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Protective Effect of Curcumin Against Paracetamol-induced Liver Damage

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Abstract: Curcumin, as well-known dietary pigment derived from the rhizome of curcuma longa L., has been shown to be a potent anti-inflammatory, antioxidant and anticarcinogenic agent. The present investigation aimed at questioning and examining the possible potential protective effect of curcumin against paracetamol-induced hepatotoxicity in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic application. Paracetamol (500 mg/kg.b.w.) administration to rats resulted in massive elevation in serum and hepatic lactate dehydrogenase (LDH) activity and TBARS as well as in serum tumor necrosis factor–alph (TNF-α) levels, with a significant decrease in serum protein thiols (Pr-SHs), blood glutathione (GSH) levels, blood superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. On the other hand, paracetamol hepatotoxicity resulted in an increased in hepatic TBARS and depletion of hepatic GSH and Pr-SHs levels as well as in hepatic SOD, GPx, GR, glutathione-s-transferase (GST) and Catalase (CAT) activities. Oral administration of curcumin at a concentration of 50 mg/kg b.w. daily for 15 days to rats treated with paracetamol produced a significant protection against-induced increase in serum and hepatic LDH activities as well as TBARS and tumor necrosis factor–alph (TNF-α) levels. Also, curcumin (50 mg/kg.B.W.) could inhibit reduce in serum Pr-SHs, blood GSH levels and enhance increase in blood SOD and GPx activities. Hepatic TBARS level was suppressed by administration of curcumin to paracetamol-treated rats. In addition, curcumin enhance increase in hepatic GSH and Pr-SHs levels as well as in hepatic SOD, GPx, GR, GST and CAT activities. These data indicate that curcumin is a natural antioxidant hepatoprotective agent against hepatotoxicity induced by paracetamol model. Thus, curcumin may have a therapeutic value in drug-induced hepatotoxicity as well as in paracetamol therapy.

Key word: LDH, TBARS, Pr-SHs , TNF-α, SGH, SOD, GPx, CAT, GST, GR, serum, liver, Paracetamol, Curcumin and hepatoprotective.

INTRODUCTION

Any organ of body is a potential target for injurious effects from chemicals but some organs are more vulnerable to adverse effects than others. The liver is often a target organ for a number of reasons. First, most toxicants enter the body *via* the gastrointestinal tract and after absorption they are carried by the hepatic portal vein to the liver. Thus the liver will be exposed to the highest concentrations of these chemicals (Reed, 1994; Lu, 1996). Chemicals encountered by other routes of exposure may also reach the liver through its blood supply from the hepatic artery as well as the portal vein (Stacey *et al*., 1993; Kulkarni and Byczkowski, 1994). Second, the liver has the ability to concentrate, biotransform and excrete chemicals, irrespective of routes of exposure (Plaa and Hewitt, 1982). It has high concentrations of xenobiotic metabolizing enzymes, mainly cytochrome P450, which render most toxicants less toxic, more water-soluble and thus more readily excretable (Murry, 1994). But in some case toxicants are activated to be capable of inducing lesions as in case of paracetamol-induced hepatotoxicity (Potter *et al*., 1973).

Paracetamol is sparingly soluble in water, freely soluble in alcohol, very slightly soluble in ether and in methylene chloride. It has been available over the counter in the most countries since the late fifties (Clissold, 1986). It is metabolized extensively by the liver via three main pathways; sulfonation, glucuronidation and oxidation (Mitchell *et al*., 1974). The first two pathways are quantitatively more important than the last, but the oxidative pathway is the culprit as far as toxicity is concerned (Jollow *et al*., 1973).oxidation of

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paracetamol occurs in the hepatic microsomes and is primarily catalyzed by cytochrome P-450 (Potter *et al.*, 1973). The process produces a highly reactive arylating compound called N-acetyl-p-benzoquinoneimine (NAPQI) (Dahlin *et al*., 1984). In human liver microsome P-4501A2, were shown to be principal catalysts of paracetamol activation (Raucy *et al*., 1989).

