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Study the effect of curcumin on hepatic DNA damage in an experimental model of hepatic fibrosis

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Keywords

- Curcumin
- DNA damage
- Hepatic fibrosis
- CCL4

Abstract

Background/aim: Curcumin, a member of the ginger family of species, has long been used to treat cases of hepatic dysfunction. Herein, we investigated the hepatoprotective and the anti-fibrotic activity of curcumin against carbon tetra chloride (CCL4)-induced hepatic damage. **Material & methods:** A total of 40 Male Wister Albino rats (200-225 g) were divided into 4 groups 10 per each. Group 1 (G1, normal control) was injected with the vehicle, olive oil, (0.1 ml/Kg body weight, i.p.) twice weekly. Group 2 was intragastrically administered curcumin (200 mg/Kg). Group 3 (CCL4) received CCL4 (0.1 ml/Kg, i.p.) twice weekly. Group 4 (CCL4 + curcumin) received both curcumin and CCL4. All treatments were given for 6 weeks. **Results:** Administration of CCL4 resulted in a significant elevation in the serum levels of ALT, AST, and the hepatic TBARS, ROS and hydroxyl proline and a significant decrease in the activities of hepatic GSH, CAT and SOD along with increased DNA damage and distorted histological structure of liver with obvious fibrosis. Administration of curcumin alleviated all these distorted parameters **In conclusion**, curcumin administration ameliorated CCL4- induced liver damage and reduced the hepatic fibrosis.

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Introduction

The liver is a multifunctional biological device with a leading role in the maintenance of body homeostasis. Therefore, when the liver is damaged due to diseases, our life may be threatened [1]. Several studies have shown the essential participatory role of inflammatory and inflammatory signaling pathways [2,3] and oxidative damage [4,5] in the pathology of liver diseases and have proved the ameliorative role of dietary antioxidants [6,7]. One popular dietary antioxidant is the curcumin, [8] which ameliorated liver injury induced by CCL4 [11]. Besides antioxidant activity, curcumin has anti-inflammatory [2] and immunoregulatory [9] which, indeed, will participate in restoring hepatic damage. It was demonstrated that curcumin ameliorates liver damage by normalizing the pro-apoptotic (Bax, caspase-3) and anti-apoptotic pathways [10]. The curcumin chemo-preventive potential is further shown by its capacity to inhibit COX-2 and i-NOS expression through NF-kB pathways, which are considered to be another improved mechanism for ameliorating liver damage [10]. Moreover, curcumin can restrict hepatic fibrosis in vitro through reduction of proliferation and apoptosis [11, 12].

The purpose of this work is to study the ameliorative effect of curcumin on hepatic fibrogenesis induced experimentally by CCL4.

Subjects and methods:

Experimental animals

A total of 40 male Wister albino rats (200-225 g and 8-10 weeks) were purchased from the Faculty of Science Tanta University. The experimental study was approved by The Ethical Animal Research Committee of Tanta University. The animals were housed at a temperature 22-24 ◦C, exposed to alternate cycles of 12 h dark/light throughout the study and fed chow ad libitum with free access to water. Animals were kept for 2 weeks for acclimatization.

Chemicals

Curcumin powder and CCL4 were obtained from Sigma-Aldrich, Egypt.

Experimental design

Animals were randomly divided into 4 groups 10 per each. Group 1 (G1, normal control) was injected with the vehicle, olive oil, (0.1 ml/Kg body weight, i.p.) twice weekly. Group 2 was intragastrically administered curcumin (200 mg/ 0.2ml/ Kg). Group 3 (CCL4) received CCL4 (0.1 ml/Kg, i.p.) twice weekly. Group 4 (CCL4 + curcumin) received both curcumin and CCL4. All treatments were given for 6 weeks [13].

Blood sample collection and biochemical assay

Blood was collected by a cardiac puncture, serum was separated by centrifugation at 4000 rpm (4 ◦C) for 15 min and was kept at – 70oC in aliquots until biochemical analysis were performed. Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) were estimated with a spectrophotometric technique by the Olympus AU 2700 auto analyzer using commercial kits according to the manufacturer's instructions and presented as IU/L [14]. Serum TNF-α was estimated [15].

