Effect of curcumin supplementation on antioxidant enzymes in liver cirrhosis: study in rats

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Abstract: This study was designed to evaluate the effects of curcumin supplementation on antioxidant enzymes in thioacetamide induced cirrhotic rats. For this purpose 24 male Albino wistar rats were divided into four groups (n=6). Group I, remained healthy control rats, Group II, received thioacetamide (at a dose of 200mg/kg b.w, twice a week i.p, for 12 weeks) in first phase and saline in second phase, Group III, received thioacetamide (200mg/kg b.w, i.p for 12 weeks, twice a week) in first phase and curcumin (50mg/kg b.w/day, i.p. for 12 weeks) in second phase and Group IV, received curcumin (50 mg/kg b.w/day, i.p. for 12 weeks) in first phase and saline in second phase. Biochemical analysis was evaluated by estimation of antioxidant enzymes (SOD) Catalase, Glutathione reductase and MDA in control and cirrhotic rats. Curcumin supplementation markedly restored the antioxidant enzymes (SOD and GSH) MDA and catalase activity. These results indicate that curcumin successively attenuates the thioacetamide induced liver cirrhosis.

INTRODUCTION

Curcumin possesses anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic and nuclear factor–κβ inhibiting properties[1,2]. Curcumin activates HSC and in this way inhibits the synthesis of collagen type I[3] and used in steatohepatitis rodent models for attenuating inflammation and hepatic fibrosis[4]. Okada reported prevention of lipid peroxidation by curcumin and Rukkumani reported amelioration of oxidative stress[5,6]. 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[7].

Curcumin is also known to upregulate heme-oxygenase I, an enzyme involves in stress response, in endothelial cells, astrocytes and in renal epithelia cells[8]. 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, and activated receptor-γ[9]. Administration of curcumin increases the activities of antioxidant enzymes and thus decreases the lipid peroxidation in rats with iron induced hepatic toxicity. It also inhibits formation of hydroxyl radicals by inhibiting the oxidation of iron (Fe^{2+}) by H_2O_2. They reported a marked reduction in iron induced lipid peroxidation in wistar rats by the administration of 300mg/Kg of curcumin for 10 days[10]. Rajakrishnan et al., 1999 reported the reversal of biochemical and histopathological changes in the kidney, liver and brain in ethanol intoxicated rats[11].

Curcumin exerts protective effects against liver damage induced by aflatoxins, erythromycin estolate, CCl_4, ethanol, iron overdose and thioacetamide[12]. Curcumin is known to have beneficial systemic and hepatic effects as it has safe ingestion and sufficient bioavailability in humans[13]. Thus, the present study is designed to examine the hepatoprotective role of curcumin on antioxidant enzymes in thioacetamide induced liver cirrhosis in experimental rats.

MATERIALS AND METHODS

Total 24 male Albino Wistar rats weighing 200-250gm were purchased from the animal house of ICCBS (International center for chemical and biological sciences, Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions before the start of experiment and caged in a quiet temperature controlled animal room (23±4ºC). Rats had free access to water and standard rat diet.

Ethical guidelines

The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for laboratory use and care in animal research (Health research extension Act of 1985).

Study design

The rats were randomly divided into four groups, each of six rats. The duration of the study was 24 weeks, divided into two phases. Thioacetamide and curcumin were administered in either phase. Thioacetamide and curcumin were purchased from Merck and the other chemicals used

in present study were purchased from BDH laboratory supplies, Fisher Scientific UK limited and Fluka AG.

Group I: the control (remained untreated).

Group II: TAA-treated

Group III: TAA+ Curcumin treated

Group IV: Curcumin treated

In Phase I, TAA-treated and TAA+Curcumin groups received TAA, dissolved in 0.9% NaCl and were injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Curcumin group received curcumin (orally at a dosage of 50mg/kg b.w/ day for 12 weeks). In phase II, TAA-treated group received saline, TAA+curcumin group received curcumin (orally at a dosage of 50mg/kg b.w/ day starting from 13th week for 12 weeks) after TAA in first phase. Curcumin group received saline in second phase. At the end of experimental period, rats from all the groups were decapitated. The blood was collected from the neck wound 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 –70ºC until analysis.

Preparation of post mitochondrial supernatant

Liver homogenate was prepared by taking 1g of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC 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 thio-barbituric acid reacting substances (TBARS) by the lipid peroxidation method[14]. Briefly, the reaction mixture consisted of 0.2ml of 8.1% sodium dodecyle14sulphate, 1.5ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2ml of 10%(w/v) of PMS. The mixture was brought up to 4.0ml 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 (15:1v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532nm 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[15]. Briefly, the assay mixture was consisted of 1.96 ml phosphate buffer (0.01M, pH 7.0), 1.0ml hydrogen peroxide (0.2M) and 0.04ml PMS (10%w/v) in a final volume of 3.0ml. 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 570nm.

