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Protective Effect of *Curcuma longa* Against CCL_4 Induced Oxidative Stress and Cellular Degeneration in Rats

[1]*Eman A. Abd Elaziz,* [2]*Zein S. Ibrahim and* [3]*Azza M. Elkattawy*

[1]Department of Pathology, [2]Department of Physiology, [3]Department of Biochemistry,
Faculty of Veterinary Medicine, Kafr El-Sheikh University, Egypt

Abstractl: *Curcuma Longa* is a member of the *Zingiberaceae* or ginger family. The plant is native to India and is the source of its culinary spice known as Turmeric and its medicinal extract called Curcumin. In the present study, the protective effect of *Curcuma longa* extract against the acute hepatotoxicity of carbon tetrachloride (CCl_4) was investigated. Intraperitoneal injection of rats with CCl_4 drastically decreased total protein, immunoglobulins (IgG, IgM and IgA), superoxide dismutase (SOD) activity and glutathione (GSH) level and increased nitric oxide (NO) production, γ glutamyl transferase (γ GT), glutamate oxaloacetate transaminase (AST), glutamate pyruvate transaminase (ALT) levels. Daily Oral administration of 80 mg/kg *curcuma longa* powder for four weeks prior to CCl_4 injection alleviated CCl_4-suppressive effect on SOD activity and GSH level and prevented CCl_4-induced NO production, γ GT, AST, ALT levels and became nearly to normal. Histopathological examination of liver tissues in the group treated with *curcuma longa* powder prior to and after CCl_4 injection showed mild degenerative changes of the hepatocytes and hepatic cells regeneration respectively, however, no multifocal necrosis was observed. Moreover, injection of rats with CCl_4 caused a significant reduction of RBCs count, PCV%, HB content and WBCs count, while oral administration of curcumin before CCl_4 injection reduced the suppressive effect of CCl_4 on RBCs count, PCV%, HB content and WBCs count. These results indicated that *curcuma longa* powder administration has a protective effect against the CCl_4-mediated hepatotoxicity and endotoxemia through down regulation of reactive oxygen species (ROS) and NO production and up-regulation of the antioxidant factors mainly GSH and SOD.

Key words: Carbon tetrachloride (CCl_4) · *Curcuma Longa* · Superoxide dismutase (SOD) · Reduced glutathione (GSH) · γ glutamyl transferase (γ GT) · Glutamate oxaloacetate transaminase (AST) · Glutamate pyruvate transaminase (ALT)

INTRODUCTION

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia and needs temperatures between 20 and 30°C and a considerable amount of annual rainfall. The *Curcuma Longa* extract is a yellow-orange polyphenol and its usual form is a dry yellow powder that is oil-soluble in its natural state. The extract is without flavor and aroma. *Curcuma Longa* extract exhibits strong antioxidant and antifibrotic affect [1]. Current traditional Indian medicine claims the use of *Curcuma Longa* L powder against biliary disease, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis [2]. Curcumin was reported to has antiinfilammatory [3], anti-immunodeficiency virus [4] and, antibacterial [5] effects. Curcumin has also been shown to inhibit hydrogen peroxide induced cell damage [6] and reduces the oxidative stress induced by ethanol and protects the liver cell *in vitro* [7]. Curcumin was reported to reverse the alteration of immunity in rat subjected to chronic mild stress such as increased serum IL6, tumor necrosis factor and reduction of natural killer cells activity in splenocytes [8].

Liver diseases constitute a major problem of world wide proportions. CCl_4 is a well known hepatotoxin that is widely used to induce acute toxic liver injury in a large range of laboratory animals. Acute hepatotoxicity occur through metabolic activation of CCl_4 to highly reactive

Corresponding Author: A.M. Elkattawy, Department of Biochemistry, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt, E-mail: elkattawyeg @yahoo.com.

substances such as reactive metabolites which induce lipid peroxidation, believed to be one of the major causes of cell membrane damage leading to a number of pathological situations [9, 10].

The liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds. Management of liver diseases is still a challenge to the modern scientific community [11]. There are few conventional drugs that can stimulate liver function and offer hepato-protection or help in the regeneration of hepatic cells [12]. Many plant-derived natural products have the potential to be hepatoprotective and therefore can be used to treat acute and chronic liver diseases. The challenge is to identify the most promising compounds and evaluate their protective mechanism [13].

