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Protective Effect of Curcumin in Rat Liver Injury Induced by Carbon Tetrachloride

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Abstract

This study was carried out to investigate the protective effects of curcumin on acute or subacute carbon tetrachloride-induced liver damage in rats.

Acute hepatotoxicity was induced by intraperitoneal injection of carbon tetrachloride after 4 consecutive days of curcumin treatment. Subacute hepatotoxicity was induced by oral administration of carbon tetrachloride twice a week during 4 weeks of curcumin treatment. In rats with acute liver injury, curcumin (100 and 200 mg kg^{-1}) lowered the activity of serum alanine aminotransferase to 52–53% ($P < 0.05$) and aspartate aminotransferase to about 62% ($P < 0.05$) those of control rats. In rats with subacute liver injury, curcumin (100 mg kg^{-1}) lowered the activity of serum alanine aminotransferase to 34% ($P < 0.01$) and alkaline phosphatase to 53% ($P < 0.05$) of control rats. The liver hydroxyproline content in the curcumin (100 mg kg^{-1})-treated group was reduced to 48% of the carbon tetrachloride control group ($P < 0.01$). Malondialdehyde levels in curcumin (100 mg kg^{-1}) treated rat liver was decreased to 67% of the control rat liver ($P < 0.01$) in subacute injury.

It was concluded that curcumin improved both acute and subacute liver injury induced by carbon tetrachloride in rats.

Curcuma longa Linn. (Zingiberaceae) is a perennial herb widely cultivated in tropical regions of Asia. Curcumin (diferuloyl methane) is a polyphenol that is contained in the rhizomes of *Curcuma longa* Linn. (turmeric) (Ammon & Wahl 1991). It is a yellow colouring spice that is widely used in Indian cooking. In the form of herbal powder turmeric, it has been used for centuries as an anti-inflammatory remedy in Asian medicine (Ammon & Wahl 1991). Traditional oriental medicine uses turmeric against biliary disorders, abdominal pains, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis.

With recent renewed interest in the pharmaceutical potential of natural products, many studies have been carried out in the exploration of the biomedical potential of turmeric and it has been suggested that its beneficial effects are due to curcumin (Ammon & Wahl 1991; Sreejayan & Rao 1994, 1997; Ruby et al 1995). It has been reported that curcumin has a hepatocyte-protective effect against carbon tetrachloride and D-galactosamine in-vitro (Kiso et al 1983; Donatus et al 1990).

In this study we investigated the protective effect of curcumin on acute liver damage and the effect of curcumin on lipid peroxidation and the fibrogenic process in rats with subacute liver damage induced by a 4-week treatment with carbon tetrachloride.

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Materials and Methods

Animals

Male Sprague–Dawley rats (200–250 g) were purchased from Dae Han Laboratory Animal Research & Co. (Choongbuk, Korea), and were allowed free access to normal standard chow diet (Jae Il Chow, Korea) and tap water. The rats were housed four or five per plastic cage and were kept under controlled conditions of $22 \pm 2^{\circ}$C, 50–60% relative humidity and 12-h light–dark cycles throughout the experiment. The rats were maintained in these facilities for 1 week before the experiment.

In the acute liver damage experiment, rats were dosed with curcumin (50, 100 or 200 mg kg^{-1} per day, in corn oil) orally for 4 consecutive days. Three hours after the last dose of curcumin, carbon tetrachloride was injected intraperitoneally (0·2 mL kg^{-1}). Twenty-four hours after the injection, rats were killed under ether anaesthesia and blood samples were collected by cardiac puncture.

In the subacute liver damage experiment, carbon tetrachloride was given orally (1 mL kg^{-1}, mixed with an equal volume of corn oil) on Mondays and Thursdays for 4 weeks during curcumin (50 or 100 mg kg^{-1} per day) treatment. Three days after the last carbon tetrachloride treatment, rats were killed under ether anaesthesia and blood and liver samples were collected.

Determination of serum biochemical parameters

Blood samples were kept at room temperature for 1 h and centrifuged at 3000 *g* for 30 min to obtain sera. Sera were kept at −20°C until further assay. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity and levels of albumin were measured with an Autodry chemistry analyser (SPOTCHEM SP4410, Arkray, Japan).

