
64. Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. *Free Rad Biol Med* 2000; **28**: 1303–12.
65. Nanji AA, Jokelainen K, Jokelainen K, *et al*. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G321–7.
66. Shapiro H, Ashkenazi M, Weizman N, *et al*. Curcumin ameliorates acute thioacetamide-induced hepatotoxicity. *J Gastroenterol Hepatol* 2006; **21**: 358–66.
67. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of turmeric (*Curcuma longa*). *J Alter Comp Med* 2003; **9**: 161–8.
68. Bruck R, Ashkenazi M, Weiss S, *et al*. Prevention of liver cirrhosis in rats by curcumin. *Liver Int* 2007; **27**: 373–8.
69. Hunter AL, Holscher MA, Neal RA. Thioacetamide-induced hepatic necrosis. I. Involvement of the mixed-function oxidase enzyme system. *J Pharmacol Exp Ther* 1977; **200**: 439–48.
70. Bruck R, Shirin H, Aeed H, *et al*. Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers. *J Hepatol* 2001; **35**: 457–64.
71. Muriel P. NF-kappaB in liver diseases: a target for drug therapy. *J Appl Toxicol* 2009; **29**: 91–100.
72. Bruck R, Aeed H, Schey R, *et al*. Pyrrolidine dithiocarbamate protects against thioacetamide-induced fulminant hepatic failure in rats. *J Hepatol* 2002; **36**: 370–7.
73. Muriel P. Some experimental models of liver damage. In: Sahu SC, ed. *Hepatotoxicity: From Genomics to In Vitro and In Vivo Models*. Chichester: Wiley, 2007a; 119–37.
74. Muriel P. Cytokines in liver diseases. In: Sahu SC, ed. *Hepatotoxicity: From Genomics to In Vitro and In Vivo Models*. Chichester: Wiley, 2007b; 371–89.
75. Friedman SL. Mechanisms of disease: mechanisms of hepatic fibrosis and therapeutic implications. *Nat Clin Pract Gastroenterol Hepatol* 2004; **1**: 98–105.
76. Pinzani M, Marra F, Carloni V. Signal transduction in hepatic stellate cells. *Liver* 1998; **18**: 2–13.
77. Gressner AM, Weiskirchen R. Modern pathogenic concepts of liver fibrosis suggest stellate cells and TGF-β as major players and therapeutic targets. *J Cell Mol Med* 2006; **10**: 76–99.
78. Punithavathi D, Venkatesan N, Babu M. Protective effects of curcumin against amiodarone-induced pulmonary fibrosis in rats. *Br J Pharmacol* 2003; **139**: 1342–50.
79. Gaedeke J, Noble AN, Border WA. Curcumin blocks multiple sites of the TGF-β signaling cascade in renal cells. *Kidney Int* 2004; **66**: 112–20.
80. Reyes-Gordillo K, Segovia J, Shibayama M, *et al*. Curcumin prevents and reverses cirrhosis induced by bile duct obstruction or CCl_4 in rats: role of TGF-β modulation and oxidative stress. *Fund Clin Pharmacol* 2008; **22**: 417–27.
81. Simpson KJ, Lukacs NW, Colleti L, *et al*. Cytokines and the liver. *J Hepatol* 1997; **27**: 1120–32.
82. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 2003; **33**: 105–36.
83. Maheswari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. *Life Sci* 2006; **78**: 2081–7.
84. Park EJ, Jeon CH, Ko G, *et al*. Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J Pharm Pharmacol* 2000; **52**: 437–40.
85. Reyes-Gordillo K, Segovia J, Shibayama M, *et al*. Curcumin protects against acute liver damage in the rat by inhibiting NF-κB, proinflammatory cytokines production and oxidative stress. *Biochim Biophys Acta* 2007; **1770**: 989–96.
86. Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripherals blood monocytes and alveolar macrophages. *Pharmacol Res* 1999; **39**: 41–7.
87. Chávez E., Alcantar LK, Moreno MG, Muriel P. Gadolinium chloride inhibits the spontaneous resolution of fibrosis in CCl_4-induced cirrhosis. *Toxicol Mech Meths* 2006; **16**: 507–13.
88. Muriel P, Moreno MG, Hernández MC, *et al*. Resolution of liver fibrosis in chronic CCl_4 administration in the rat after discontinuation of treatment: effect of silymarin, silibinin, colchicine and trimethylcolchicinic acid. *Basic Clin Pharmacol Toxicol* 2005; **96**: 375–80.
89. Kowluru AR, Kanwar M. Effects of curcumin on retinal oxidative stress and inflammation in diabetes. *Nutr Metabolism* 2007; **4**: 1–8.
90. Muriel P. Peroxidation of lipids and liver damage. In: Baskin SI, Salem H, eds. *Oxidants Antioxidants and Free Radicals*. Washington, DC: Taylor and Francis, 1997; 237–57.
91. Pari L, Murugan P. Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. *J Basic Clin Physiol Pharmacol* 2005; **16**: 257–74.
92. Hironaka K, Sakaida I, Matsumura Y, *et al*. Enhanced interstitial collagenase (matrix metalloproteinase-13) production of Kupffer cells by gadolinium chloride prevents pig serum-induced rat liver fibrosis. *Biochem Biophys Res Commun* 2000; **267**: 290–5.
93. Sakaida I, Hironaka K, Terai S, Okita K. Gadolinium chloride reverses dimethylnitrosamine (DMN)-induced rat liver fibrosis with increased matrix metalloproteinases (MMPs) of Kupffer cells. *Life Sci* 2003; **72**: 943–59.
94. Swarnakar S, Ganguly K, Kundu P, *et al*. Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. *J Biol Chem* 2005; **280**: 9409–15.

95. Hozumi A, Nishimura Y, Nishiuma T, *et al*. Induction of MMP-9 in normal human bronchial epithelial cells by TNF-alpha via NF-kappa B-mediated pathway. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: 1444–52.

96. Kang HC, Nan JX, Park PH, *et al*. Curcumin inhibits collagen synthesis and hepatic stellate cell activation in-vivo and in-vitro. *J Pharm Pharmacol* 2002; **54**: 119–26.