The aim of this study was to investigate the ability of curcumin (a phytopolyphenol pigment isolated from the plant *curcuma longa*) to protect and improve the alterations due to CCl_4-hepatotoxicity in rats.

MATERIALS AND METHODS

Animals: 60 male Wistar albino rats (100 - 120g) were used in the present study. The animals were acclimatized for one week before the beginning of the experiment and were fed with standard animal feed and water *ad* libitum and divided into six groups of 10 rats each.

Group 1 (G1): (control) was given 1ml of corn oil per os daily for four weeks.

Group 2 (G2): Was given curcumin only in a dose 80 mg/kg per os dissolved in corn oil daily for four weeks.

Group 3 (G3): Was given curcumin in a dose 80 mg/kg per os dissolved in corn oil daily for four weeks thereafter injected intra-peritoneally with 0.4 ml/kg CCl_4 as a single dose 48 hours before the end of experiment .

Group 4 (G4): Was injected intra-peritoneally with 0.4 ml/kg CCl_4 as a single dose at the first day of experiment and was given curcumin from the second day of experiment in a dose 80 mg/kg per os dissolved in corn oil daily till the end of four weeks.

Group 5 (G5): Was injected with intra-peritoneally with 0.4 ml/kg CCl_4 as a single dose at the first day of experiment and given 1ml of corn oil per os daily .

Group 6 (G6): This group was given 1ml of corn oil per os daily and injected intra-peritoneally with 0.4 ml/kg CCl_4 as a single dose 48 hours before the end of experiment

Sampling and Biochemical Analysis: Samples of the whole blood were collected from each animal via the retro-orbital venous plexus after fasting for 12 hours (h). The first blood sample was collected on heparin for estimation of superoxide dismutase (SOD) activity [14] and reduced glutathione (GSH) level [15]. The second sample was collected in centrifuge tube and left to coagulate then centrifuged at 3,000 r.p.m. for 15 minutes. The collected sera were used for biochemical analysis of γ-Glutamyl transferase (γ-GT) [16], Glutamic-oxaloacetic transaminase (AST) and Glutamic -pyruvic transaminase (ALT) [17], Also, nitric oxide [18] and α-L- Fucosidase tumour marker [19] were measured. Immunoglobulins (IgG,IgM and IgA) [20], the red blood cells count, white blood cells count, packed cell volume (PCV %) and blood indices [21] and the hemoglobin contents [22] were determined.

Histopathological Examination: For microscopic evaluation, livers were fixed in 10% neutral phosphate-buffered formalin solution. Following dehydration in ascending series of ethanol (70-100%), tissue samples were cleared in xylene and embedded in paraffin and tissue section of 5 μm were stained with haematoxyline and eosin (H&E), for histopathological examination [23].

Statistical Analysis: Values expressed as mean ± S.E. were compared using ANOVA test. Differences were considered significant when $P \leq 0.05$. The obtained data were analyzed by one way ANOVA test using SAS computer program [24].

RESULTS

Effect of Curcumin on Oxidant and Anti-oxidant Biomarkers: As summarized in table 1, results indicate a significant ($P \leq 0.05$) decrease in the activities of antioxidant enzyme (SOD) as well as GSH level and a significant ($P \leq 0.05$) increase in the oxidant nitric oxide in CCl_4 injected groups (G5, G6) as compared to that seen in G1 and G2. The daily oral administration of rats with curcumin 4 weeks before and after CCl_4 injection alleviated the CCl_4-induced suppressive effect on antioxidant enzymes and decreased the CCl_4-induced increase in nitric oxide in G3 and G4 as compared to that seen in (G1, G2).

Table 1: Oxidant and Antioxidant biomarkers in hepatotoxic rats induced by CCl4 and treated with Curcumin.

Groups Criteria	G1 Control	G2 Curcumin (80mg/kg.)	G3Curcumin (80mg/kg.) ThenCCl4 (0.4ml /kg) before collection 48h	G4 CCl4 (0.4ml /kg) 1st day then Curcumin (80mg/kg.)	G5 CCl4 (0.4ml /kg) 1st day	G6 CCl4 (0.4ml /kg) before collection 48h
Superoxid dismutase (SOD)(ug/dl)	91.356^{a}± 2.379	91.978^{a}± 1.586	83.398^{b}± 1.025	73.370^{c}± 2.519	64.840^{d}± 2.779	67.440^{cd}± 2.561
Reduced glutathione (GSH)(mg/dl	89.156^{a}±0.589	90.178^{a}±0.721	84.798^{b}± 0.618	81.37^{c}± 1.335	71.240^{e}±0.976	78.440^{d}±1.749
Nitric oxide (NO) (µmol/L)	28.356^{e}±0.760	27.778^{e}±0.619	35.798^{d}±1.122	38.370^{c}±1.190	44.280^{a}±0.780	41. 640^{b}±0.534

