

Hepatoprotective role of curcumin in rat liver cirrhosis

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Abstract: The present research work was designed to evaluate the effects of curcumin supplementation on various biochemical parameters in rats with thioacetamide (TAA) induced liver cirrhosis. For this purpose 24 male Albino Wistar rats were randomly distributed into four groups (n=6). Group I served as control, Group II and Group III received thioacetamide 200mg/kg b.w, i.p, twice a week for 12 weeks in first phase. In second phase Group II received saline and Group III received curcumin 50mg/kg b.w/day, i.p for 12 weeks, in second phase, Group IV received curcumin 50mg/kg b.w/day, i.p, for 12 weeks, in first phase and saline in second phase. Evaluation of histopathological and biochemical parameters was carried out by liver histopathology and estimation of total and direct bilirubin, liver specific enzymes, antioxidant enzymes, MDA level, plasma and intraerythrocyte sodium and potassium respectively. Histopathology of liver showed highest degree of fibrosis and nodule formation, significant alteration in biochemical parameters indicated development of severe liver cirrhosis. Curcumin treatment showed reduced amount of fibrosis and significant reduction in level of liver biomarkers, reversal of antioxidant enzymes (SOD and GSH), MDA level, catalase activity and regain of electrolyte homeostasis. These findings confirm the protective role of curcumin in liver cirrhosis..

Keywords: Cirrhosis, curcumin, TAA, liver enzymes, antioxidant enzymes, electrolytes.

INTRODUCTION

Curcumin, obtained from a plant of ginger family, rhizome of turmeric (*Curcuma longa*), is a yellow coloring principal ingredients of curry powder. Curcumin (diferuloylmethane) is the major active ingredient of turmeric and has been used for years in indo-chinese medicines for the treatment of wound healing, skin problems, digestive disorders and liver diseases (Grant and Schneider., 2000). Curcumin possesses anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic and nuclear factor- κ B inhibiting properties (Araujo and Leon. 2001) (Surh *et al.*, 2001). Curcumin activates HSC and in this way inhibits the synthesis of collagen type I (Kang *et al.*, 2002) and used in steatohepatitis rodent models for attenuating inflammation and hepatic fibrosis (Leclercq *et al.*, 2004). Okada reported prevention of lipidperoxidation by curcumin and Rukkumani reported amelioration of oxidative stress (Okada *et al.*, 2001) (Rukkumani *et al.*, 2004). Iqbal *et al.*, 2003 reported the property of curcumin to enhance the expression of enzymes involved in xenobiotic detoxification reactions in the kidney and liver of mice such as glutathione reductase, glutathione S-transferase and NADPH (Iqbal *et al.*, 2003). Curcumin is also known to upregulate heme-oxygenase I, an enzyme involves in stress response, in endothelia cells, astrocytes and in renal epithelia cells (Hill-Kapturczak *et al.*, 2002). Activities of many kinases are down regulated by curcumin. Curcumin inhibits many transcription factors such as activator and signal transducers of transcription proteins, β -Catenin, activated receptor- γ (Shishodia *et al.*,

2007). Administration of curcumin increases the activities of antioxidant enzymes and thus decreases the lipidperoxidation in rats with iron induced hepatic toxicity. It also inhibits formation of hydroxyl radicals by inhibiting the oxidation of iron (Fe²⁺) by H₂O₂. They reported a marked reduction in iron induced lipid peroxidation in wistar rats by the administration of 300mg/Kg of curcumin for 10 days (Reedy and Lokesh., 1994). Rajakrishnan *et al.*, 1999 reported the reversal of biochemical and histopathological changes in the kidney, liver and brain in ethanol intoxicated rats (Rajakrishnan *et al.*, 1999). Curcumin exerts protective effects against liver damage induced by aflatoxins, erythromycin estolate, CCL₄, ethanol, iron overdose and thioacetamide (Yadira and Pablo. 2004). Curcumin is known to have beneficial systemic and hepatic effects as it has safe ingestion and sufficient bioavailability in humans (Sharma *et al.*, 2004). Thus, the purpose of our research is to evaluate hepatoprotective aspect of curcumin in rats with thioacetamide induced liver cirrhosis.

MATERIALS AND METHODS

For the study 24 Albino Wistar male rats (200-250gm) were purchased from International center for chemical and biological sciences, Karachi, Pakistan. Before the start of experiment rats were acclimatized to laboratory conditions and caged in a quite temperature controlled animal room.