Liver tissue preparation and antioxidant/oxidative stress

All animals were anesthetized with 45 mg/kg of sodium pentobarbital. Rats were sacrificed and the liver from each rat was excised immediately after perfusion and rinsed with an ice-cold saline solution. Each liver tissue was homogenized with 20 times volume of liver weight (100 mg tissue in 2.0 mL buffer) in ice cold 0.05 M potassium phosphate buffer (pH 7.4) and treated separately for different measurements. Tissue homogenate was used for the measurement of hydroxyproline in the liver tissues as previously described by Jamall et al. [16]. The activity of SOD was analyzed by assay kits according to the manufacturer's protocol [17]. Hepatic GSH was determined using the method described by Eyer and Podhradsky [18]. ROS formation was evaluated using a modified method of Li et al. [19]. Thio Barbituric Acid Reactive Substance (TBARS), a marker of lipid peroxidation, was estimated using the method of Shapiro et al. [20].

Histopathology and DNA fragmentation

The ventral median lobe of the liver was fixed in 10% neutral buffered formalin. Sections were cut and stained with hematoxylin and eosin and observed under a light microscope for histopathological study. DNA Fragmentation was performed using agarose gel electrophoresis in the genetic coding center at Tanta Universal Teaching Hospital according to the method described by Kasibhatla et al. [21].

Statistical analysis

All values were expressed as mean ± SD. SPSS version 16.0 was used for statistical analysis. Data were statistically analyzed using one-way ANOVA for multiple group comparison. Significance was set at $p \leq 0.05$.

Results:

The serum levels of ALT and AST were significantly higher in the CCL4 group compared with both the control and curcumin groups. Curcumin administration significantly reduced ALT and AST in the CCL4 treated group compared with CCL4 group. No significant changes were observed when comparing the curcumin group with the control group (Table 1).

Similarly, hepatic TBARS and ROS formation was significantly higher in the CCL4 group as compared with both the control and curcumin groups. Curcumin administration significantly reduced TBARS and ROS formation in the CCL4 treated group compared with CCL4 group. No significant changes were observed when comparing the curcumin group with the control group (Table 1).

Hepatic GSH, CAT and SOD were decreased significantly in the CCL4 group. However, they were significantly increased by curcumin administration. Regarding GSH, no significant change was observed when comparing control, curcumin and CCL4- treated groups (Table 1). CAT and SOD were significantly lower in CCL4-treated group compared with both the control and curcumin groups. Regarding hydroxyproline content and serum TNF-α, there was a significant increase in CCL4 and CCL4 treated group compared with the control group. Curcumin administration significantly reduced hydroxyproline content and TNF-α in the CCL4

treated group compared with CCL4 group. No significant changes were observed when comparing the curcumin with the control group (Tab.1).

DNA extracted from hepatic tissues of the CCL4 group showed apoptotic DNA fragmentation. DNA extracted from hepatic tissues of CCL4 + the curcumin group showed reduction of apoptotic DNA fragmentation (Fig. 5 &6).

Our results had been supported by the histopathological findings of the CCL4 group which showed hepatocellular necrosis, thickening of blood vessels with cellular infiltrations, fibrosis and severe damage in the hepatic architecture. Curcumin administration improved dramatically these disturbed histopathological changes.

Tab. 1: Liver enzymes, lipid peroxidation, antioxidant activity, ROS production and hydroxyl proline levels in the studied groups.

Parameter	Control	Curcumin	CCL4	CCL4+curcumin	F Value
AST (IU/L)	42.34±1.69	42.31±2.11	266.1±27.18 *#	104.6±16.89 *#¥	432.84
ALT (IU/L)	60.7±4.20	61.1±3.80	301.7±23.39 *#	157.6±14.43 *#¥	657.50
CAT Units /mg protein	14.8±0.65	16.5±0.84	7.06±0.68 *#	12.43±3.05 *#¥	62.190
SOD Units /mg protein	101.9±16.80	105±16.92	42.1±7.1 *#	72.1±12.6 *#¥	44.608
Serum (TNF-α) (pg ml $^{-1}$)	10.50 ±1.86	9.10±2.03	54.56±10.83 *#	19.32± 4.6 *#¥	301.22
Hydroxy proline µg/100 mg tissue	19.96±1.84	10.88±1.83	65.81±8,14 *#	23.1±3.85 *#¥	308.80
GSH (µ mol/g tissue)	5.14±0.60	5.34±0.52	0.96±0.47 *#	4.71±0.45 #¥	159.06
TBARS n mol/g tissue	19.55±1.50	19.4±1.44	34.06±5.61 *#	23.9±2.37 *#¥	45.548
ROS formation (mean fluorescence intensity)	196.8±13.24	197.4±14.90	568.7±62 *#	347.1±28.36 *#¥	244.58