NAPQI is normally rapidly conjugated with GSH and is excreted eventually as the cysteinyl conjugate or the corresponding mercapturic acid (Mitchell *et al*., 1973). As long as the rate of formation of this toxin is not greater than the maximal rate of synthesis of GSH there will be no damage to the cell or organ (Mitchell et al., 1973).Hepatic synthesis of GSH, which is directly suppressed within the first few hours following ingestion of hepatotoxic dose of paracetamol, is overwhelmed and manifestations of toxicity appear when GSH level falls below 30% of normal (Makin and Williams, 1997). When more NAPQI if formed than can be conjugated to GSH, the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins (Vermeulen *et al*., 1992).

Finally, some investigators have suggested that hepatic macrophages may have a pathogenic role as inhibition of their function status attenuates the degree of paracetamol-induced hepatic injury by a mechanism that is independent of NAPQI production (Laskin *et al.,* 1995).

Many plant extracts and plant products have been shown to have significant antioxidant activity (AL-Howiriny *et al.,* 2005, Hussein, 2009& 2010), which may be an important property of medicinal plants associated with the treatment of several ill fated diseases including liver toxicity. Thus, herbal plants are considered a useful means to prevent and/or ameliorate certain disorders, such as diabetes, atherosclerosis, hepatotoxicity and other complications (AL-Howiriny *et al.*, 2005; Hussein, 2008). Among these herbal resources, curcumin, is the main yellow phenolic material present in the rhizomes of turmeric (Curcuma longa L.) and is widely used as a food coloring agent (Govindarajan, 1980). Curcumin is a β-diketone compound which contains two ferulic acid molecules linked via a methylene bridge at the carbon atoms of the carbonyl groups (Sharma, 1979). Various curcumin-related phenols (curcuminoids) have also been found in edible plants, especially Zingiberaceae plants (Toda *et al.,* 1985; Masuda *et al*., 1992; Masuda *et al*., 1999). Extracts of rhizomes of turmeric have been widely used in Indian medicine and they are considered to be efficacious in the treatment of liver disorders and certain pyrogenic infections (Nadakarni, 1954). Curcumin exhibits anti-inflammatory and antiviral effects and it is also considered as a potent scavenger of reactive oxygen and nitrogen species (Joe *et al.,* 2004). Some of these activities are responsible for the ability of curcumin to protect DNA against free radical-induced damage and to protect hepatocytes against various toxins (Sahu and Washington, 1992).

In vitro and *in vivo* tests have been reported with curcumin to determine, for example, its, antimutagenic, anticarcinogenic and anticholestatic activities (Sahu and Washington, 1992; Ahmed and Mannaa, 2004). But there are no reports of the hepatoprotective effect of curcumin against hepatotoxicity induced by paracetamol.

The present study was undertaken to investigate the ability of oral administration of curcumin to alleviate the adverse effects of paracetamol on liver.

MATERIALS AND METHODS

Chemicals:

Paracetamol was provided as gift from El-Nile Pharmaceutical Company (Cairo, Egypt). When intended to be used *in vivo* experiments, paracetamol was suspended in 0.5 % tween 80 and orally administrated in dose of 500 mg/kg.B.W. (Kostrubsky *et al.*, 1995).
Tween 80 was produced by Prolabo, Farance.

Extract:

Crude curcumin was extracted from turmeric using soxhlet apparatus and ethyl alcohol as a solvent. The extract was then evaporated till dryness under reduced pressure. The extracted fraction was completely evaporated in a vacuum oven at a temperature not exceeding 40 ^{0}C until a constant weight was obtained. Hundred grams of turmeric yield 6.25 grams crude curcumin. Crude curcumin contains 77% pure curcumin, 19% monodemethoxy curcumin and 4% bisdemethoxy curcumin (Deters *et al.,* 2000).