Ethical guidelines

The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for laboratory use and

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care in animal research (Health research extension Act of 1985).

Study design

Experimental rats were divided into four groups (n=6). The study duration was consisted of 24 weeks, divided into two phases. Thioacetamide and curcumin were administered in either phase. Thioacetamide was purchased from Merck, curcumin and other chemicals used in present study were purchased from Fluka AG, Fisher Scientific UK limited and BDH laboratory supplies.

Group I: The control (remained untreated).

Group II: TAA-treated

Group III: TAA+ Curcumin treated

Group IV: Curcumin treated

In phase I, TAA-treated rats and TAA+curcumin treated rats were given thioacetamide, dissolved in 0.9% NaCl, injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. In phase II, TAA-treated rats were given saline while TAA+curcumin treated rats were given curcumin (orally at a dosage of 50mg/kg b.w/day starting from thirteenth week for twelfth week) after receiving TAA in first phase. Curcumin-treated rats were given curcumin (orally at a dosage of 50mg/kg b.w/day starting from thirteenth week for twelfth week) in phase I and saline in phase II. At the end of experimental period, rats from all the four groups were sacrificed. The blood from neck wound was collected in the lithium heparin coated tubes and centrifuged to collect plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at -70°C until analysis.

Estimation of ALT and total and direct bilirubin

Plasma alanineaminotransferase (Retiman and Franhel, 1957) and total and direct bilirubin (Sherlock, 1951) were estimated by using commercially prepared reagent kits from randox.

Preparation of post mitochondrial supernatant

1 gram of liver tissue was taken in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10, 500g for 20 minutes at 4°C to get post mitochondrial supernatant which was used to assay SOD, Catalase, MDA, and glutathione reductase activity.

Estimation of thiobarbituric acid substances:

The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the lipid

peroxidation method (Okhawa *et al.*, 1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of catalase

Catalase activity was assayed by the method of Sinha (Sinha *et al.*, 1979). Briefly, the assay mixture was consisted of 1.96 ml phosphate buffer (0.01M, pH 7.0), 1.0 ml hydrogen peroxide (0.2 M) and 0.04 ml PMS (10% w/v) in a final volume of 3.0 ml. 2 ml dichromate acetic acid reagent was added in 1 ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570 nm.

Estimation of SOD

Superoxide dismutase levels in the cell free supernatant were measured by the method of (Kono *et al.*, 1978). Briefly, 3ml of solution A (0.1 M EDTA containing 50 mM Na₂CO₃, pH 10.0), 0.5 ml of solution B (90µM NBT nitro blue tetrazolium dye) and 0.1 ml of solution C (0.6% Triton X-100 in solution A), 0.1 ml of solution D (20 mM Hydroxyl amine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) require in one minute.

Estimations of glutathione reductase

Activity of glutathione reductase was estimated by continuous spectrophotometric rate determination method (Calberg and Mannervik, 1985). In a clean glass test tube, 0.3 mL of 10% BSA, 1.5mL of 50mM potassium phosphate buffer (pH 7.6), 0.35mL of 0.8mM β-NADPH and 0.1mL of 30mM oxidized glutathione was taken and finally added 0.1mL of homogenate, mixed well by inversion. Absorbance was recorded at 340nm at 25°C for 5 minutes on kinetic spectrophotometer PRIM 500 (Germany) with automatic aspiration and thermostat. The activity was calculated using the molar coefficient for NADPH of 6.22 µmol⁻¹×cm⁻¹ and expressed in unit/gram tissue.

Estimation of intraerythrocyte sodium and potassium

Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the magnesium chloride (112mmol /L), centrifugation at 450g at 4°C for 5 minutes and aspiration of the supernatant was done (Fortes and Starkey, 1977). Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then used for the estimation of intraerythrocytes sodium and potassium.

STATISTICAL ANALYSIS

Results are presented as mean $\pm$ SD. Statistical significance and difference from control and test values were evaluated by student's t-test. P-values of **P<0.01 and *P<0.05 were considered significant.

RESULTS

Effect of thioacetamide and curcumin treatment on body weight in control and treated rats

Decreased body weight was observed after chronic administration of TAA in TAA and TAA + curcumin groups. Rats of TAA + curcumin group regained their body weight after curcumin treatment in second phase. Rats of TAA group continuously lost their body weights. Rats of curcumin group and control group gained their body weights throughout the treatment (fig1).