Determination of hydroxyproline content of livers

The hydroxyproline content of livers was determined by the method of Jamall et al (1981). In brief, liver specimens were weighed and completely hydrolysed in 6 M hydrochloride. An aliquot of the samples was derivatized using chloramine T solution and Erhlich reagent and measured at 558 nm. A standard calibration curve was prepared using *trans*-4-hydroxy-L-proline (Sigma Chemical Co., USA).

Determination of lipid peroxidation levels in liver

Lipid peroxidation was quantified by the thiobarbituric acid method (Plaa & Charbonneau 1994) using the S9 fraction of liver homogenates as an enzyme source; all manipulations were made rapidly on ice to avoid peroxidation. The S9 fraction of the homogenate was reacted with thiobarbituric acid solution in a boiling water bath and then extracted with n-butanol. The organic phase containing coloured complex was collected for fluorescence measurement. 532 nm and 553 nm were used for excitation and emission wavelengths, respectively. A calibration curve was prepared using 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., USA), a chemical releasing malondialdehyde in acidic conditions.

Statistical analysis

The results were evaluated by one-way analysis of variance and Tukey's multiple comparison test. *P* values of less than 0·05 were considered to indicate a significant difference.

Results and Discussion

In acutely liver-damaged rats, serum levels of ALT and AST were increased to 397% and 340% that of normal rats ($P < 0{\cdot}001$). Curcumin treatment (100 or 200 mg kg^{-1}) significantly lowered serum AST and ALT levels in rats with acute liver damage (Table 1). In rats treated with 50 mg kg^{-1} curcumin, serum ALT and AST were lowered to 55% and 74% that of carbon tetrachloride-treated control rats, but there were no significant differences. There were no significant differences in the activity of serum ALT and AST activities in rats treated with curcumin (50, 100 or 200 mg kg^{-1}) alone or treated with vehicle (corn oil) alone when compared with untreated normal rats (Table 1).

In subacute liver damage, serum levels of ALT and ALP in carbon tetrachloride-treated control rats were increased to 1653 ($P < 0{\cdot}01$) and 1041% ($P < 0{\cdot}01$), respectively (Table 2). The serum AST activity was increased to 840%'' and the serum albumin level was decreased to 78% of normal rats, but the difference was not significant. In rats treated with 100 mg kg^{-1} of curcumin suspended in corn oil, serum levels of ALT and ALP were significantly decreased to 34% ($P < 0{\cdot}01$) and 53% ($P < 0{\cdot}05$), respectively, of carbon tetrachloride-treated control rats (Table 2). The albumin level in rats treated with curcumin (100 mg kg^{-1} per day, suspended in corn oil) was increased to 120% when compared with that of 4-week carbon tetrachloride-treated rats, but there was no significant difference. In rats treated with 50 mg kg^{-1} curcumin, serum AST and ALT levels were lower than those of the carbon tetrachloride-treated control rats, but there

Table 1. Effect of curcumin treatment on levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum of rats with acute liver damage.

Group		n	ALT (IU L^{-1})	AST (IU L^{-1})
Normal		4	46 ± 9	97 ± 4
Carbon tetrachloride		8	182 ± 62***	330 ± 89**
Curcumin	50 mg	8	100 ± 73*	243 ± 100
	100 mg	8	97 ± 48†	211 ± 36†
	200 mg	8	95 ± 43†	210 ± 84†

Results are mean ± s.d. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$, compared with normal rats; †$P < 0{\cdot}05$, compared with rats treated with carbon tetrachloride.

Table 2. Serum biochemical values in carbon tetrachloride-intoxicated rats treated with curcumin for 4 weeks.