Animals:

Adult albino rats weighing around 180-200gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatized to animal house conditions. Animals were provided with standard diet and water *adlibtum*. Rats were kept under constant environmental condition and observed daily throughout the

experimental work. The study was approved with the Committee for the purpose of control and supervision of experimental animals in Atomic Energy Authority (AEA) and the registration of AEA guidelines were followed for animal handling and treatment

Experimental Set Up:

The animals were divided into five groups with seven animals in each.

Group I: Normal control (was given similar volume of 1% tween 80)

Group II: Was treated with crude curcumin (50mg/kg b.w.) suspended in tween 80 orally in a single daily dose for 15 days (Choudhary *et al.*, 1999).

Group III: Was given paracetamol (500 mg/kg b.w. in tween 80) in a single daily dose for the last 5 days of the experimental period (Kostrubsky *et al.,* 1995).

Group IV: Was pretreated with crude curcumin (50mg/kg b.w.) alone for 10 days then received both curcumin (50mg/kg b.w.) and paracetamol (500 mg/kg b.w.) for other 5 days (prophylactic I).

Group V: Was simultaneously given curcumin (50mg/kg.b.w.) and paracetamol (500 mg/kg b.w.) for 5 days followed by crude curcumin (50mg/kg b.w.) alone for other 10 days (prophylactic II).

I-Treatment of Blood Samples:

After 15 days of treatment blood samples were withdrawn from the retro-orbital vein of each animal and each sample was collected into 2 tubes, heparinized and non-heparinized. The non heparinized blood samples were allowed to coagulate and then centrifuged at 1000 xg for 20 min. The separated sera were used for the estimation of serum activity of LDH and TBARS, Pr-SHs, total protein levels. The heparinized blood samples were divided into 2 aliquots. The first aliquot was used for determination of GPx activity.

The second aliquot was haemolyzed using bidistilled water and the haemolysate of each sample was divided into two portions was treated with chloroform/ethanol (3:5 V/V) mixture to precipitate and the resultant supernatant was used for the determination of SOD activity. The second portion was deproteinized with meta-phosphoric acid and the clear supernatant was used for the estimation of GSH level. Haemoglobin levels were determined in the heparinized blood samples and used in the calculation of the enzyme activity.

II-Preparation of Liver Samples:

Animals were killed by cervical dislocation, and then livers were rapidly removed. A part of each liver was weighed and homogenized, using glass homogenizer (Universal Lab. Aid MPW-309, mechanika precyzyjna, Poland), with ice-cooled saline to prepare 25% W/V homogenate. The homogenate was divided into three aliquots. The first one was deproteinized with ice-cooled 12% trichloroacetic acid and the obtained supernatant, after centrifugation at 1000 xg, was used for the estimation of GSH. The second aliquot was centrifuged at 1000 xg and the resultant supernatant was used for estimation of LDH activity, TBARS, Pr-SHs, total protein and albumin levels. The third aliquot of homogenate was used to prepare a cytosolic fraction of the liver by centrifugation at 10500 xg for 15 min at 4 ^{0}C using a cooling ultra-centrifuge (Sorvall comiplus T-880, Du Pont, USA), and the clear supernatant (cytosolic fraction) was used for the determination of SOD, GPx, GR, GST and CAT activities.

Biochemical Assays:

Colorimetric determination of serum and hepatic LDH, TBARS, Pr-SHs and GSH levels in blood and hepatic were done by the methods described by (Buhl and Jackson, 1978; Uchiyama and Mihara, 1987; Koster, *et al*., 1986 and Chanarin, 1989), respectively. Lipid peroxidation products were estimated in serum and liver by the determination of the level of TBARs that were measured as malondialdehyde (MDA). Quantitative estimation of blood and liver homogenate SOD and GPx activities were carried out (Paglia and Valentine, 1967; Marklund and Marklund, 1974), respectively. Quantitative determination of tumor necrosis factor–alph (TNF-α) was done by ELISA according to the method of Corti *et al.,* (1992) using kit produced Diaclone Research, France. GSH level in liver homogenate were estimated by spectrophotometer method according to the method of Sedlak and Lindsay, 1968 using Oxis Research kit. Blood haemoglobin was determined using a test reagent kit according to the method of Van Kampen and Zijlstra, (1961). The protein content of liver tissue was measured by applying the method of Lowry *et al*., (1951). Superoxide dismutase (SOD) activity in liver homogenate was assayed by red formazan dye reduction produced by Suttle, 1986 using 50 µl diluted homogenate. Liver glutathione peroxidase (GPx) activity was determined using reduced glutathione and cumene hydroperxide as substrate using 20 µl diluted homogenate by the modified method of (Paglia and Valentine 1967). Catalase (CAT), glutathione reductase (GR) and glutathione-S-transferase (GST) activities were measured

according to the method of (Tukahara *et al*., 1960; Horn, 1965 and Habig *et al*., 1974), respectively.

Statistical Analysis:

All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for seven separate determinations.

Results:

Table 1 and 2 shows the concentration of serum and liver LDH, TBARS, Pr-SHs and serum TNF-α of control and experimental groups of rats. Paracetamol (500 mg/kg.), orally given to rats markedly increased serum and hepatic LDH activity. Also, the levels of serum and hepatic TBARS and serum TNF-α in paracetamol injected rats were significantly higher than control rats, whereas paracetamol injected rats-treated with the curcumin after and before paracetamol [prophylactic I (group 4) and prophylactic II(group 5)] groups restored the altered values to the near normalcy. The decreased concentration of serum and hepatic Pr-SHs was observed in paracetamol injected rats. Administration of curcumin after and before paracetamol (prophylactic I and prophylactic II) groups tends to bring the Pr-SHs level to near normal. The effect was more pronounced in the groups of rats administered with curcumin after paracetamol (prophylactic I) group 4.

Table 3 and 4 shows the concentration of blood and liver reduced glutathione (GSH), activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) of control and experimental groups of rats. The decreased concentration of blood and hepatic GSH was observed in paracetamol control rats. Administration of curcumin (prophylactic I and prophylactic II) groups tends to bring the GSH level to near normal. The activities of SOD and GPx in liver were significantly lower in paracetamol injected rats compared to paracetamol injected rats-treated with curcumin (prophylactic I and prophylactic II) groups. The effect was more pronounced in the paracetamol injected rats-treated with curcumin (prophylactic I) group 4.

Table 5 shows the concentration of liver activities of catalase (CAT), glutathione-s-transferase (GST) and glutathione reductase (GR) (NADPH-dependent and NADH-dependent) of control and experimental groups of rats. The activities of CAT, GST, NADPH-dependent GR and NADH-dependent GR in liver was significantly lower in paracetamol control rats compared to paracetamol injected rats-treated with curcumin (prophylactic I and prophylactic II) groups. The effect was more pronounced in the paracetamol injected rats-treated with curcumin (prophylactic I) group 4.

Table 1: Levels of lactate dehydrogenase (LDH), lipid peroxides (TBARS), protein thiols (Pr-SHs) and tumor necrosis actor–alph (TNF-α) in serum of control and experimental groups of rats.

Groups	LDH(U/l)	TBARS(nmol/ml)	Pr-SHs(µmol/l)	TNF-αP(g/ml)
Normal Control1 % tween 80	186.64 ± 10.28	2.53 ± 0.19	536.3± 24.76	61.5 ± 5.41
Curcumin50 mg/kg.b.w.	154.77 ± 8.73*	1.91± 0.08*	601.82± 27.90*	55.8± 3.69*
Paracetamol500 mg/kg b.w.	510.34 ± 36.51*	8.73 ± 1.23*	480.46 ± 21.84*	79.68± 4.45*
Curcumin + Paracetamol (Prophylactic I)	265.63 ± 11.47*	3.11 ± 0.16*	521.24 ± 29.75*	63.92 ± 5.11*
Paracetamol + Curcumin (Prophylactic II)	380.71 ± 19.04*	5.81 ± 0.22*	490.44 ± 22.55*	68.33 ± 6.15*

Values are given as mean ± SD for groups of seven animals each. Values are statistically significant at *$P<0.05$. Curcumin and paracetamol treated rats were compared with normal control rats. Experimental groups (4 &5) were compared with paracetamol treated rats.