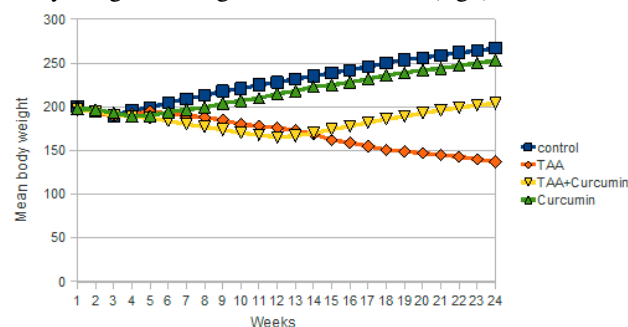


Fig.1: Effect of thioacetamide and Curcumin treatment on body weight in control and treated rats:

Effect of thioacetamide and Curcumin treatment on liver weight and liver to body weight ratio in control and treated rats

Significant increase in liver weight and liver-body weight ratio was observed in thioacetamide group after 12 week administration of thioacetamide as compare to control (6.33 ± 1.31 p<0.01) (0.028 ± 0.003 p<0.01) (Table-1). Whereas a decrease in liver weight and liver to body weight ratio was found in TAA + curcumin group as compare to control (5.534 ± 0.45 p<0.01) (0.03 ± 0.004 p<0.01) respectively. Curcumin treated group had reduced

liver weight and liver to body weight ratio (5.15 ± 0.54 P<0.01) (0.026 ± 0.002 p<0.05) (table 1).

Effect of thioacetamide and Curcumin treatment on plasma total and direct bilirubin and plasma ALT activity in control and treated rats

Table 2 represents a significant elevation in level of total bilirubin in TAA-treated rats as compare to control (3.6 ± 0.1 p<0.01) whereas supplementation of curcumin in TAA+curcumin treated group brought those elevated levels to normal concentration as compare to control (0.72 ± 0.01 p<0.01). Curcumin treated group showed normal range of total bilirubin as compare to control group (0.61 ± 0.03 p<0.05). Rats of TAA-treated group showed increased level of direct bilirubin as compare to control (3.8 ± 0.03 p<0.01) whereas the supplementation of curcumin in TAA+curcumin group significantly reduced those higher levels to normal levels as compare to control (1.40 ± 0.01 p<0.05). Level of direct bilirubin was not affected in curcumin-treated group (table 2). TAA-treated group showed increased plasma alanineaminotransferase level as compare to control (960.3 ± 30.19 p<0.01). TAA + curcumin showed markedly reduced plasma ALT level as compare to control (280 ± 11.6 p<0.01). Plasma ALT level was not affected by curcumin supplementation in curcumin-treated group as compare to control (210 ± 9.6) (table 2).

Effect of thioacetamide and curcumin treatment on hepatic concentration of Glutathione reductase in control and treated rats

TAA-treated group showed a significant reduction in hepatic concentration of glutathione reductase as compare to control (0.80 ± 0.01 p<0.01) (table 3). Glutathione reductase was also significantly increased by curcumin supplementation in curcumin-treated group as compare to control (0.89 ± 0.02 p<0.01).

Effect of thioacetamide and curcumin treatment on hepatic concentration of MDA in control and treated rats

TAA-treated rats showed increased level of MDA as compare to control (130 ± 2.1 p<0.01). TAA+curcumin-treated rats showed a significant reduction in level of MDA as compare to control (60.1 ± 1.5 p<0.01) whereas curcumin-treated group showed no effect on MDA level as compare to control (58.4 ± 1.5) (table 3).

Effect of thioacetamide and curcumin treatment on hepatic concentration of superoxide dismutase in control and treated rats

TAA-treated group showed a marked reduction in superoxide dismutase activity as compare to control (500 ± 2.3 p<0.01). TAA + curcumin treated group, after curcumin supplementation, showed a marked elevation in SOD activity as compare to control (760 ± 4.5 p<0.01).