***** Significant compared with control group
Significant compared with curcumin treated group
¥ Significant compared with CCL4 toxic group

Histological Examination: Control group revealed normal hepatocellular architecture (Fig. 1A&1B). Curcumin treated group with no deviation from the normal control group (Fig. 2A&2B). CCL4 group showed hepatocellular necrosis, thickening of blood vessels with cellular infiltrations, fibrosis and severe damage in the hepatic architecture. Dilatation of central vein, blood sinusoid and portal venule are seen ballooning. Degeneration of hepatocytes is seen with vacuolated cytoplasm and indistinct cell boundaries. (Fig. 3A&3B). CCL4 + curcumin group showed extensive proliferation of bile ductules with marked regeneration of hepatocytes and minimal fibrosis (Fig. 4A&4B).

Control group

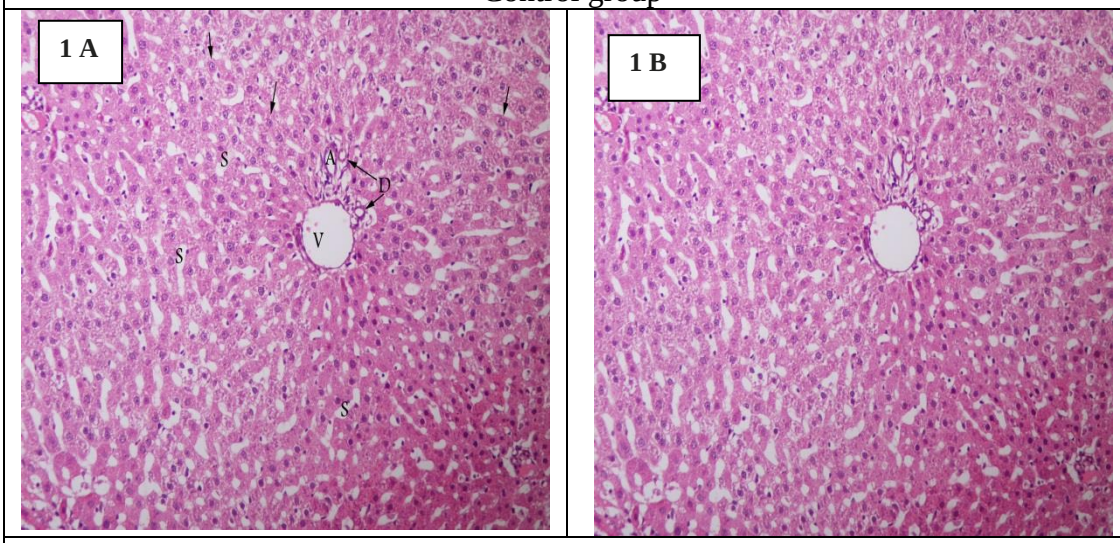

Fig. 1 Control group with normal hepatocellular architecture.(CV ;Central Vein , S; Sinusoids)

