The Preventive and Therapeutic Role of Curcumin in Liver Cirrhosis

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Abstract: Hepatic fibrosis or cirrhosis is emerging as a treatable complication of chronic liver disease, following significant progress in understanding its underlying mechanisms. Efforts have focused on the hepatic stellate cells (HSC), as these cells can undergo, "activation" into proliferation and fibrogenic myofibroblast-like cells during liver injury. Antifibrotic therapies could become important in treating the millions of patients with chronic fibrosing liver disease. Curcumin the major polyphenolic compound in tumeric has been shown to attenuate hepatic damage. So, the present study was designed to assess the efficacy of curcumin intake in preventing thioacetamide-induced hepatic cirrhosis and portal hypertension (manifested as splenomegaly). Four groups of rats were used throughout this study. **Group I (Control group):** rats received the solvent at identical amount and duration. Liver cirrhosis was induced in Groups II, III, and IV by thioacetamide (TAA; 200mg/kg, ip) twice weekly for 12 weeks. **Group II (Cirrhosis group):** untreated group. **Group III (Prevention group):** rats received curcumin (300 mg/kg/day, by gavage for 12 weeks) concomitantly with TAA. **Group IV (Treatment group):** rats were given curcumin for 6 weeks after TAA discontinuation. Specimens from the livers were processed for paraffin sections and stained with Hx&E and Masson Trichrome stain. Alpha smooth muscle actin expressed immunohistochemically by HSC were considered a marker of their activation to myofibroblast. Image analyzer was used to analyze the results morphometrically. Also, statistical analysis of the results was determined by ANOVA test. Histological findings proved that the curcumin protected the liver structure in TAA-induced liver cirrhosis rats. The curcumin treatment almost normalized these effects in the histoarchitecture of liver. Indeed, there was remarkable reduction in fibrosis extent and a decrease of stellate infiltration in rats concomitantly treated with curcumin compared to non treated group. Curcumin had no effect on pre-established liver cirrhosis. In conclusion, this study showed that curcumin has protective effects from hepatic cirrhosis in rats that were proven by histopathological analysis. As curcumin is safe for consumption by humans, it may have a beneficial role in chemical-induced hepatic damage although this finding needs further study to know the active constituents appearing to protect rat liver against cirrhosis.
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Key words: Curcumin, Liver, cirrhosis, immunohistochemistry

1. Introduction

Liver disease, including chronic hepatitis, non-alcoholic steatosis, fibrosis/cirrhosis, hepatocellular carcinoma (HCC), afflicts over 10% of the world's population**.** The most significant risk factor for the development of HCC is the presence of cirrhosis, regardless of its etiology. Hence, preventive and therapeutic inventions are necessary to halt or slow down the progression of chronic hepatitis to cirrhosis, HCC and death [(1).]

In Saudi Arabia, HCC is one of the most common malignancies. Its incidence is expected to increase dramatically in the Kingdom in the next 30 years. Hepatitis B and hepatitis C appear to be the two most important risk factors for HCC in Saudi Arabia [(2)]**.** Also, the rising prevalence of obesity in the Saudi Arabia is associated with an alarming increase in Non-alcoholic steatohepatitis (NASH) leading to advanced fibrosis and cirrhosis [(3)]**.**

Certain forms of hepatic injury lead to a chronic, partially self-perpetuating inflammation and an attempt at tissue regeneration progress in some patients to liver cirrhosis. Chronic tissue injury and inflammation lead to activation of hepatic stellate cells (HSC), which become contractile and deposit collagen in the space of Disse. This can eventually lead to disruption of the functional structure of hepatic lobules and to increased resistance to portal blood flow [(4)].

Increased collagen deposition between hepatocytes and sinusoids and the diminution of the size of endothelial fenestrae lead to the capillarization of sinusoids. Constriction of sinusoids by contractile HSC and capillarization increase resistance to blood flow and contribute to the development of portal hypertension**.** Complications of portal hypertension are the main causes of mortality in patients with cirrhosis [(5)]**.**

Although nucleoside analogues (e.g. lamivudine, adefovir dipivoxil) and interferon have been clinically proven medicines for curing chronic viral hepatitis, there are still no effective therapeutic drugs for fibrosis. Such unfavorable scenarios have

drawn considerable attention from pharmaceutical industries and healthcare professionals [6].

The rhizome of turmeric (Curcuma longa), a plant belonging to the ginger family, is widely used as a food colouring and is one of the principal ingredients in curry powder. Turmeric has long been used in alternative medicine as an anti-inflammatory to treat digestive and liver disorders, and for the treatment of skin diseases and wound healing [7].

The main active ingredient in turmeric is curcumin (diferuloylmethane) [7], administration of which is therapeutic in rodent models of a number of intestinal, pancreatic and liver diseases [8]. Anti-inflammatory, anti-oxidant and nuclear factor-κB (NF-κB) inhibiting properties may underlie curcumin's beneficial effect in these conditions [9]. It has been reported that curcumin prevents collagen type I formation by activated HSC [10] and reduces hepatic fibrosis and inflammation in rodent models of steatohepatitis [11]. Moreover, **Shapiro et al.,**[12] mentioned that Curcumin ingestion is safe in humans, and is sufficiently bioavailable to produce beneficial systemic and hepatic effects.

Utilizing the chronic administration of thioacetamide (TAA) to rats as a model of liver cirrhosis, the present study was designed to assess the efficacy of curcumin intake in preventing and /or treating thioacetamide-induced hepatic cirrhosis and portal hypertension (manifested as splenomegaly).