Table 2: Activity of lactate dehydrogenase (LDH) and levels of lipid peroxides (TBARS) and protein thiols (Pr-SHs) in liver of control and experimental groups of rats.

Groups	LDH(U/g protein)	TBARS(nmol/g protein)	Pr-SHs(µmol/g protein)
Normal Control 1 % tween 80	301.32 ± 15.47	265.73± 12.19	71.63± 6.17
Curcumin 50 mg/kg.b.w.	283.46 ± 10.17*	195.35± 15.76*	98.55± 4.64*
Paracetamol500 mg/kg b.w.	736.35 ± 40.51*	810.69 ± 32.84*	53.75 ± 5.95*
Curcumin + Paracetamol (Prophylactic I)	324.66 ± 17.98*	299.74 ± 10.63*	88.36 ± 7.17*
Paracetamol + Curcumin (Prophylactic II)	354.84 ± 21.91*	310.84 ± 11.70*	69.79 ± 4.94*

Values are given as mean ± SD for groups of seven animals each. Values are statistically significant at *$P<0.05$. Curcumin and paracetamol treated rats were compared with normal control rats. Experimental groups (4 &5) were compared with paracetamol treated rats.

Table 3: Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of control and experimental groups of rats.

Groups	GSH(mg %)	SOD(U/g Hb)	GPx(U/g Hb)
Normal Control 1 % tween 80	53.24 ± 2.69	13.90± 1.54	156.81± 4.67
Curcumin50 mg/kg.b.w.	86.37 ± 4.25*	19.47± 1.95*	217.19± 8.76*
Paracetamol500 mg/kg b.w.	29.46 ± 2.66*	7.33 ± 1.51*	113.88± 6.42*
Curcumin + Paracetamol (Prophylactic I)	49.58 ± 3.85*	14.38 ± 1.74*	148.62± 7.58*
Paracetamol + Curcumin (Prophylactic II)	36.09± 4.38*	11.96± 1.66*	123.71 ± 8.22*

Values are given as mean ± SD for groups of seven animals each. Values are statistically significant at *P<0.05. Curcumin and paracetamol treated rats were compared with normal control rats. Experimental groups (4 &5) were compared with paracetamol treated rats. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the haemoglobin concentration.

Table 4: Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in liver of control and experimental groups of rats.

Groups	GSH(mg/g protein)	SOD(U/g protein)	GPx(U/g protein)
Normal Control 1 % tween 80	9.19 ± 1.37	120.11± 3.97	261.77± 19.58
Curcumin50 mg/kg.b.w.	15.63± 1.16*	151.17± 6.51*	301.86± 15.35*
Paracetamol500 mg/kg b.w.	4.16 ± 0.49*	37.93± 4.32*	127.37± 18.64*
Curcumin + Paracetamol (Prophylactic I)	8.73± 0/98*	125.41± 7.62*	247.17± 16.84*
Paracetamol + Curcumin (Prophylactic II)	7.36± 1.28*	115.82± 5.44*	236.94± 22.31*

Values are given as mean ± SD for groups of seven animals each. Values are statistically significant at *P<0.05. Curcumin and paracetamol treated rats were compared with normal control rats. Experimental groups (4 &5) were compared with paracetamol treated rats. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration.

Table 5: Activities of glutathione-s-transferase, catalase and glutathione reductase in liver of control and experimental groups of rats.