Curcumin group

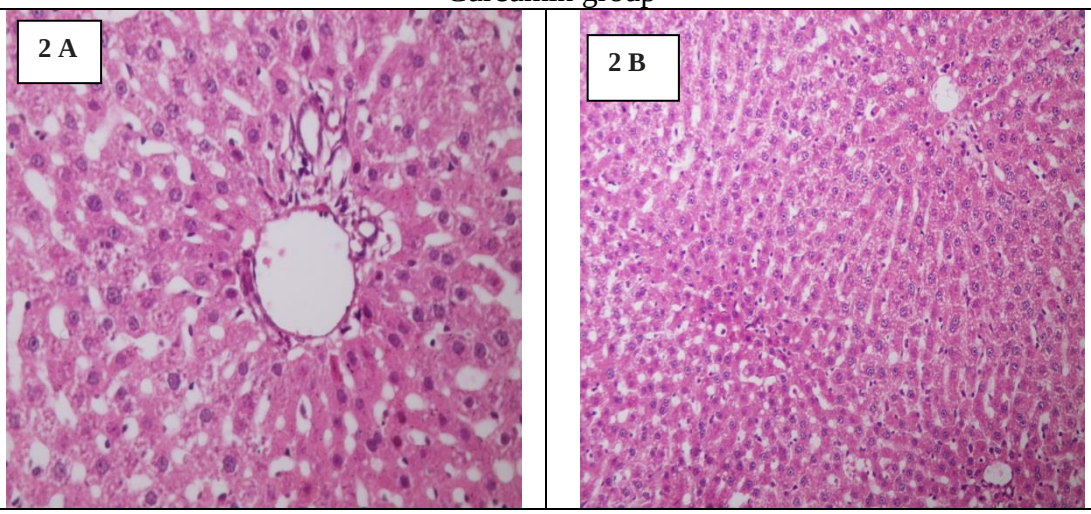

Fig. 2 Curcumin treated group with normal hepatocellular architecture. (CV ;Central Vein , S; Sinusoids) (H&E, Mic. Mag. A 400 X and B 200X)

CCL4 group

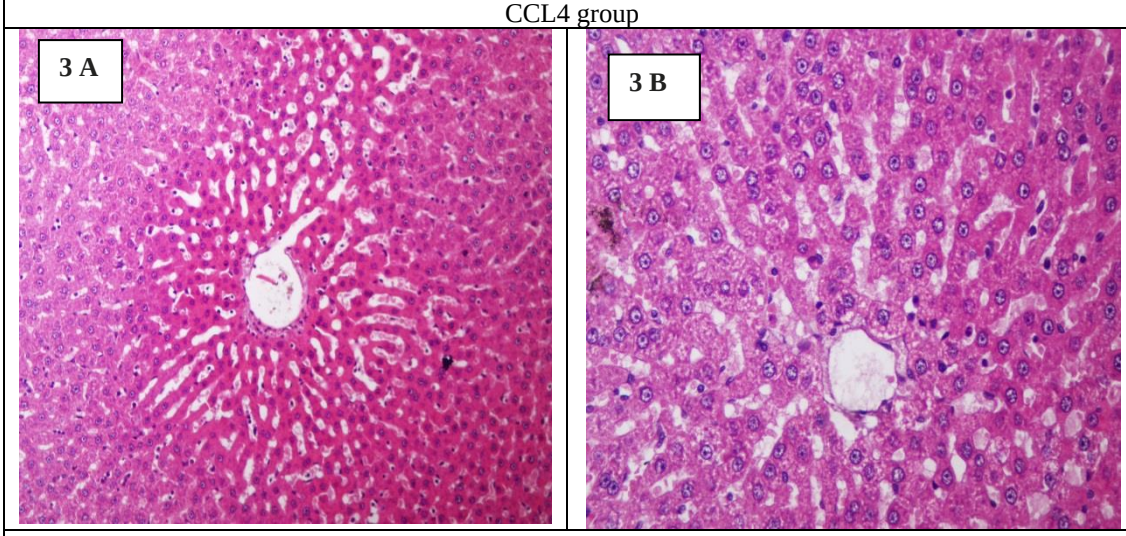

Fig. 3 CCL4 group showed hepatocellular necrosis, thickening of blood vessels with cellular infiltrations, fibrosis and severe damage in the hepatic architecture. Dilatation of central vein, blood sinusoid and portal venule are seen ballooning. Degeneration of hepatocytes is seen with vacuolated cytoplasm and indistinct cell boundaries (H&E, Mic. Mag. A 200 X and B 400X).