2. Materials and methods:

A) Test drugs:

1. Thioacetamide (Sigma-Aldrich, St. Louis, USA).
2. Curcumin, glycerol formal and cremohore (Sigma-Aldrich, St. Louis, USA). Curcumin was freshly prepared in glycerol formal, cremohore and water (5:2:2).

B) Animal groups:

Male adult albino rats weighting 250-300 gm were purchased from the animal house of King Fahd Research Center. They were kept under normal laboratory conditions, and given free access of food and water. They were left for acclimatization for one week before starting the experiment.

The rats were divided into 4 main groups, each consisting of 12 animals:

Group I (Control group): Rats received only the solvent for the same period of time without induction of cirrhosis.

Group II (Cirrhosis Group): Liver cirrhosis was induced by intraperitoneal (i.p.) injection of Thioacetamide (TAA) (200 mg/kg) twice a week for 12 weeks [4].

Group III (Prevention Group): In this group liver cirrhosis was induced by intraperitoneal (i.p.) injection of Thioacetamide (TAA) (200 mg/kg) twice a week for 12 weeks. Rats concomitantly received daily intragastric curcumin (300mg/kg, dissolved in solvent described above and given in a final volume of 2 ml per rat) [4].

Group IV (Treatment Group): This experiment was designed also to determine whether curcumin would have a beneficial effect on established liver cirrhosis. Such an effect may support a mechanism that includes direct anrti-fibrotic or fibrinolytic effects. Rats was given bi-weekly i.p. TAA 200mg/kg for 12 weeks followed by 300mg/kg/day curcumin given by gavage for another 6 weeks after TAA discontinuation.

At the end of the study, the rats were sacrificed, their livers were removed and the spleen weights were measured. Thereafter, livers were prepared for histological study.

Ratio of spleen weight relative to body weight:

Characteristic hemodynamic changes of liver cirrhosis including portal hypertension and hyperdynamic circulation are accompanied by a significant increase in spleen weight. So, the average weights of the animals were recorded on the same days of sacrifice and the wet weight of the spleen were determined and compared to the body weight.

Histological study:

By the end of the experiment, rats from each group were sacrificed; livers were removed and washed with saline. Parts of the tissue were fixed in 10% buffered formol saline and processed for 5um paraffin sections for H & E and Masson trichrome staining [13].

Immunohistochemical study:

Immunostaining for alpha smooth muscle actin was performed on paraffin sections from the livers of all groups. This was done using a primary anti serum to alpha smooth muscle actin (DAKO Corp. Denmark) followed by biotinylated horse antimouse antiserum, avidin-biotin complex and DAB as the chromogen. Smooth muscle was used as positive control specimens. On the other hand, one of the liver specimens was used as negative control by omitting the step of applying the primary antibody. A positive reaction was expressed as a dark brown color in the cytoplasm of hepatocyte stellate cell indicating its activation into myofibroblast.

Morphometric studies:

Quantitation was performed with Leica image analysis computer system using the software Qwin

500 (England). It was used to study and compare the area percent of activated hepatocyte stellate cells in alpha smooth muscle actin immuno-stained slides among the different groups used in this study.

Moreover, image analysis was used to quantify the liver fibrosis by measuring the area percent of collagen fibers on specimens stained with Masson's trichrome in all groups.

The area percent of the stainings were measured inside a standard measuring frame of a known area. Using the mouse of the computer, a small area of the stained alpha smooth muscle actin or collagen fibers was selected and masked by a binary color. Then the area of the binary color was measured and expressed as an area percent in relation to the area of the standard measuring frame. This procedure was repeated in 10 microscopic fields (original magnification, X200) for each rat and their mean values were finally obtained.

Statistical analysis of the results:

Results of the present study were presented as the mean ± SD. The significance of differences among different groups was determined by ANOVA test according to **Mould [14].** Results were considered significant when probability P is <0.5.

3. Results

Ratio of spleen weight (SW) relative to body weight (BW):

There was a significant increase in SW/BW ratio in cirrhosis group (P<0.05). Pretreatment of cirrhotic rats with curcumin significantly decreased SW/BW ratio compared to cirrhotic group throughout the study period as shown in table (1). However, there was no significant change between curcumin treated cirrhotic and cirrhotic rats (table 1).

Table (1): the ratio of spleen weight (SW) relative to body weight (BW) in all studied groups:

Group	Body weight (g) Mean ± SD	Spleen weight (g) Mean ± SD	Spleen weight/body weight (%)
Group I (Control group)	229 ± 5.25	0.51± 0.03	0.22
Group II (Cirrhosis Group)	185 ± 6.31*	1.31± 0.05*	0.71 *
Group III (Prevention Group)	220 ± 3.95#	0.82±0.08 #	0.37 #
Group IV (Treatment Group)	191 ± 6.15*	1.22±0.04*	0.64 *

Values are represented as mean ± SD Significance was considered P<0.05

*** Significant change compared to Group I (Control group) # Significant change compared to Group II (Cirrhosis Group)**

Histological results:

Group I (Control group) (Fig.1):

In group I (the control group), the hepatic lobules showed almost normal histological architecture of liver cells arranged in the form of liver cords, radiating from the central veins. The liver cords were separated from each other by blood sinusoids which were lined by endothelial cells and von Kupffer cells. The hepatocytes were polygonal cells with granular and eosinophic cytoplasm and their nuclei were mostly large, rounded with prominent nuclei. The connective tissue was demonstrated as a thin layer of collagen fibers in the wall of the central vein and the portal tracts.