Table1: Effect of thioacetamide and curcumin treatment on liver weight, liver to body weight ratio in control, thioacetamide-treated, thioacetamide+curcumin-treated and curcumin-treated rats

Groups	Liver Weights	Relative Liver Weights
Control	6.33±1.31	0.028±0.003
TAA-treated	6.82±0.71**	0.39±0.002**
TAA+curcumin	5.534±0.45**	0.03±0.004**
curcumin-treated	5.15±0.54**	0.026±0.002*

Table 2: Effect of thioacetamide and curcumin treatment on total and direct bilirubin and ALT activity in control, thioacetamide-treated, thioacetamide+curcumin-treated and curcumin-treated rats

Parameters	Control	TAA-treated	TAA+curcumin treated	curcumin-treated
Total bilirubin(unit/L)	0.60±0.04	3.6±0.2**	0.75±0.01**	0.61±0.03*
Direct bilirubin(unit/L)	1.35±0.03	3.8±0.03**	1.40±0.01*	1.34±0.01*
Alanin-amino transferase(unit/L)	210±9.6	960.3±30.19**	280±11.6**	210±8.5

Table 3: Effects of thioacetamide and curcumin treatment on hepatic concentration of glutathione reductase, superoxide dismutase, malondialdehyde and catalase in control, thioacetamide, thioacetamide+curcumin and curcumin-treated rats

Parameters	Control	TAA-treated	TAA+curcumin treated	curcumin-treated
Glutathione Reductase (unit/gm of tissue)	0.91±0.02	0.052±0.01**	0.80±0.01**	0.89±0.02**
Superoxide dismutase unit/gm of tissue.	890±2.0	500±2.3**	760±4.5**	890±3.2
Malondialdehydenmol/gm of tissue.	58.1±3.4	130.2±2.1**	60±1.5**	58.4±1.5
Catalase nmol/gm of tissue.	7.2±0.01	42.3±0.01**	7.5±0.16**	7.2±0.01

Table 4: Effect of thioacetamide and curcumin treatment on plasma and intraerythrocyte sodium and potassium in control, thioacetamide-treated, thioacetamide+curcumin-treated and curcumin-treated treated rats

Parameters	Control	TAA-treated	TAA+curcumin treated	curcumin-treated
Intraerythrocyte Na ⁺ mmol/L	3.78±0.06	2.9±0.01**	3.5±0.01**	3.68±0.03**
Intraerythrocyte K ⁺ mmol/L	53.28±1.1	42.33±1.5**	50.2±2.3**	53.37±3.1*
Plasma Na ⁺ mmol/L	140.8±1.2	122±1.0**	103±0.6**	105±0.8**
Plasma K+mmol/L	5.16±0.3	4.3±0.2**	5.72±0.1**	5.78±0.24**

n=6 Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide and curcumin and curcumin treated groups by t-test *P<0.05, **P<0.01.

Table 5: Histological examination of liver of thioacetamide and curcumin treatment on control, thioacetamide-treated, thioacetamide+curcumin treated and curcumin treated rats

Group	Amount of fibrosis	Disorganization of liver architecture	Stage of nodule formation and disorientation of vascular architecture
Control	-	-	-
Thioacetamide	+++++	+++	+++
Thioacetamide+curc-umin	++	+	00

Curcumin-treated groups showed no significant change as compared to control group (890 ± 3.2).

Effect of thioacetamide and curcumin treatment on hepatic concentration of catalase in control and treated rats

TAA-treated group showed significantly increased concentration of catalase as compare to control (42.3±p<0.01). TAA+curcumin treated group after curcumin supplementation in second phase showed a significant reduction in catalase concentration as compare to control (7.5±0.16 p<0.01). Alone curcumin had no

effect on catalase concentration in curcumin-treated group as compare to control (7.2±0.01) (table 3).

Effect of thioacetamide and curcumin treatment on intra-erythrocytes sodium and potassium in control and treated rats

Decreased level of intraerythrocyte sodium was observed in TAA group (2.9±0.01 P<0.01), whereas curcumin supplementation significantly increased intraerythrocyte sodium in TAA+curcumin group as compare to control (3.5±0.01 p<0.01). Alone Curcumin supplementation resulted in significant reduction in intraerythrocyte

sodium level in curcumin-treated group as compare to control (3.68 ± 0.03 $p < 0.01$) (table 4).