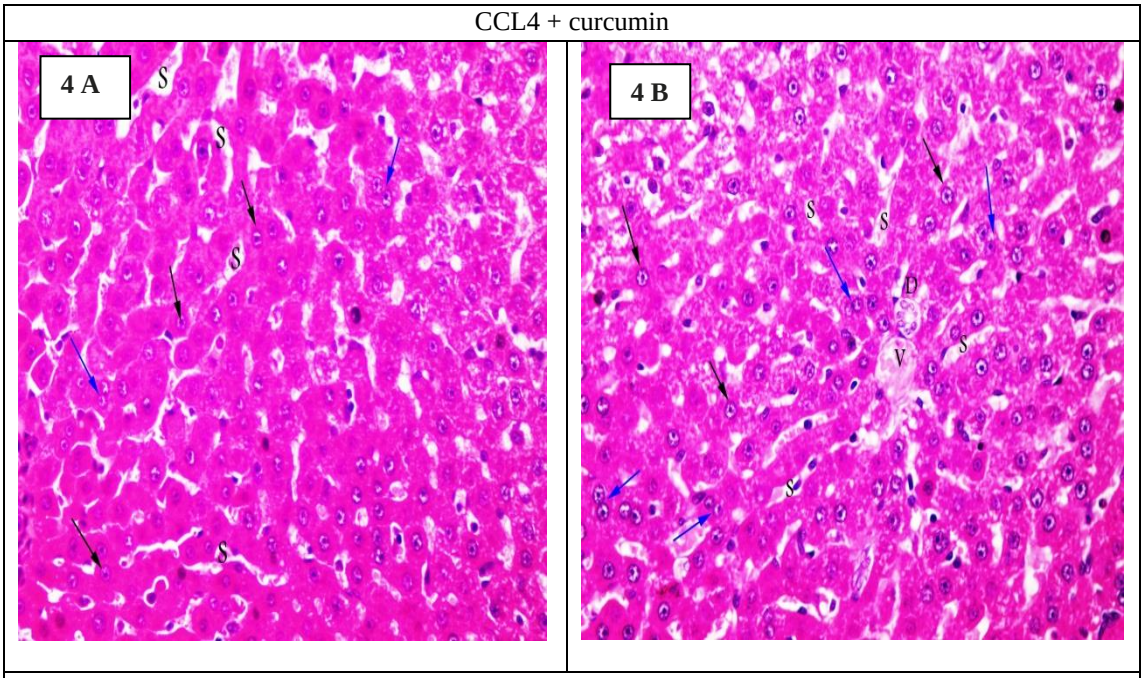

Fig. 4 CCL4 + curcumin group showed extensive proliferation of bile ductules with marked regeneration of hepatocytes and minimal fibrosis (H&E, Mic. Mag. 200 X).