Values which have different letters are significantly different from each other at P≤ 0.05

Table 2: Liver function test and a-L-fucosidase biomarkers in hepatotoxic rats induced by CCl4 and treated with Curcumin.

Groups Criteria	G1 Control	G2 Curcumin (80mg/kg.)	G3Curcumin (80mg/kg.) ThenCCl4 (0.4ml /kg) before collection 48h	G4 CCl4 (0.4ml /kg) 1st day then Curcumin (80mg/kg.)	G5 CCl4 (0.4ml /kg) 1st day	G6 CCl4 (0.4ml /kg) before collection 48h
γglutamyl transferase (γGT) (U/L)	28.356^{e} ±0.760	27.778^{e}±0.619	35. 798^{d}±1.122	38.370^{c}± 1.190	44.280^{a}±0.780	41.640^{b}±0.534
Glutamate oxalo-acetate transaminase (AST), (U/ml)	33.200^{c}± 2.200	32.600^{c}±2.293	41. 400^{b}± 1.630	45.200^{ab}± 2.311	50.200^{a}± 2.107	48.200^{a}± 2.107
Glutamate pyruvate transaminase (ALT) (U/ml)	39.200^{e}±0.860	38.200^{e}±0.969	44.600^{d}±0.509	49.200^{c}±0.583	54.400^{a}±0.509	51.400^{b}±0.748
a-L-fucosidase(U/L)	0.992^{a}±0.118	0.972^{a}±0.126	1.082^{a}±0.189	1.096^{a}±0.194	1.170^{a}±0.203	1.090^{a}±0.200

Values which have different letters are significantly different from each other at P≤ 0.05

Table 3: Total protein and Immunoglobulin biomarkers in hepatotoxic rats induced by CCl4 and treated with Curcumin.

Groups Criteria	G1 Control	G2 Curcumin (80mg/kg.)	G3Curcumin (80mg/kg.) ThenCCl4 (0.4ml /kg) before collection 48h	G4 CCl4 (0.4ml /kg) 1st day then Curcumin (80mg/kg.)	G5 CCl4 (0.4ml /kg) 1st day	G6 CCl4 (0.4ml /kg) before collection 48h
Immunoglobulin (IgG) mg/dl	740.346^{a} ±17.126	730.252^{a}±19.447	612.356^{b}± 43.950	602.334^{b}± 46.052	574.114^{b}± 43.718	593.280^{b}±48.947
Immunoglobulin (IgM) (mg/dl)	177.868^{a}±7.601	178.828^{a}±7.504	163.504^{ab}±7.241	162.546^{ab}±7.405	153.436^{ab}±7.356	155.306 ^{b}±6.801
Immunoglobulin (IgA)(mg/dl)	43.004^{a}±0.930	42.6980^{a}±1.751	38.636^{ab}± 1.343	36.420^{b}± 2.481	30.640^{c}± 1.367	34.952^{bc}±0.002

Values which have different letters are significantly different from each other at P≤ 0.05

Table 4: The Blood picture in hepatotoxic rats induced by CCl4 and treated with Curcumin :