Group II (Cirrhosis Group) (Figs.2, 3):

Histological examination of the livers of group II (the cirrhotic group) rats showed disorganization of the normal lobular pattern with the formation of well-defined pseudolobules. Variable grades of degeneration and necrosis were demonstrated starting centrally around the central veins then progressed to all zones of the hepatic lobules (centrilobular). The affected hepatocytes were vacuolated or apoptotic having darkly eosinophilic cytoplasm with pyknotic nuclei. Mononuclear cellular infiltration was found between the hepatocytes and marked within the portal areas. Apparent hyperplasia of bile ductules were also observed in some cases. Moreover, thick bundles of collagen fibers were bridging the expanded portal areas and surrounding the lobules.

Group III (Prevention Group) (Fig.4):

No uniform pattern was found in the livers of group III (prevention group). Confined areas showed variable grades of degeneration of hepatocytes among apparently normal ones. It varied from vacuolization up to complete destruction of these cells. The degenerated areas were mostly centrilobular and surrounded by minimal mononuclear cellular infiltration. Moreover, expanded portal tracts with dilatation and congestion of their portal veins were seen in focal areas of liver lobules. However, Masson trichrome stain showed increased connective tissue and deposition of collagen fibers in portal areas and around central veins.

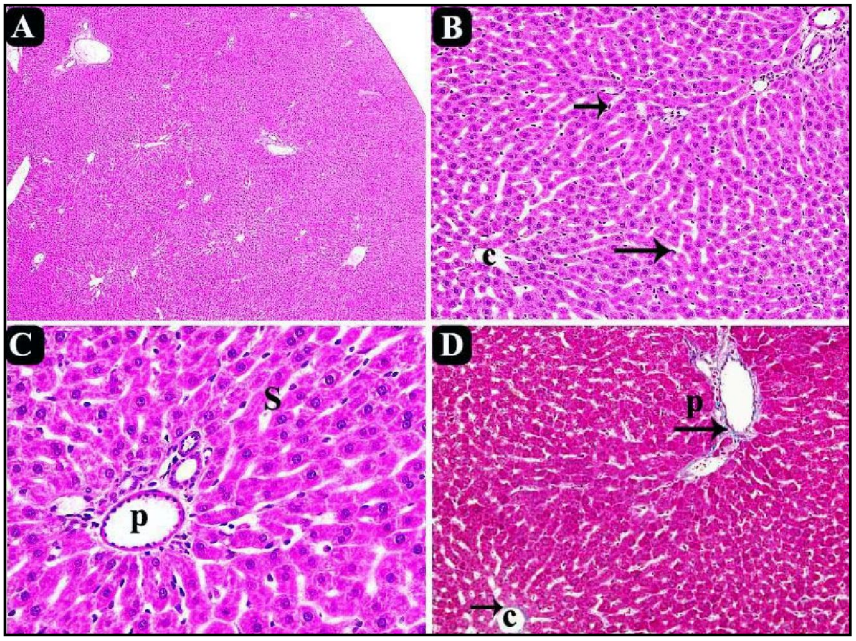

Fig. 1: **Photomicrographs of sections in the liver of a control rat (Group I) showing:**

A. Normal hepatic architecture (H&E; X40)

B. Classic hepatic lobules with branching and anastomosing cords of hepatocytes radiating from a central vein (C). Note the blood sinusoids are lined by endothelial cells and von Kupffer cells (arrows) (H&E; X100)

C. Distinct hepatocytes separated by sinusoidal spaces (S) around a portal area (P). The hepatocytes are polygonal cells with granular eosinophilic cytoplasm and nuclei with prominent nucleoli (H&E; X400)

Normal distribution of connective tissue (arrows) is confined to the portal area (P) and around the central vein (C) (Masson trichrome; X100)

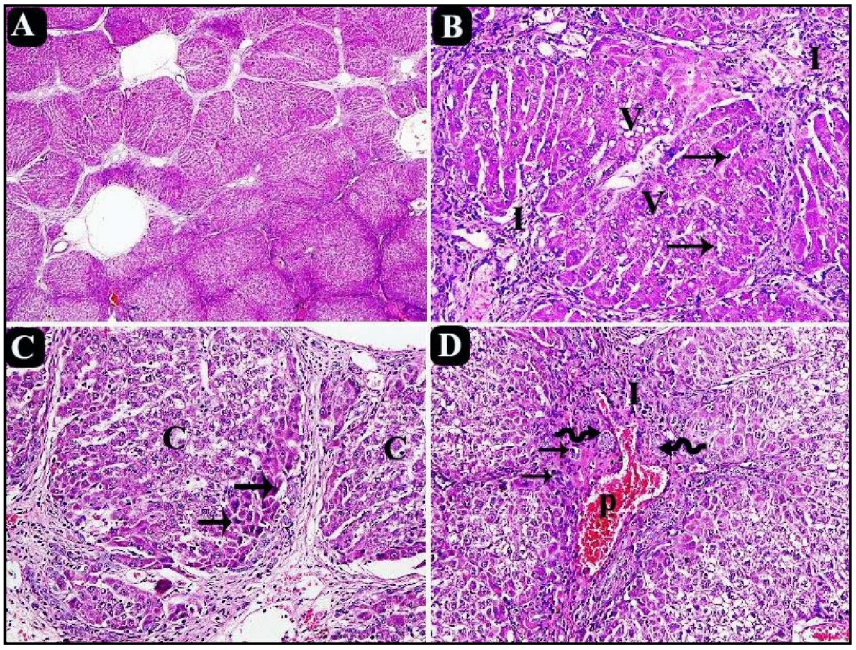

Fig. 2: Photomicrographs of sections in the liver of a rat from Group II (cirrhosis group) demonstrating:

A. Distorted hepatic architecture the formation of well- defined pseudolobules by thick fibrous septa (H&E; X40)

B. Vacuolar degeneration of hepatocytes (V). Mononuclear infiltrating cells (I) and prominent von Kupffer cells (arrows) are seen in between hepatocytes (H&E; X100)

C. Centrilobular degeneration and necrosis (C). Necrotic hepatocytes show darkly eosinophilic cytoplasm and pyknotic nuclei (arrows). (H&E; X100)

D. A dilated congested portal vein (P) and obliteration of blood sinusoids by degenerated hepatocytes. Note the presence of apoptotic cells (arrow) and Mononuclear infiltrating cells are present within the portal areas (I). Proliferation of bile ductules is prominent(curved arrows) (H&E; X100)

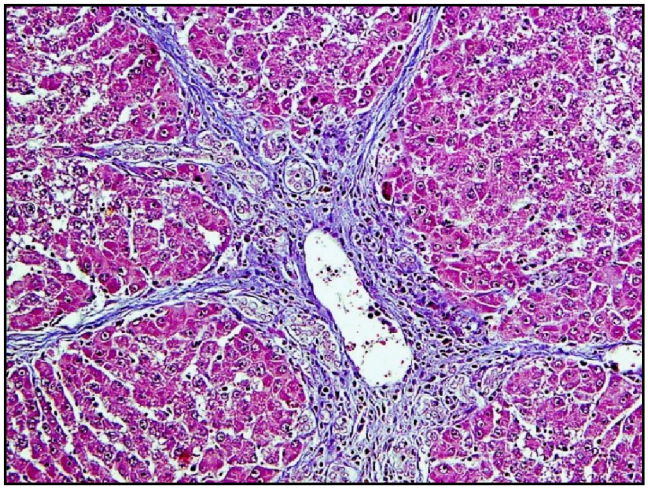

Fig. 3: A photomicrograph of a section in the liver of a rat from Group II (cirrhosis group) demonstrating the advanced expansion of a portal tract and bundles of collagen fibers surround the hepatic lobules (Masson trichrome; X 200).

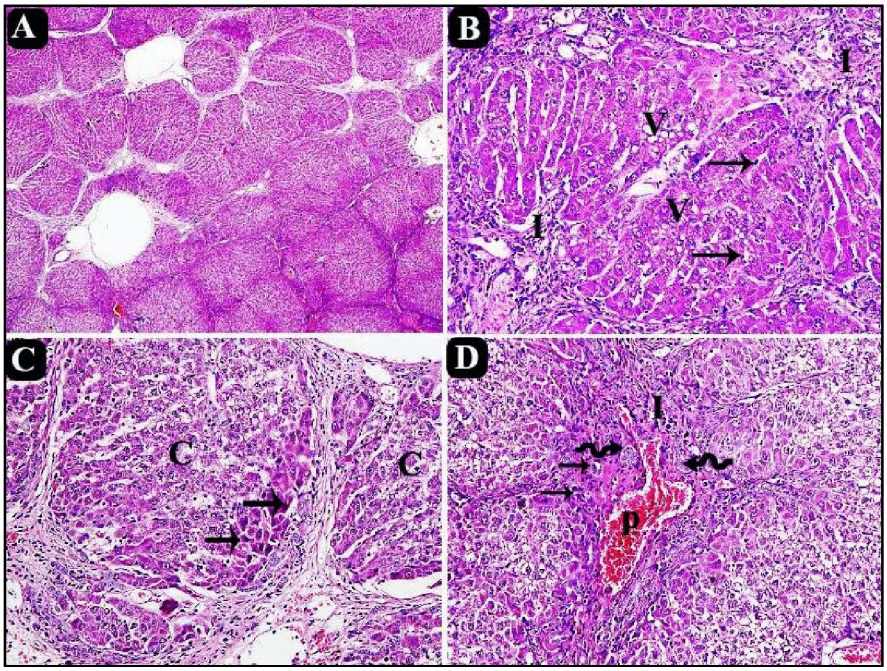

Fig. 3: Photomicrographs of sections in the liver of a rat from Group II (cirrhosis group) demonstrating:

E. Distorted hepatic architecture the formation of well- defined pseudolobules by thick fibrous septa (H&E; X40)

F. Vacuolar degeneration of hepatocytes (V). Mononuclear infiltrating cells (I) and prominent von Kupffer cells (arrows) are seen in between hepatocytes (H&E; X100)

G. Centrilobular degeneration and necrosis (C). Necrotic hepatocytes show darkly eosinophilic cytoplasm and pyknotic nuclei (arrows). (H&E; X100)

H. A dilated congested portal vein (P) and obliteration of blood sinusoids by degenerated hepatocytes. Note the presence of apoptotic cells (arrow) and Mononuclear infiltrating cells are present within the portal areas (I). Proliferation of bile ductules is prominent(curved arrows) (H&E; X100)

Group IV (Treatment Group) (Fig.5):

In group IV (treated group), curcumin treatment had no beneficial effect in reducing fibrosis in our model. The liver sections revealed cirrhosis-like structural patterns characterized by mixed-sized fibrotic nodules surrounded by fibrous tissue septae with an apparent increase in bile ductules. Moreover, hepatic parenchyma showed mononuclear cellular infiltration, hepatocyte apoptosis, cytoplasmic vacuolation and centrilobular degeneration and necrosis. Displayed thick bundles of collagen fibers surround the lobules and resulted in distorted tissue architecture (Fig 5).