			(GR) (nmol/ mg protein)	
Groups	(GST)	(CAT)	NADPH-dependent GR	NADH-dependent GR
Normal Control 1 % tween 80	8.31±0.52	98.61±3.81	2.71±0.11	0.63±0.04
Curcumin 50 mg/kg.b.w.	10.64±0.61*	122.53±5.12*	3.14±0.3*	0.84±0.03*
Paracetamol 500 mg/kg b.w.	4.56±0.34*	47.37±4.16*	1.12±0.02*	0.34±0.01*
Curcumin + Paracetamol (Prophylactic I)	9.43±1.2*	113.74±7.43*	2.90±0.14*	0.76±0.04*
Paracetamol + Curcumin (Prophylactic II)	8.17±0.96*	107.15±4.17*	2.63±0.08*	0.70±0.05*

Values are given as mean ± SD for groups of seven animals each. Values are statistically significant at *P<0.05. Curcumin and paracetamol treated rats were compared with normal control rats. Experimental groups (4 &5) were compared with paracetamol treated rats. Activity is expressed as: units per min per mg of protein for GST; μmoles of hydrogen peroxide decomposed per min per mg of protein for catalase.

Discussion:

The present study was conducted to evaluate the beneficial effects of crude curcumin on antioxidant status in paracetamol - induced hepatotoxicity rats. The preliminary studies conducted by this work revealed the non-toxic nature of curcumin on normal rats. Paracetamol causes acute centrilobular hepatic necrosis in rats and other animal species (Piperno *et al*., 1978). Hepatic necrosis following massive paracetamol administration is well documented (Waters *et al*., 2001). Drastic elevation in the activity of serum and liver cytosolic LDH were shown in the current study after administration of paracetamol (500 mg/kg.b.w). Paracetamol toxicity was reported to associate with increased released of LDH in experimental animals (Blazka *et al*., 1996). Increase in cytosolic LDH activity by paracetamol might be due to the intracellular accumulation of Ca^{2+}, which results in activation of phosphofructokinase and anaerobic glycolysis leading to lactate formation (Landowne and Ritchie, 1971). Loss of Ca^{2+} homeostasis as a result of oxidative damage and increase in intracellular Ca^{2+} has been reported to a late and perhaps irreversible final stage in the process of cell death for paracetamol (Strubelt and Younes, 1992). Curcumin administration controlled serum and hepatic LDH. However, it did not normalize the LDH level completely as it remained lesser than paracetamol injected rats.

Increased lipid peroxidation, as evidenced by the elevated levels of thiobarbituric acid reactive substances (TBARS) in serum and hepatic tissues was demonstrated in the present study. These results are in harmony with those of other investigators who reported the association between paracetamol toxicity and lipid peroxidation (Wendel *et al*., 1996). Toxic dose of paracetamol can modulate the lipid composition and fluidity

of biomemberanes, by activation of Ca^{2+}-phospholipase that results in diminished structural integrity and by alteration of membrane bound enzymes such as Ca^{2+} - ATPase activity (Ray *et al*., 1996). In the present study, serum and hepatic TBARS levels were significantly lower in the curcumin – treated groups compared to the paracetamol treated group. The above result suggests that the curcumin may exert antioxidant activities and protect the tissues from lipid peroxidation.

Serum and hepatic protein thiols (Pr-SHs) contents were markedly decreased after paracetamol administration, as shown in the current investigation. Similar results were reported for paracetamol (Kyle *et al*., 1990). The loss of Pr-SHs is held to be a critical event in the genesis of lethal injury by an acute oxidative stress (Di Monte *et al*., 1984). Such depletion is presumed to be a direct oxidation of the thiol groups of contiguous amino acids with the formation of protein-protein disulphides (Di Monte et al., 1984). The cytotoxic effects of paracetamol have been attributed to depletion of Pr-SHs (Kyle *et al*., 1990). The covalent binding of NAPQI accounts for only a part of Pr-SHs depletion induced by paracetamol (Birge et al., 1988), while the remainder is said to result from direct oxidation of Pr-SHs, presumably by NAPQI itself (Kyle *et al*., 1990).