Estimation of SOD

Superoxide dismutase levels in the cell free supernatant were measured by the method[16]. Briefly 1.3ml of solution A (0.1M EDTA containing 50mM Na_2CO_3, pH 10.0), 0.5 ml of solution B (90µm NBT nitro blue tetra zolium dye) and 0.1ml of solution C (0.6%Triton X-100 in solution A), 0.1ml of solution D (20mM 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

GSH activity was determined by continuous spectrophotometric rate determination[17]. 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 ß-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 25ºC 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 $\mu mol^1 \times cm^1$ and expressed in unit/gram tissue.
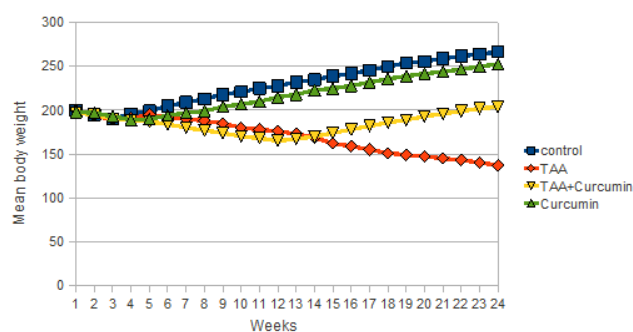
Figure 1: Effect of thioacetamide and curcumin treatment on body weight in control and treated rats.

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 (figure 1).

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

Increased liver weight and liver-body weight ratio was observed in TAA group after 12 week administration of TAA as Compare to control (6.33 ± 1.31 $P<0.01$) (0.028 ± 0.003 $P<0.01$) (Table 1) whereas reduction in the liver weight and liver to body weight ratio was observed 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 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**

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

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

Hepatic concentration of glutathione reductase was significantly reduced in TAA-treated group as compared to control (0.052 ± 0.001 $P<0.01$). TAA + curcumin group, after curcumin supplementation, showed increased level of glutathione reductase as compare to control (0.80 ± 0.01 $P<0.01$) (Table 2). Glutathione reductase was almost normal in curcumin group as compared 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

Level of MDA was markedly increased in TAA group as compare to control (130 ± 2.1 $P<0.01$). Curcumin administration in TAA + curcumin group decreased the concentration of MDA as compared to control (60.1 ± 1.5 $P<0.01$) while rats of curcumin group showed normal range of MDA level as compared to control (58.4 ± 1.5 $P<0.01$) (Table 2).

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

Table 2 showed a significant decrease in SOD activity in TAA group as compare to control (500 ± 2.3 $P<0.01$). TAA + curcumin group, after curcumin supplementation, showed a significant reduction in SOD activity (760 ± 4.5 $P<0.01$). SOD activity was almost normal in curcumin group (890 ± 3.2 $P<0.01$.

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

Concentration of catalase was significantly increased in TAA group (42.3 ± 0.01 $P<0.01$) as compare to control. Administration of curcumin in second phase in TAA+curcumin group brought these higher levels to normal limits (7.5 ± 0.16 $P<0.01$) as compare to control. Activity of catalase was normal (7.2 ± 0.01 $P<0.01$) in curcumin group (Table 2).

Table 2: 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
GR (U/gm)	$0.91\pm$ 0.02	$0.052\pm$ 0.01*	$0.80\pm$ 0.01*	$0.89\pm$ 0.02*
SOD (U/gm)	$890\pm$ 2.0	$500\pm$ 2.3*	$760\pm$ 4.5*	$890\pm$ 3.2*
MDA (nmol/gm)	$58.1\pm$ 3.4	$130.2\pm$ 2.1*	$60\pm$ 1.5*	$58.4\pm$ 1.5*
Catalase (nmol/gm)	$7.2\pm$ 0.01	$42.3\pm$ 0.01*	$7.5\pm$ 0.16*	$7.2\pm$ 0.01*

n=6, Values are mean±SD. Significant difference among control, TAA-treated, TAA+curcumin treated and curcumin-treated groups by student's t-test **$P<0.05$, *$P<0.01$.

DISCUSSION

In the present study, increase in liver oxidative stress was indicated by a marked elevation in TBARS levels and a reduction in GSH and SOD levels in liver in thioacetamide treated rats. Antioxidant defense mechanism is affected by the reactive oxygen species (superoxide anion, H_2O_2, hydroxyl radical) generated in the process of lipid peroxidation, initiated by the administration of

hepatotoxin which results in the reduction of reduced glutathione concentration and a decrease in the activity of SOD[18]. Curcumin acts as a potent scavenger for a number of reactive oxygen species[19]. 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[20]. In present study, the antioxidant role of curcumin was confirmed by the reversal of the level of SOD, MDA, GSH and Catalase (table 2). 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[21]. 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[22]. 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[23]. Haim Shapiro reported that co-administration of curcumin in a dose of 300mg/Kg/day for 12 weeks to thioacetamide treated rats appeared in a marked improvement of all the changes found in cirrhotic group[24]. In present study, curcumin supplementation to thioacetamide treated rats resulted in the reversal of altered levels of antioxidant enzymes, in the level of MDA and in body weight indicates that curcumin successfully attenuates liver cirrhosis in rats.

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