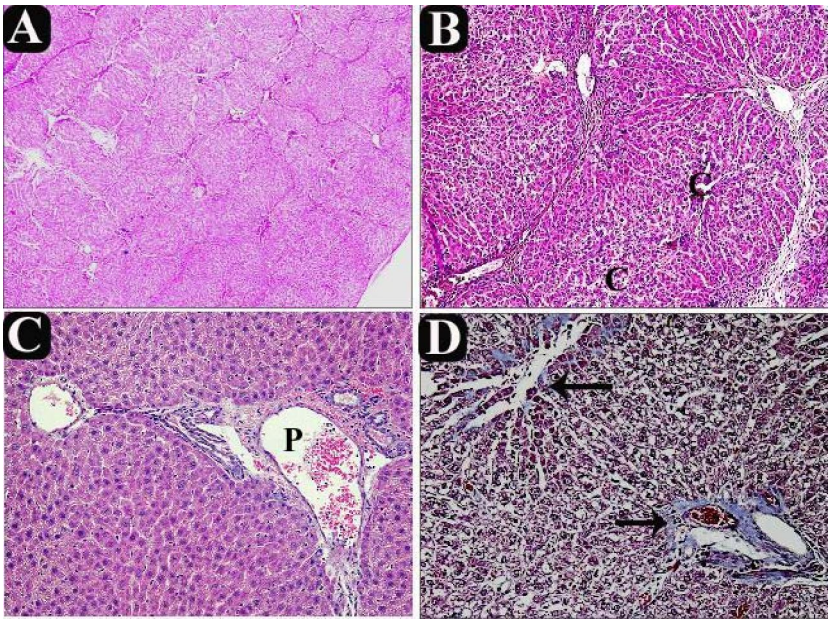

Fig. 4: Photomicrographs of sections in the liver of a rat from Group III (prevention group) demonstrating:

A. Minimal Distortortion of normal lobular architecture (H&E; X40)

B. Degenerated centrilobular areas surrounded by minimal mononuclear cellular infiltration(C) (H&E; X100)

C. Expanded portal tract with dilatation and congestion of their portal vein (P). The hepatocytes are apparently normal with vesicular nuclei and granular acidophilic cytoplasm (H&E; X200).

D. The collagen fibers in the portal tract and those surrounding the central vein are moderately increased (arrow) (Masson trichrome; X 200).

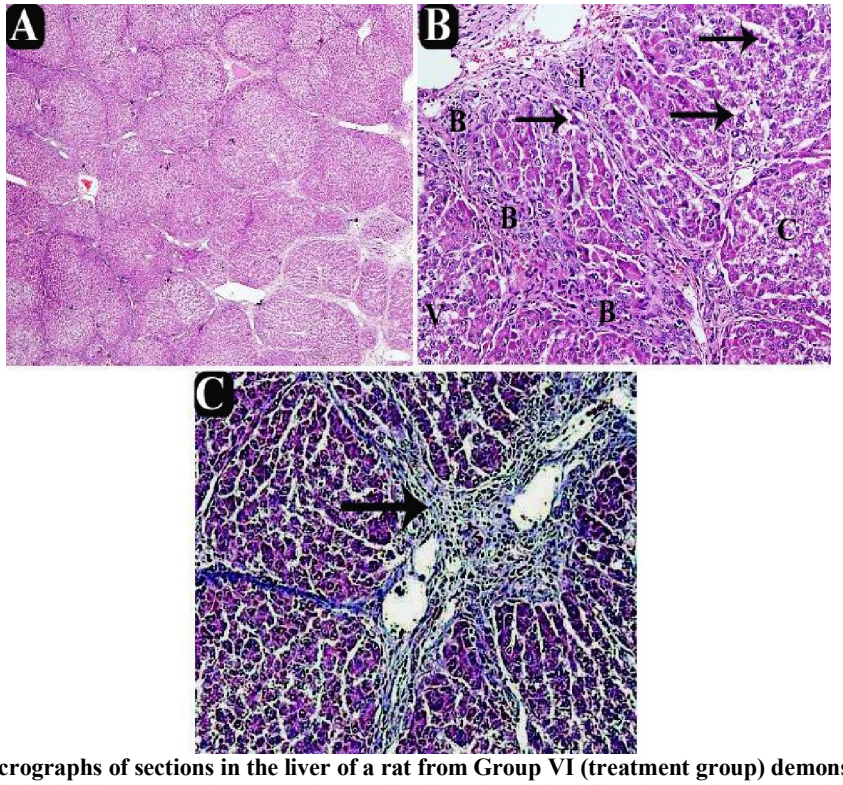

Fig. 5: Photomicrographs of sections in the liver of a rat from Group VI (treatment group) demonstrating:

A. Cirrhosis-like structural patterns characterized by mixed-sized fibrotic nodules surrounded by fibrous tissue septae (H&E; X40)

B. An apparent increase in bile ductules (B), mononuclear cellular infiltration(I), hepatocyte apoptosis (arrow), cytoplasmic vacuolation (V) and centrilobular (C) degeneration and necrosis (H&E; X200)

C. Thick bundles of collagen fibers (arrow) in the portal area and extend to surround the lobules (Masson trichrome; X 200).