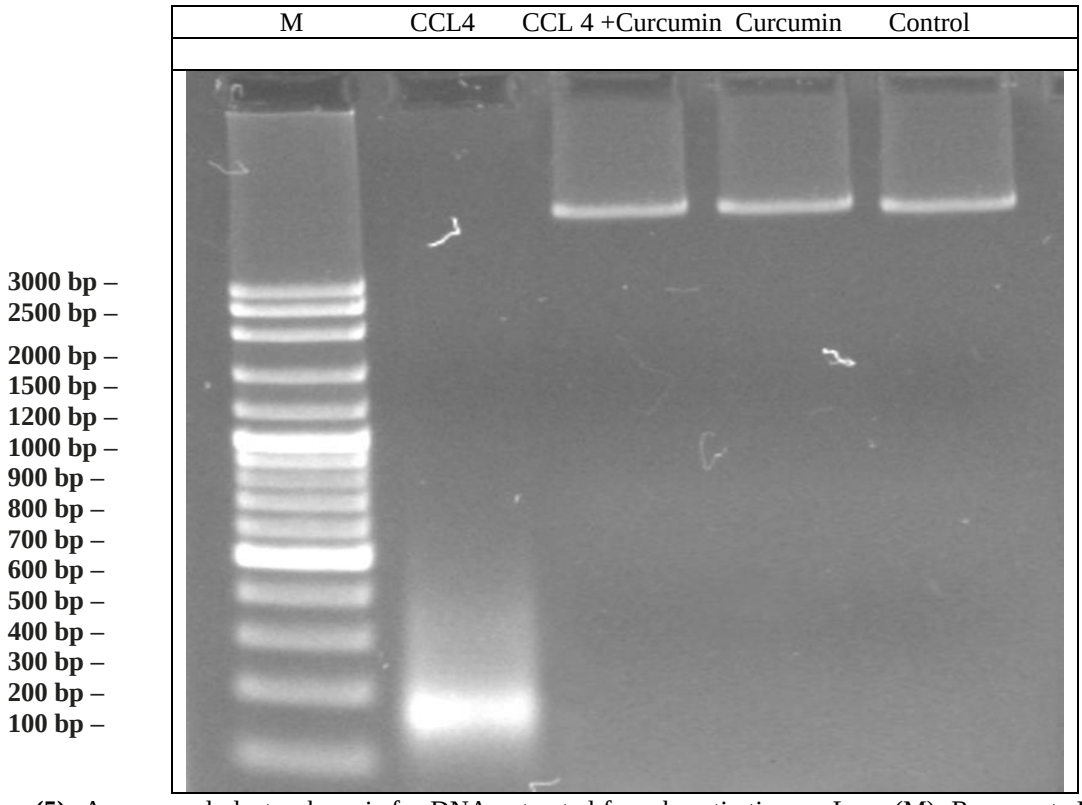

Fig. (5): Agarose gel electrophoresis for DNA extracted from hepatic tissue . Lane (M): Represents DNA ladder (100-3000 bp).Lane (CCL4): Represents DNA extracted from hepatic tissues of the CCL4 group and shows apoptotic DNA fragmentation approximately at 200 bp. Lane (CCL4 + Curcumin): Represents DNA extracted from hepatic tissues of CCL4 + the curcumin group and shows reduction of apoptotic DNA fragmentation. Lane (Curcumin): Represents DNA extracted from hepatic tissues of the curcumin treated group and shows no apoptotic DNA fragmentation. Lane (Control): Represents DNA extracted from hepatic tissues of the control group and shows no apoptotic DNA fragmentation.