Group	ALT ($IU\,L^{-1}$)	AST ($IU\,L^{-1}$)	ALP ($IU\,L^{-1}$)	Alb ($g\,dL^{-1}$)
1	36 ± 5	91 ± 8	311 ± 58	5·26 ± 0·45
2	34 ± 6	83 ± 13	288 ± 94	5·54 ± 0·43
3	35 ± 6	83 ± 5	292 ± 58	5·41 ± 0·39
4	44 ± 8	100 ± 30	397 ± 187	5·75 ± 0·36
5	37 ± 8	94 ± 14	406 ± 15	5·86 ± 0·23
6	595 ± 302**	764 ± 848	3236 ± 1521**	4·08 ± 0·69
7	312 ± 128††	506 ± 174	3839 ± 1284**	4·34 ± 0·40
8	201 ± 110††	244 ± 105	1711 ± 437†,‡	4·91 ± 0·91

ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; ALP, serum alkaline phosphatase; Alb, serum albumin. Rats were treated as follows: group 1: untreated; group 2: corn oil (p.o., daily); group 3: corn oil (p.o., daily and twice a week simultaneously); group 4: curcumin ($50\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and corn oil (p.o., twice a week) simultaneously; group 5: curcumin ($100\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and corn oil (p.o., twice a week) simultaneously; group 6: corn oil (p.o., daily) and carbon tetrachloride ($1\,mL\,kg^{-1}$, p.o.) mixed with an equal volume of corn oil, simultaneously; group 7: curcumin ($50\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and carbon tetrachloride ($1\,mL\,kg^{-1}$, p.o.) mixed with an equal volume of corn oil, simultaneously; group 8: curcumin ($100\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and carbon tetrachloride ($1\,mL\,kg^{-1}$, p.o.) mixed with an equal volume of corn oil simultaneously. $^{**}P < 0{\cdot}01$ compared with groups 1–5. $^{\dagger}P < 0{\cdot}05$, $^{\dagger\dagger}P < 0{\cdot}01$ compared with group 6. $^{\ddagger}P < 0{\cdot}01$, compared with group 7. Results are mean ± s.d.

were no significance. In rats treated with vehicle (corn oil) or curcumin alone, the serum biochemical parameters were not significantly different when compared with those of untreated normal rats (Table 2).

In subacutely liver-damaged rats, the liver hydroxyproline content was increased to 277% in carbon tetrachloride-treated rats ($P < 0{\cdot}01$) when compared with normal rats (Table 3). In rats treated with $100\,mg\,kg^{-1}$ of curcumin, the hydroxyproline content was decreased to 48% that of carbon tetrachloride-treated control rats ($P < 0{\cdot}001$) (Table 3). In rats treated with $50\,mg\,kg^{-1}$ of curcumin, the hydroxyproline content was decreased to 76% that of carbon tetrachloride-treated control rats, but this was not significant. In rats treated with vehicle (corn oil) or curcumin (50 or $100\,mg\,kg^{-1}$) alone, the levels of hydroxyproline were not significantly different from that of normal rats (Table 3).

The malondialdehyde level in the livers of carbon tetrachloride-treated control was increased to 203% ($P < 0{\cdot}01$) that of normal, showing that 4 weeks of carbon tetrachloride treatment significantly increased lipid peroxidation in the liver (Table 3). In curcumin ($100\,mg\,kg^{-1}$)-treated rats, the malondialdehyde level was reduced to 67% that of normal rats ($P < 0{\cdot}01$). In the livers of rats treated with $50\,mg\,kg^{-1}$ of cucurmin, the malondialdehyde level was reduced to 92% that of normal rats, but this was not significant (Table 3). In rats treated with curcumin (50 or $100\,mg\,kg^{-1}$) or vehicle (corn oil) alone, the malondialdehyde values in liver were not significantly different from that of untreated normal rats (Table 3).

Until now, the hepatoprotective effects of curcumin have been focused on acute liver damage in-vivo or in-vitro (Kiso et al 1983; Donatus et al 1990). This study was carried out to investigate the protective effect of curcumin in subacute, as well as acute, liver damage. Rats were treated with carbon tetrachloride for 4 weeks to induce subacute liver damage. It was found that curcumin at a dose of $100\,mg\,kg^{-1}$ partially prevented fibrotic processes and liver damage induced by 4 weeks of carbon tetrachloride administration.

Table 3. Hydroxyproline and Thiobarbituric acid reactive substance levels in livers of carbon tetrachloride-intoxicated rats treated with curcumin.