Groups Criteria	G1 Control	G2 Curcumin (80mg/kg.)	G3Curcumin (80mg/kg.) ThenCCl4 (0.4ml /kg) before collection 48h	G4 CCl4 (0.4ml /kg) 1st day then Curcumin (80mg/kg.)	G5 CCl4 (0.4ml /kg) 1st day	G6 CCl4 (0.4ml /kg) before collection 48h
RBCSmillion/UL	7.136^{a}±0.139	7.036^{a}±0.131	6.000^{b}±0.196	5.518^{c}±0.241	4.986^{cd}±0.122	5.074^{d}±0.103
PCV%	38.004^{a}±1.088	38.076^{a}± 0.989	34.058^{b}± 1.202	31.100^{bc}±1.493	28.420^{c}±0. 699	29.700^{c}±0.363
Hb(gm%)	12.850^{a}±0.355	12.834^{a}±0.340	10.324^{b}±0.174	9.880^{bc}±0.128	8.902^{d}±0.231	9.404^{cd}±0.208
MCV(cubic micron)	53.150^{a}±2.227	54.226^{a}±1.118	57.082^{a}±2.964	56.456^{a}±1.859	56.904^{a}±2.564	58.636^{a}±1.418
MCH(pico gram)	17.838^{a}±0.397	18.282^{a}±0.698	17.292^{a}±0.715	18.046^{a}±0.842	17.916^{a}±0.717	18.560^{a}±0.478
MCHC%	33.950^{a}±1.483	33.842^{a}±1.554	30.496^{a}±1.368	31.998^{a}±1.268	31.442^{a}±1.378	31.690^{a}±0.878
WBCSthousand/UL	8.328^{a}±0.649	8.756^{a}±0.302	6.540^{b}±0.600	4.814^{c}±0.359	3.970^{c}±0.368	5.118^{c}±0.082

Values which have different letters are significantly different from each other at P≤ 0.05

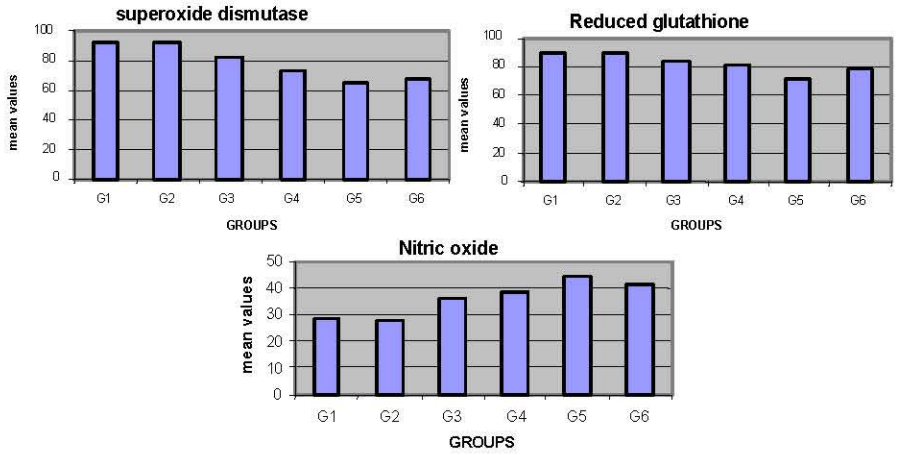

Fig. 1: Oxidant and Antioxidant biomarkers in hepatotoxic rats induced by CCl_4 and treated with Cureumin

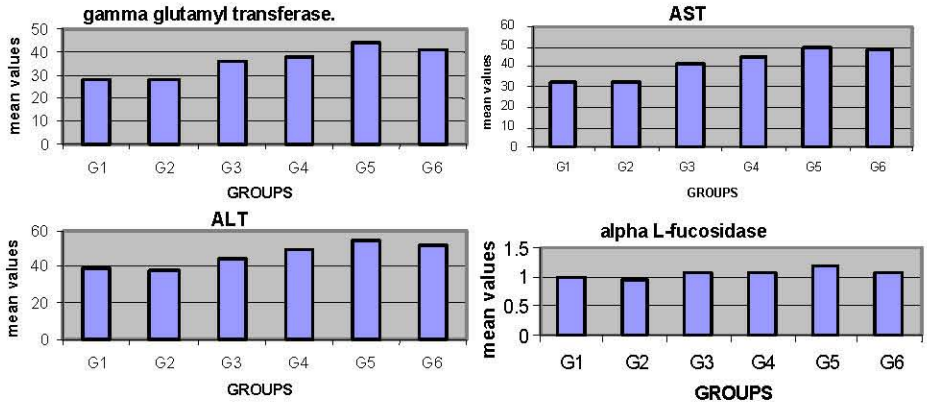

Fig. 2: Liver function test and α-L-fucosidase biomarkers in hepatotoxic rats induced by CCl_4 and treated with Cureumin

Effect of Curcumin on Liver Function Test: As summarized in table 2 results showed that injection of rats with CCl_4 significantly ($P \leq 0.05$) increased γ GT, AST,ALT in (G5, G6) as compared to that seen in G1 and G2. While daily oral administration of curcumin prevented the increase in hepatic enzymes and tend to return the levels to normal (G3, G4) compared to that seen in G1 and G2. The result obtained in case of tumor marker α-L-fucosidase was non significant increase in G3, G4, G5 and, G6 as compared to that seen in G1 and G2