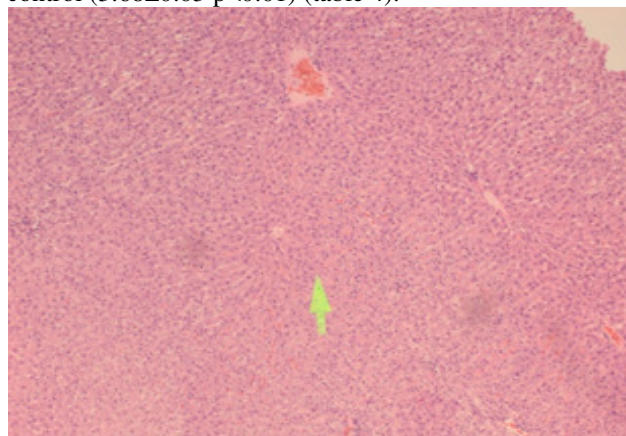


Fig.2A: Normal liver histology from control rats

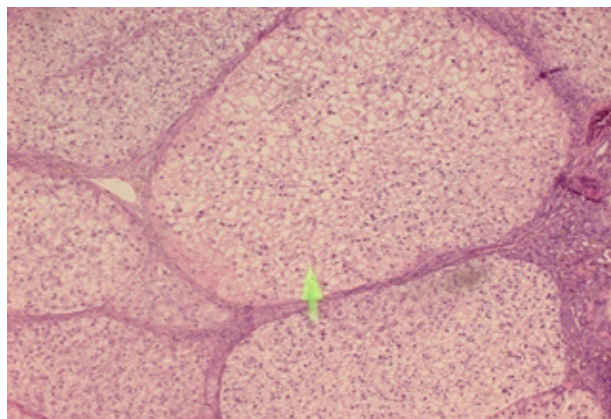


Fig. 2B: shows histological abnormalities after 12 week administration of thioacetamide in TAA treated rats.

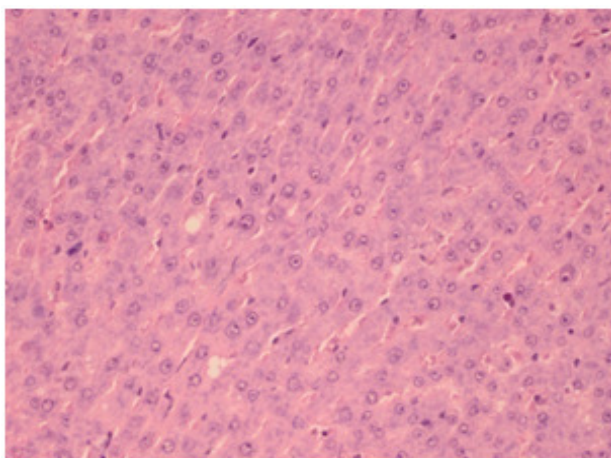


Fig. 2C: Shows effect of Curcumin treatment which reduces degree of fibrosis in TAA+Curcumin-treated rats.

Decreased intraerythrocyte potassium level was observed in TAA group as compare to control (42.33 ± 1.5 $P < 0.01$). Whereas increased intra-erythrocyte potassium level was observed in TAA+curcumin group (50.2 ± 2.3 $P < 0.01$) as compare to control. Alone curcumin supplementation resulted in increased level of

intraerythrocyte potassium in curcumin treated group (53.37 ± 3.1 $P < 0.05$) (table 4).

Effect of thioacetamide and curcumin treatment on plasma sodium and potassium in control and treated rats

Plasma sodium was decreased in TAA group (122.3 ± 1.0 $p < 0.01$) as compare to control whereas it was also decreased in TAA + curcumin group (103 ± 0.6 $P < 0.01$). Curcumin group also showed a significant reduction in plasma sodium (105 ± 0.8 $P < 0.01$) (table 4). Table 4 Showed decreased plasma potassium in TAA group (4.3 ± 0.2 $p < 0.01$) $P < 0.01$) as compare to control group whereas curcumin treatment in TAA + curcumin treated group significantly increased plasma potassium as compare to control (5.7 ± 0.1 $p < 0.01$). Curcumin group showed slightly increased concentration of plasma potassium (5.78 ± 0.24 $p < 0.01$) as compare to control.

Histology of liver in control and treated rats

After 12 week administration of thioacetamide in TAA-treated rats, histological examination showed last stage of liver cirrhosis, amount of fibrosis was (++++++)maximum. Supplementation of curcumin in TAA + curcumin group reduces the amount of fibrous tissue and the stage of nodule formation was (00) minimum (fig 2, table.5).