Immunohistochemical and Morphometric Results

Alpha smooth muscle actin (α SMA) immunoexpression on hepatic stellate cells were expressed in the form of darkly and lightly stained brown areas in their cytoplasm and was considered a marker of their activation to myofibroblast. Image analyzer was used to analyze the results morphometrically.

Hepatic stellate cells were very rarely immunostained for α SMA in sections of group I (control rats) (Fig.6) and the mean area percent of their immunoreactions was 2.5±2.2 (table 2, Fig.7).

In group II (cirrhotic rats), α SMA immunoexpression was seen increased in the hepatic stellate cells and the darkly stained areas of the immunoreactions appeared more prominent when compared to control group (Fig.6). Moreover, the mean area percent and the mean optical density of α SMA immunoexpression indicated a highly significant increase in comparison to control rats (table 2, Fig.7).

In group III (prevention rats), an obvious decrease in α SMA immunoexpression was noticed and apparent lightly stained areas that slightly increased when compared to control rats. A remarkable difference was noticed in the immunoexpression between this group and groups II and IV (Fig.6). The mean area percent and the mean optical density of α SMA immune expression indicated a highly significant decrease in comparison with groups II and IV (table2, Fig.7).

In group VI (treated rats), α SMA immunoexpression and the existence of darkly stained immunoreactive areas in curcumin treated rats appeared nearly similar to group II. The mean area percent and the mean optical density of α SMA immunoexpression indicated a highly significant increase in this group versus control rats; however nonsignificant difference was found in comparison to rats of group II (table2, Fig.7).

Liver fibrosis was quantified by computer image analysis on specimens stained with Masson's trichrome. Statistical analysis of the image analyzer data revealed that the mean area percent (MAP) of the connective tissue (CT) in cirrhotic non treated (group II) and cirrhotic curcumin treated (group IV) rats was higher than that of the control group (group I). This was statistically significant. Meanwhile, there was no significant difference in the MAP between both (group III) and control group (table3, Fig.8).

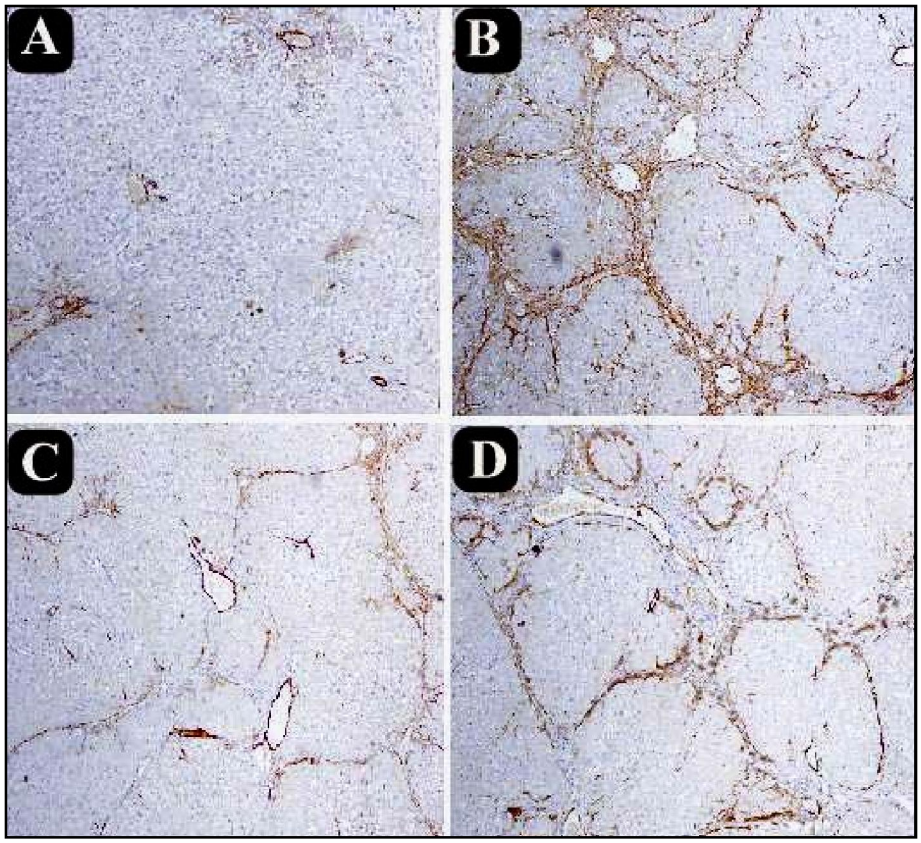

Fig.6. Alpha smooth muscle actin (α SMA) immunoexpression: (A) Group I (control rats), showing very weak immunostaining; (B) Group II, showing strong α SMA expression in cirrhotic livers; (C) low density of α SMA immunostaining in Group III (prevention group); (D) abundant α SMA immunostaining levels in Group VI (treatment group). Magnification ×200

Table (2): the mean area percent and the optical density of α smooth muscle actin (αSMA) immune-expression on hepatic stellate cells in control and experimental groups:

Groups	Mean area % of (α SMA) immune-expression	Mean optical density of (αSMA) immune-reaction
Group I (control Group)	2.5±2.2	0.156±0.026
Group II (cirrhotic Group)	35.5±2.2*	0.423±0.047
Group III (prevention Group)	8.0±2.4#	0.240±0.17
Group IV (treatment Group)	23.5±3.2*	0.392±0.34

Values are represented as mean ± SD Significance was considered P<0.05

* Significant change compared to Group I (Control group) # Significant change compared to Group II (Cirrhosis Group)

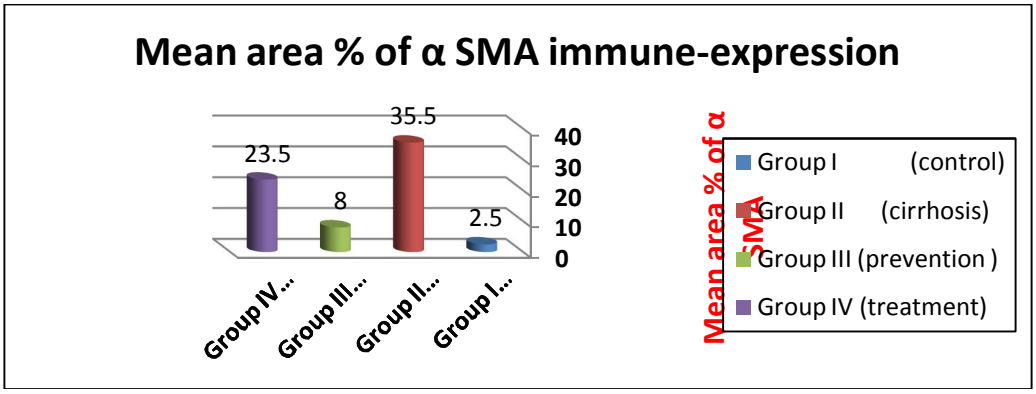

Fig. 7: the mean Area % of α SMA immune-expression in all studied groups.

Table (3): the mean Area % of connective tissue (CT) in all studied groups:

Group	Mean Area% of CT
Group I (Control)	4.56 ± 2.12
Group II (Cirrhosis)	32.32 ± 1.52*
Group III (Prevention)	7.96 ± 1.22 #
Group VI (Treatment)	29.55 ± 3.58*

Values are represented as mean ± SD Significance was considered P<0.05

* Significant change compared to Group I (Control group)

Significant change compared to Group II (Cirrhosis Group)

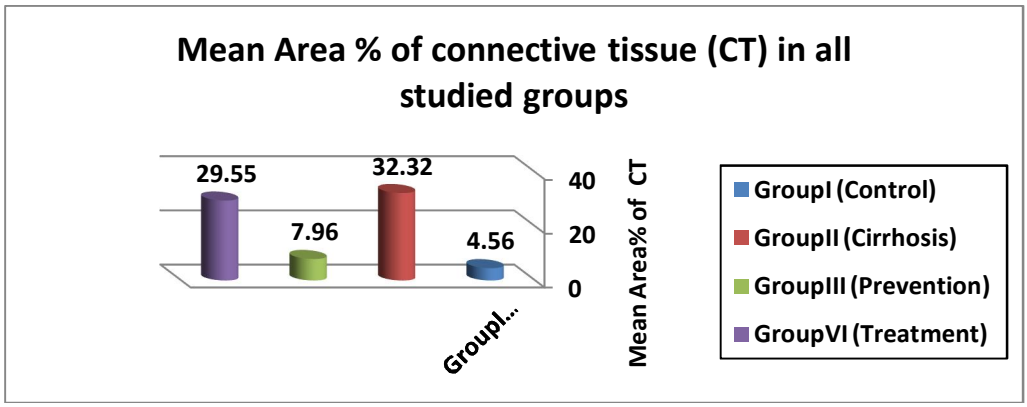

Fig. 8. the mean Area % of connective tissue (CT) in all studied groups.

4. Discussion

To date, there is no cure for cirrhosis, which is a leading worldwide cause of death [15]. 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.

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 [16].

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.

Hepatic stellate cells (HSC) have been considered the most important cell-type involved in hepatic fibrogenesis. Proliferation and differentiation of hepatic stellate cells into myofibroblast-like cells has been related to the development of liver fibrosis. The alpha-actin expressed by hepatic stellate cells was considered a marker of their activation to myofibroblast-like cell. Utilizing the chronic administration of thioacetamide (TAA) to rats as a model of liver cirrhosis [17] the aim of this study was to unravel the mechanism of curcumin's effect on hepatic fibrosis: is it a direct effect on HSC and collagen formation or an indirect effect by the prevention of inflammation and necrosis. Also this study highlighted the potential importance of biologically active compounds derived from dietary agents that could be explored further for the prevention and / or treatment of liver cirrhosis