Effect of Curcumin on Total Protein and Immunoglobulin: As summarized in table 3, the obtained result showed that a significant ($P \leq 0.05$) decrease in total protein and immunoglobulins in G5 and G6 as compared to that seen in G1 and G2. When rats orally administered every day with curcumin 4 weeks before and after CCl_4 injection, curcumin alleviate the CCl_4-induced reduction of total protein and immunoglobulins in groups G3 and G4 towards normal as compared to that seen in G1 and G2.

Effect of Curcumin on Blood Parameters: As summarized in table 4, injection of rats with CCl_4caused a significant ($P \leq 0.05$) reduction of RBCs count, PCV%, HB content and WBCs count in G5 and G6 as compared to G1 and G2. Daily oral administration of curcumin 4 weeks before and after CCl_4 injection reduced the suppressive effect of CCl_4 on RBCs count, PCV%, HB content and WBCs count in G3 and G4 as compared to G1 and G2. MCV showed non significant increase in G3, G4, G5 and G6 as compared to that seen in G1 and G2. MCH and MCHC% showed non significant decrease in G3, G4, G5 and G6 as compared to that seen in G1 and G2.

Histopathological Findings: Oral administration of curcumin at a dose of 80 mg/k.g B.W daily for 4 weeks (G2) did not affect the parenchyma of the liver of rat comparing with control group (G1) in which the hepatocytes were intact and observed in fairly radial position in relation to the central vein (Fig. 4).

CCl_4 injection at a dose of 0.4 ml/kg B.W as a single dose by intraperitoneal route (G6) 48 hr before the end of

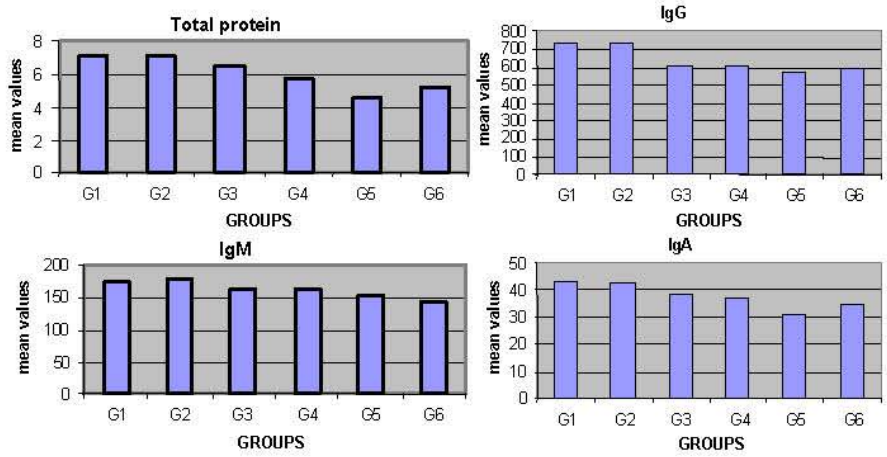

Fig. 3: Total protein and Immunoglobulin biomarkers in hepatotoxic rats induced by CC14 and treated with Cureumin

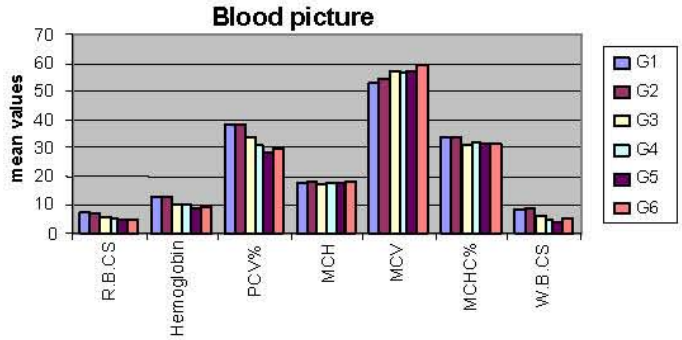

Fig. 4: The Blood picture in hepatotoxic rats induced by CC14 and treated with Cureumin