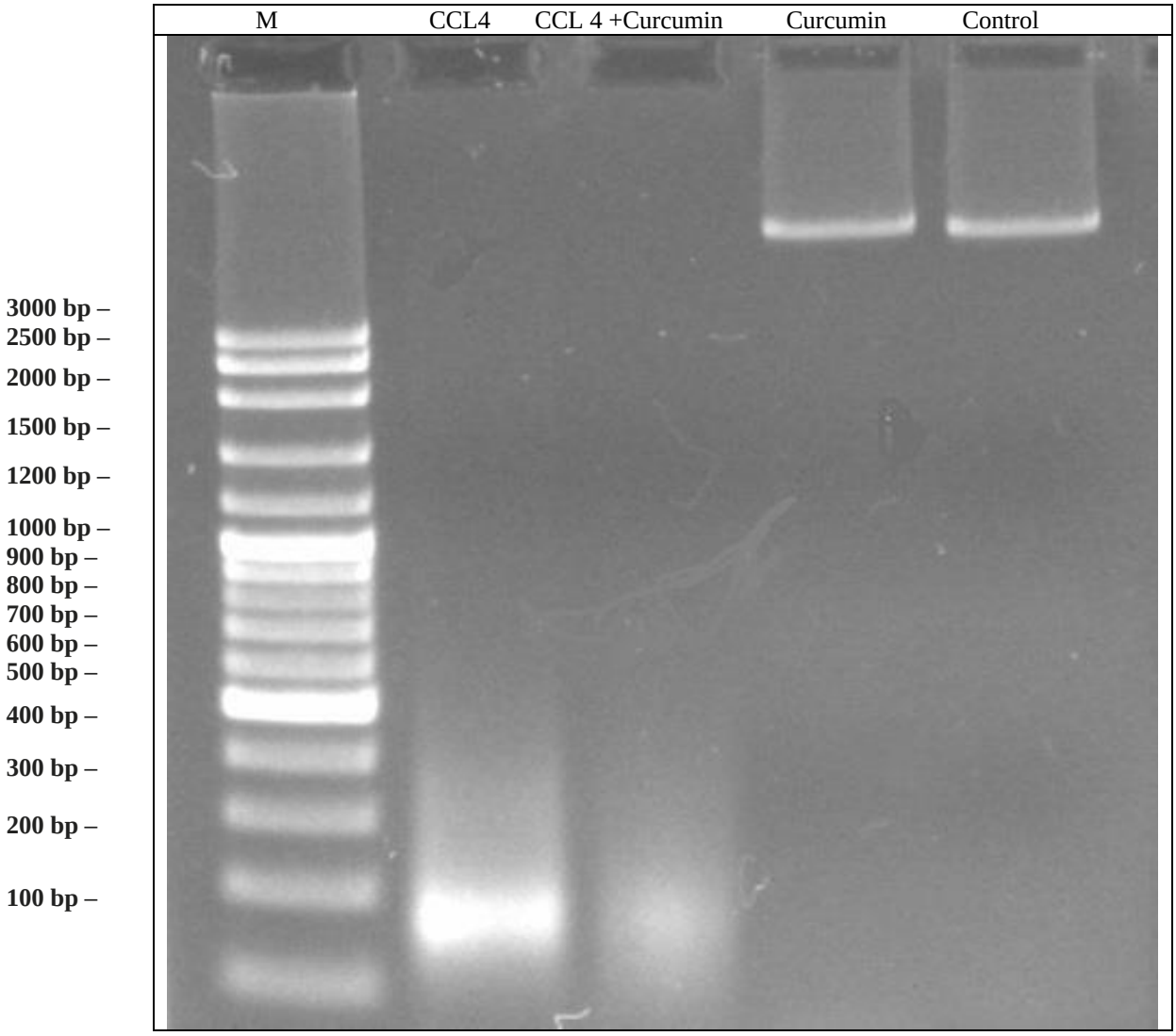

Fig. (6): Agarose gel electrophoresis for DNA extracted from hepatic tissue . Lane (M): Represents DNA ladder (100-3000 bp). Lane (CCL4): Represents DNA extracted from hepatic tissues of CCL4 group and shows apoptotic DNA fragmentation approximately at 200 bp. Lane (CCL4 + Curcumin): Represents DNA extracted from hepatic tissues of CCL4 + the curcumin group and shows reduction of apoptotic DNA fragmentation. Lane (Curcumin): Represents DNA extracted from hepatic tissues of the curcumin treated group and shows no apoptotic DNA fragmentation. Lane (Control): Represents DNA extracted from hepatic tissues of the control group and shows no apoptotic DNA fragmentation.

DISCUSSION:

In the present study, CCL4 produced a significant elevation of ALT, AST levels compared with the control and curcumin groups. Increased serum ALT and AST levels have been attributed to the damage of hepatic tissue, as these enzymes are localized in the cytoplasm and only transfer to blood following cellular damage. Curcumin administration has reduced the high liver enzymes. The results obtained from histopathological examination are compatible with these data. Similar results were obtained by [22] who attribute this effect to the powerful antioxidant activity of curcumin. Curcumin, as a potent cytochrome P450 inhibitor, can normalize antioxidant enzymes and non-enzymatic antioxidants such as GSH [23]. Administration of CCL4 led to oxidative damage of hepatic tissues as revealed by increased levels of lipid peroxidation marker MDA along with a

significant decrease in GSH content and the activity of antioxidant enzymes (SOD and CAT). Administration of curcumin ameliorated this hepatic damage [24]. In consistent with our findings, previous studies reported a potent antioxidant effect for curcumin and owed this effect to the ability of curcumin to induce antioxidant enzymes (SOD, CAT) and GSH formation [7, 25, 26]. The actual mechanism by which the curcumin works as an antioxidant is still unclear. However, some studies attributed this effect to curcumin content of phenolic hydroxyl groups [27], β-diketone [28], and ortho-alkoxy group [29] in addition to its ability to interact with the thiol group of glutathione and thioredoxin1 [30]. Fu and his group had studied the effect of curcumin on the hepatic injury caused by CCL4 and had postulated that curcumin was of great value in minimizing the hepatic biochemical and histological parameters [13]. This study revealed a significant elevation of serum TNF-α in the CCL4 group compared with the control and curcumin groups. The anti-inflammatory effect of curcumin is partially mediated by the inhibition of IkB kinase activity leading to the suppression of NF-kB activation. They also found that the anti-inflammatory effect could be improved if other alkyl or alkoxyl groups are present at positions 3 and 5 on the phenyl ring [31]. Administration of CCL4 for 6 w was enough to induce hepatic fibrosis as evidenced by the high level of hydroxy proline (HP) and histopathological findings. Treatment by curcumin reduced hepatic fibrosis and this is consistent with anti-fibrotic activity of curcumin [32]. The latter was attributed to its antioxidant effect and its inhibitory effects on NF-kB and TGF-β [33]. As a profibrogenic cytokine, TGF-β plays a crucial role in the development of liver fibrosis [34] mainly through stimulation of extracellular matrix production, and activation of trans-differentiation of hepatic stellate cells to myofibroblasts [35]. Curcumin improves fibrosis induced by bile duct ligation through down-regulation of TGF-β and inhibition of oxidative stress. In other organs, curcumin inhibits the profibrotic role of TGF-β and limits the progress of fibrosis in lung and kidney [36 -37]. Histologically, curcumin mitigated steatosis and inflammation in the hepatic tissues mainly due to its antioxidant and anti-inflammatory effect. Similarly, curcumin effectively mitigates nonalcoholic fatty liver diseases via its antioxidant and anti-inflammatory actions [33]. In conclusion, curcumin administration ameliorated CCL4-induced liver damage in rats. Curcumin reduced liver fibrosis-induced experimentally. Further clinical studies are needed to provide data on the clinical use of curcumin as an anti-fibrotic agent.

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