In addition, the metabolism of paracetamol also generates GSSG, a product that reacts with Pr-SHs to form GSH mixed disulphides (Kyle *et al.*, 1990). In the present study, the elevation of Pr-SHs levels in serum and liver was observed in the curcumin rats. This indicates that the curcumin can either increase the biosynthesis of Pr-SHs or reduce the oxidative stress.

Serum tumor necrosis factor–alph (TNF-α) content was markedly increased after paracetamol administration, as shown in the current investigation. These results are in harmony with those of Lashin *et al*., 1986 who shown that hepatocytes treated with paracetamol release factors, which activate of proinflammatory cytokines as TNF-α and interlukins. The hepatoprotective effect on curcumin was demonstrated through correcting the value of serum α-GST that were significant raised by paracetamol administration. This result indicated that curcumin has a membrane stabilizing effect (Reddy and Lokesh, 1996). Additionally, there is growing evidence that the hepatoprotective effect of extract takes place directly at the level of hepatocytes by lowering intracellular levels of cholesterol and cytotoxic bile acids (Asai and Miyazawa, 2001).

GSH has a multifactorial role in antioxidant defense. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Wohaieb and Godin 1987). Liu *et al.,* 1993, suggested that the decrease in blood and liver GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress and tissue injury. Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased hepatic GSH content. In the present study, the elevation of GSH levels in blood and liver was observed in the curcumin treated rats.

This indicates that the curcumin can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen (Mc Crod *et* al., 1976), hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes (Sozmen *et a*l., 2001). The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide (Chance *et al*., 1952). Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase.

GPx plays a primary role in minimizing oxidative damage. Glutathione peroxidase (GPx), an enzyme with selenium and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione (Bruce *et al*., 1982).

Reduce activities of SOD and GPx in blood and liver have been observed in paracetamol-treated rats. Several authors reported the decrease in SOD and GPx activities in paracetamol-treated animals (O'Brien *et al.*, 2000; Ahmed and Khater, 2001). Our results were asserted by the finding of Reddy and Lokesh 1994) who demonstrated a significant increase in the activities of hepatic glutathione peroxidase, superoxide dismutase and catalase accompanied with marked decrease in hepatic peroxidation by treatment with curcumin. Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Searle and Wilson 1980). This decrease in CAT activity could result from inactivation by glycation of enzyme (Yan and Harding 1997). Reduced activities glutathione-s-transferase (GST), catalase (CAT) and glutathione reductase (GR) in the liver have been observed in paracetamol-treated rats and this may result in a number of deleterious effects may be due to the accumulation of superoxide radicals and hydrogen peroxides (Wohaieb and Godin 1987). Whereas, the curcumin-treated groups showed a significant increase in the hepatic SOD, GPx, GST, CAT and GR activities of the paracetamol-treated rats.

This means that the extract can reduce the potential glycation of radicals and improve the activities of antioxidant enzymes. It is most likely that the potential effect of the curcumin in reducing the lipid peroxide level is a consequence of the modulatory influence of the curcumin on the biotransformation enzymes of detoxification. Protective effects of curcumin against paracetamol-induced lipid peroxidation and liver damage have not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind. In this context, other workers also reported a decrease in the activity of these antioxidant enzymes (SOD, GPx, GST, CAT and GR) in the serum, blood and liver of paracetamol-treated rats (Piper *et al*., 1998).

In conclusion, the findings of the present study confirm a role for ROS activation in the pathogenesis of paracetamol -induced liver damage, and indicate that a blockade of their formation/activation by curcumin has a potent anti-inflammatory effect. As ingestion of turmeric/curcumin appears to be safe in humans (Chainani, 2003), our findings support a role for this compound as a hepatoprotectant.

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