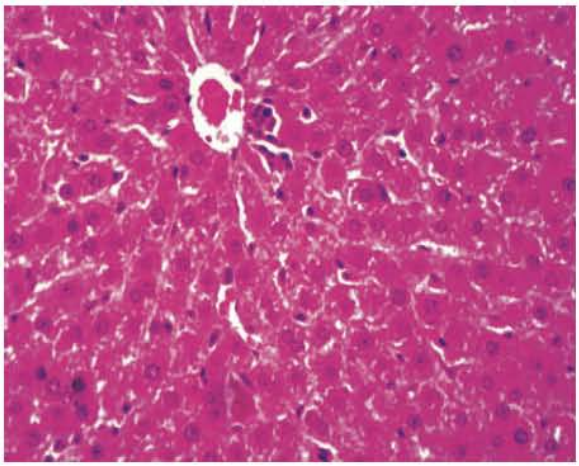

Fig. 4: Liver (G2), showing intact hepatocytes arranged in fairly radial position in relation to the central vein. HE, 200.

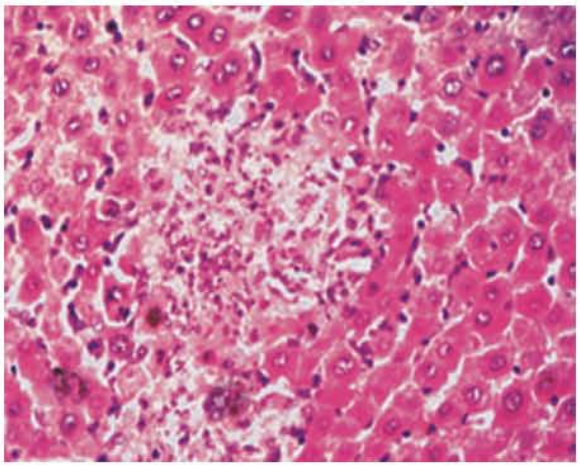

Fig. 5: Liver (G6), revealed big areas of necrosis more or less centrolobular in position and showing inflammatory cell reaction particularly at the periphery. HE, 200

experiment revealed big areas of necrosis more or less centrolobular in position and showing inflammatory cell reaction particularly at the periphery (Fig.5). Also, there was dilatation of blood sinusoids with marked activation of sinusoidal cells and single hepatic cell necrosis which occasionally involved most of the cell cord (Fig. 6). Examination of liver of some animals in this group showed centrolobular big vacuoles in hepatic cells which revealed signet ring appearance of fatty change (Fig7).

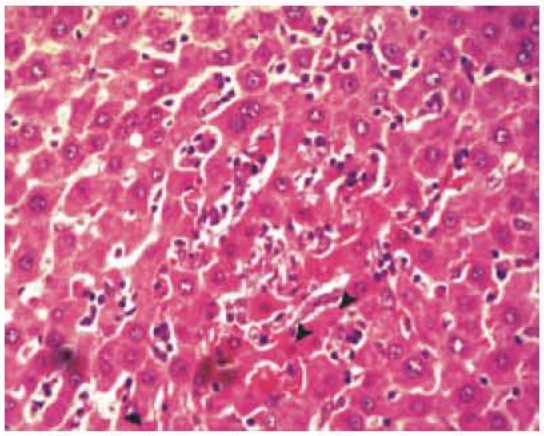

Fig. 6: Liver (G6), showing single hepatic cell necrosis and dilatation of blood sinusoids with marked activation of sinusoidal cells. HE, 200

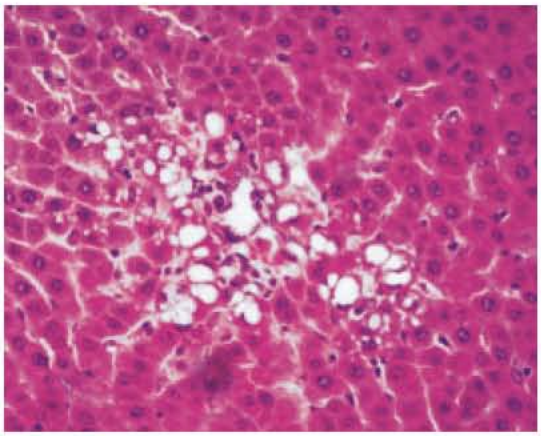

Fig. 7: Liver (G6), showing big vacuoles in hepatic cells compress the nuclei at the periphery of cells giving the appearance of signet ring appearance of fatty change. HE, 200