DISCUSSION

Thioacetamide, by the action of hepatic cytochromes, is converted into effective hepatotoxins, although it is not toxic itself (Hunter *et al.*, 1977). Such effective hepatotoxins generate strongly reactive compounds which become the cause of liver injury (Bruck *et al.*, 2004). Fulminant hepatic failure and early death are resulted from high dose administration of thioacetamide (Bruck *et al.*, 2002). Whereas liver cirrhosis resulted from a long term lower dose administration of thioacetamide (Pines *et al.*, 1997). In our study, chronic administration of thioacetamide in a dose of 200mg/kg b.w, twice a week resulted in the induction of definite cirrhotic changes in albino Wistar rats which was indicated by the increased levels of total bilirubin and ALT activity (table 2), showing the toxic effects of thioacetamide on bile ductular system (Yamada and Fausto, 1998). Results of our present study are in agreement with the findings of Torres (Torres, 1996) and Strugill (Strugill and Lambert, 1997). These increased activities can be attributed to discharge of these enzymes from injured hepatic cells into blood streams due to altered permeability of liver membrane (Shohda *et al.*, 2009). Supplementation with curcumin to thioacetamide treated rats resulted in normalization of bilirubin level and alanine-aminotransferase activity (table 2), Thioacetamide administration in animals resulted in induction of mechanisms which leads to the development of fulminant hepatic failure such as increased generation of reactive oxygen species and lipidperoxides by the liver (Sun *et al.*,

2000). NF- κ B stimulation and the resultant formation of proinflammatory molecules (Rahman *et al.*, 2003). Bruck *et al* reported development of liver cirrhosis in rats by the 12 weeks (biweekly), i.p. administration of thioacetamide indicated by gross macroscopic appearances, histopathology of liver, content of hepatic hydroxyproline and an increase in spleen weight, the activation of hepatic stellate cells was shown by liver which was determined by enhanced expression of α -smooth muscle actin and expression of type I collagen gene (Bruck *et al.*, 1977). In present study, the elevation of ALT and bilirubin were coupled with significant increase in liver oxidative stress as indicated by a marked elevation in TBARS level and a reduction in GSH and SOD level in liver in thioacetamide treated rats. Antioxidant defense mechanism is affected by the reactive oxygen species (superoxide anion, H₂O₂, hydroxyl radical) generated in the process of lipidperoxidation, initiated by the administration of hepatotoxin which results in the reduction of reduced glutathione concentration and a decrease in the activity of SOD (Srilaxmi *et al.*, 2010). Curcumin acts as a potent scavenger for a number of reactive oxygen species (Maheshwari *et al.*, 2006). The mechanism of antioxidant action of curcumin is because of its specific conjugated structure, comprises of two methylated phenols and an enol form of β -diketone. The ability of curcumin to act as a chain breaking antioxidant and trapping of free radicals is due to this structure (Masuda *et al.*, 2001). In present study, the antioxidant role of curcumin was confirmed by the reversal of the level of SOD, MDA, GSH and Catalase (table 3). Our results are in agreement with Oetari *et al.*, 1996 who reported role of curcumin in reducing oxidative stress by preventing hepatic lipoperoxides formation which was indicated by decreased levels of TBARS in rats acutely intoxicated with thioacetamide (Oetari *et al.*, 1996). Curcumin has the ability to scavenge free radicals because of which it prevents ROS formation and increases endogenous antioxidant activity. Many antioxidant enzymes of liver such as catalase, SOD and glutathione system are induced or activated by curcumin (Joe *et al.*, 2004; Sharma *et al.*, 2001; Iqbal *et al.*, 2003). Bruck *et al.*, 2007 found that anti-inflammatory and antioxidant properties of curcumin are involved in the protection of liver cirrhosis induced by chronic administration of thioacetamide (Bruck *et al.*, 2007). Shapiro *et al.* (2006) reported that co-administration of curcumin in a dose of 50mg/Kg/day for 12 weeks to thioacetamide treated rats appeared in a marked improvement of all the changes found in cirrhotic group.

CONCLUSION

In present study, curcumin supplementation to thioacetamide treated rats resulted in the reversal of altered levels of bilirubin and ALT activity, in the level of antioxidant enzymes, in the level of MDA, in

electrolyte homeostasis and in body weight indicates that curcumin successfully attenuates liver cirrhosis in rats.

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