Group	n	Hydroxyproline ($\mu g\ (g\,liver)^{-1}$)	Thiobarbituric acid reactive substance ($pmol\ (mg\,protein)^{-1}$)
1	6	281 ± 28	274 ± 86
2	4	273 ± 35	304 ± 36
3	4	302 ± 31	328 ± 50
4	4	305 ± 43	314 ± 20
5	4	295 ± 30	260 ± 58
6	6	777 ± 209**	556 ± 62**
7	6	589 ± 192*	512 ± 26**
8	6	373 ± 130††	374 ± 83†

Results are mean ± s.d. Rats were treated as follows; group 1: untreated; group 2: corn oil (p.o., daily); group 3: corn oil (p.o., daily and twice a week simultaneously); group 4: curcumin ($50\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and corn oil (p.o., twice a week) simultaneously; group 5: curcumin ($100\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and corn oil (p.o., twice a week) simultaneously; group 6: corn oil (p.o., daily) and carbon tetrachloride ($1\,mL\,kg^{-1}$ p.o.) mixed with an equal volume of corn oil, simultaneously; group 7: curcumin ($50\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and carbon tetrachloride ($1\,mL\,kg^{-1}$, p.o.) mixed with an equal volume of corn oil, simultaneously; group 8: curcumin ($100\,mg\,kg^{-1}$, p.o., daily) suspended in corn oil and carbon tetrachloride ($1\,mL\,kg^{-1}$, p.o.) mixed with an equal volume of corn oil, simultaneously. $^{*}P < 0{\cdot}01$, compared with groups 1–5. $^{**}P < 0{\cdot}001$, compared with groups 1–5. $^{\dagger}P < 0{\cdot}01$, $^{\dagger\dagger}P < 0{\cdot}001$ compared with group 6.

A survey conducted by the National Nutritional Monitoring Bureau (Hyderabad, India) indicated that a daily intake of 0·1–3·8 g of turmeric and 4 g of turmeric per adult was reported after analysing 6 different curry powders (Sambaiah et al 1982). The results of this study suggested that the effective dose of curcumin required for protection against carbon tetrachloride-induced liver damage was 100 mg kg^{-1} in rats. As curcumin accounts for 2–8% of the dry weight of turmeric (Bhavant Shankar et al 1980; Ruby et al 1995), 1·25–5·0 g kg^{-1} of turmeric will give 100 mg kg^{-1} of curcumin. This dose of turmeric is 19–75 times higher than the estimated daily intake of turmeric of 4 g per adult, when the body weight of an adult is assumed to be 60 kg. The amount of curcumin used in this study was 500–2000 times the acceptable daily intake (ADI) of 0–0·1 mg kg^{-1} recommended by the FAO/WHO Expert Committee (Food Additives Fifty-first Meeting, Geneva, 1998).

High doses of curcumin or turmeric in the diet are reported to be non-toxic and non-mutagenic (Ammon & Wahl 1991). When rats were fed with turmeric at doses that were 2–125 times those normally consumed by humans (estimated to be 4 g per adult daily) or were fed with curcumin at 1000–20 000 times the FAO/WHO recommendation, there were no adverse effects on growth, feeding efficiency ratio, erythrocytes, leukocytes or on the levels of blood constituents (Sambaiah et al 1982). No mortality was observed in any of these groups and no histological abnormality was noticed in the gastro-intestinal tract, liver, spleen or kidney (Sambaiah et al 1982). In fact, curcumin is used in large doses as shown in a number of reported clinical studies which reported use of doses of 400–1200 mg/day of curcumin in patients with rheumatoid arthritis or inflammation without any side-effects during the study period (Deodhar et al 1980; Ammon & Wahl 1991). Turmeric has also been used traditionally in Asia in doses of 6–20 g daily in adults (Shokayakuzi-Kenkyosho 1981).

In summary, this study demonstrates that curcumin can effectively inhibit the liver injury caused by either acute or subacute carbon tetrachloride treatment as monitored by serum biochemical parameters and hydroxyproline content and lipid peroxides levels in the liver. Further study is needed to reveal the exact mechanism of the inhibition of the liver fibrogenic process by curcumin.

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