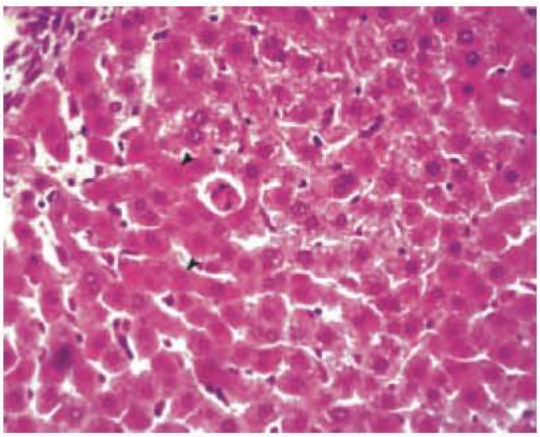

Fig. 8: Liver (G3), showing slight degenerative changes of the hepatice cells and single cell necrosis. HE, 200

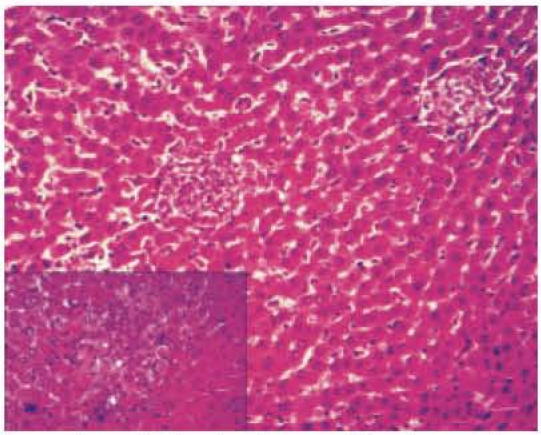

Fig. 9: Liver (G5), showing multifocal necrotic areas, which showed destructed hepatic cells with nuclear changes in the form of condensation of chromatin mass and fragmented nuclei. HE, 100. inset showing focal necrotic area with inflammatory cells reaction. HE, 200

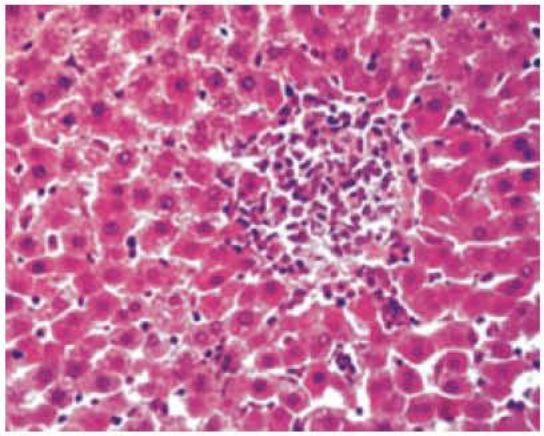

Fig. 10: Liver (G5), showing a necrosed area invaded and surrounded by inflammatory cellular reaction giving the picture of granuloma like lesion. HE, 200

Oral administration of curcumin at a dose of 80 mg /kg B.W daily for 4 weeks prior to CCl_4 injection (G3), showed slight degenerative changes of hepatic cells and the presence of single cell necrosis (Fig. 8).

Injection of 0.4 ml/kg B.W CCl_4 intra-peritoneal route as a single dose at the first day of the experiment showed multifocal necrotic area (Fig.9) that revealed destructed hepatic cells with nuclear changes in the form of condensation of chromatin mass and fragmented nuclei. In some cases, the necrotic areas were surrounded and invaded by inflammatory cellular reaction giving the picture of granuloma like lesion (Fig. 10).

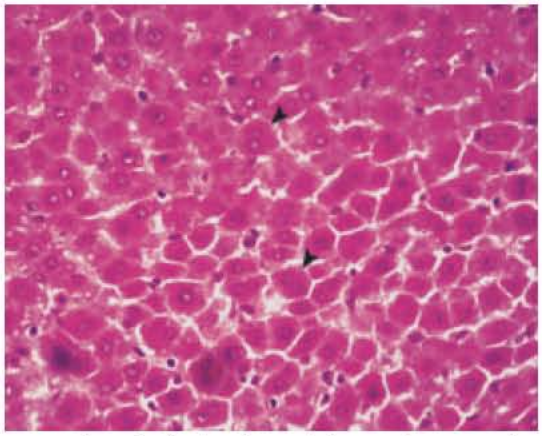

Fig. 11: Liver (G4), showing evidence of regeneration of hepatic cells which manifested by increased number of binucleated cells, slight cytomegally of hepatocytes with cloudy cytoplasm, hyperchromatic nuclei with haphazardly arranged hepatocytes. HE, 200