Toxic injury occurs in the liver more often than that in any other organ. When a drug is used widely, drug-induced liver injury has become a serious health problem in contemporary society, and then research on the mechanism of drug-induced liver injury is very useful in therapy and prevention of drug-induced liver injury [18]. Thioacetamide is known hepatotoxic, which produces hepatic necrosis in high doses by producing free radicals during TAA metabolism resulting in oxidative stress mediated acute hepatitis and induces apoptosis of hepatocytes in the liver [19]. It has been reported that long-term taken of TAA induced cirrhosis in rats; on account of this, it is proven that thioacetamide through cytochrome p-450 pathway is converted into a highly toxic metabolite N-acetyl-p-benzoquinone imine (NAPBI). Meanwhile, (NAPBI) is accompanied with glutathione and excreted in the urine as conjugates. The acute hepatic necrosis induced by TAA, which activates cytochrome p450 and produces a highly reactive NAPBQI that, by the way, combines with sulpha-hydryl groups of proteins and causes a rapid reduction of intracellular glutathione. Therefore, increases the oxygen free radical causing an oxidative stress and initiates apoptosis; consequently, the elevated liver enzymes (ALT, AST) are an indicator of cellular liver necrosis [20]. In addition, TAA interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury resulting in a rise in serum liver markers [21]. TAA toxic metabolite free radicals induced oxidative stress in the hepatic cells. It is responsible for many changes occur for hepatocytes such as cell permeability changes, rise in intracellular concentration of Ca++, and effects on mitochondrial activity, which leads to cell death [22].

The beneficial effects in the present study can therefore be attributed to the amelioration of TAA metabolites' toxicity, rather than their formation. The objective of the present study was to assess whether dietary curcumin could attenuate the development of cirrhosis and portal hypertension (manifested as splenomegaly) in rats due to chronic TAA administration, and to further explore the mechanisms responsible for curcumin's anti-fibrotic effect. Rats that received bi-weekly, i.p. injections of TAA for 12 weeks developed hepatic cirrhosis, manifested by liver histopathology and increased spleen weight. Their livers also robustly exhibited the primary components of HSC activation, i.e. increased expression of α smooth muscle actin. The abnormal reconstruction of the lobular architecture, the appearance of widespread fibrosis in addition and nodular lesions of the hepatic parenchyma are the main characteristics of liver cirrhosis [23]. Continuous accumulation of extracellular matrix (ECM) results in hepatofibrosis; collagen is the main component of the ECM in fibrotic tissue [24]. Mean area percent of

collagen fibers, was used as an indicator for evaluating the extent of liver fibrosis [25]. In the present study, these indices and mediators of fibrosis and cirrhosis were significantly lower in the rats that were administered curcumin concomitantly with TAA. Curcumin (300 mg/kg/day) concomitantly with TAA significantly lowered collagen accumulation as evidenced by the percentage area of fibrotic tissue. Although curcumin treatment (group VI) after TAA discontinuation partially inhibited the fibrotic effect, there was no statistically significant difference between group VI and TAA group (group II) based on quantitative morphometric analysis results. Therefore, curcumin treatment may be more beneficial as a protective than treatment with respect to antihepatofibrotic properties. It is generally recognized that HSC activation plays a critical role in the process of hepatic fibrogenesis, and αSMA is a marker of activated HSCs [26]. In the present study, immunohistochemical observations of αSMA indicated that Curcumin concomitantly with TAA noticeably suppressed the activation of HSCs. Evidence has been published that indicates oxidative stress influences a series of liver diseases including hepatic fibrogenesis [27]. In fact, the histopathological changes were virtually prevented by curcumin. This was in accordance with the work of Bruck et al.,[4].

Our histological findings prove that the curcumin protected the liver structure in TAA-induced liver cirrhosis rats. Indeed, there was remarkable reduction in fibrosis extent and a decrease of stellate infiltration in rats concomitantly treated with curcumin compared to non-treated group. Histological studies confirmed the hepatoprotective effect of curcumin. TAA treated rat liver sections showed degeneration of hepatocytes, necrosis of cells and mononuclear cellular infiltration. The curcumin treatment almost normalized these effects in the histoarchitecture of liver. Therefore, from this study the curcumin could be a hepatoprotective against thioacetamide induced liver damage in rats. The antioxidant capabilities of the phenolic compounds are important for the human body to destroy the free radicals that exist in our body. Many of the polyphenols such as flavonoids have been identified as powerful antioxidants; moreover, play a significant role in the treatment of many diseases, including liver cirrhosis [28].

To further explore potential anti-fibrotic actions of curcumin, we examined whether curcumin would have a beneficial effect on established liver cirrhosis. Such an effect may support a mechanism that includes direct antifibrotic or fibrinolytic activity. However, curcumin administration for 6 weeks to rats that already received TAA for 12 weeks and were confirmed to be cirrhotic did not improve liver histology, or the spleen weights, indicating that curcumin had no effect on pre-established liver cirrhosis. These data are consistent with the results in HSC that showed no effect of curcumin on area percent of their immunoreactions to α SMA. The failure of curcumin to inhibit HSC proliferation and profibrogenic activity in vitro, observed in the work of Bruck et al., [4] which was not in accordance with another study Kang et al., [10] study.

In conclusion, this study showed that curcumin has protective effects from hepatic cirrhosis in rats that were proven by histopathological analysis. As curcumin is safe for consumption by humans, it may have a beneficial role in chemical-induced hepatic damage although this finding needs further study to know the active constituents appearing to protect rat liver against cirrhosis.

Conclusion and recommendations

In conclusion, this study showed that curcumin has protective effects from hepatic cirrhosis in rats that were proven by histopathological analysis. As curcumin is safe for consumption by humans, it may have a beneficial role in chemical-induced hepatic damage although this finding needs further study to know the active constituents appearing to protect rat liver against cirrhosis.

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