When rats were administered Curcumin daily for 4 weeks after CCl_4 injection (G4), microscopic examination of the liver revealed slight alteration in hepatic cells as in group 3. In some cases, liver showed evidence of regeneration of hepatic cells which manifested by increased number of binucleated cells, evidence of slight cytomegally of hepatocytes with cloudy cytoplasm, hyperchromatic nuclei with haphazardly arranged hepatocytes (Fig.11).

DISCUSSION

The present study investigated the effects of supplementation with curcumin on hepatic antioxidant status in rats with CCl_4-induced liver injury. The free radicals, from both endogenous and exogenous sources, are implicated in the etiology of several degenerative diseases such as coronary artery disease, stroke, rheumatoid arthritis, diabetes and cancer [25]. CCl_4 hepatotoxicity depends on its biotransformation by the cytochrome P-450 system mainly CYP2E1 and CYP2B into two free radicals. The first, trichloromethyl free radical (CCl_3) is formed from the metabolic conversion of CCl_4 [26] and reacts very rapidly with O_2 to forms a second metabolite, trichloromethyl peroxy free radical (CCl_3OO^-) or abstract hydrogen atoms to form chloroform [27]. The previously mentioned free radicals initiate the peroxidation of membrane poly-unsaturated fatty acids [28]. The lipid peroxidation process results in the generation of ROS like the superoxide anion O^-_2, H_2O_2 and the hydroxyl radical, OH^-. ROS affect the antioxidant defense mechanisms, decrease the intracellular concentration of reduced glutathione (GSH) and reduces the activity of SOD [29].

The results of the present study showed that the activities of antioxidant enzyme SOD and GSH level were significantly decreased in the rats of groups G5, G6 injected with CCl_4 as a single dose. This decrease of the antioxidant enzyme SOD and GSH may be attributed to the exhaustion of these antioxidant factors in a trial to scavenge excesses production of ROS caused by the toxic effect of CCl_4 [30]. The reduction of the antioxidant factors can be also due to decreasing the hepatocytes ability to produces these antioxidant factors due to cell damages. This is confirmed by histopathological finding of the liver in these groups (G5, G6) which showed significant hepatic damage in the form of multifocal necrotic areas. The necrotic areas were surrounded and invaded by inflammatory cells. This was stated to be due to increase of chemokines production [31] which leads to aggregations of mono nuclear inflammatory cells and activation of sinusoidal cells in the liver in present study. Moreover; histopathological examination of the liver showed fatty change. This result is in accordance with that reported by Hsu *et al* [32] who reported that administration of CCl_4 to rats resulted in acute hepatic necrosis and fatty changes with foamy degeneration. This fatty changes may be caused by over production of ROS mainly NO [33]. CCl_4 administration increases the iNOS mRNA expression and hence NO production. Inducible NO synthase generated NO not only directly contributes to tissue damage but also up-regulates the inflammatory response [34]. The protective effect of curcumin against CCl_4-induced tissue damage may be attributed to its ability to suppress NO production. Chan *et al* [35] reported that *in vivo* oral treatment of curcumin reduces iNOS mRNA expression in the liver of lipopolysaccharide (LPS)-injected mice by 50%-70%.

In this study, CCl_4 injection to rats resulted in significant increased levels of γ-GT and amino transferase enzymes (AST and ALT), this in agreement with Hewawasam *et al* [36] who reported that this significant increase due to CCl_4 cause hepatic damage. α-L fucosidase (α-Lf) is a lyzosomal enzyme present in all mammalian cells that rises in primary hepatocellular carcinoma at early stage [37] but our result there was a non significant increase in α-L fucosidase (α-Lf) owing to the short duration in exposure to CCl_4 toxicity .

It is worth stating that in group of rats which received curcumin only, the liver parenchyma was normal and the biochemical and physiological parameter were normal in agreement with a study by Prakash *et al* [38]

who reported a normal histological appearance of liver of rats after administration of curcumin per os daily at a dose of 100mg /kg B.W for successive 30 days. In this study, daily oral administration of rats with curcumin 4 weeks prior CCl_4 injection alleviated the Ccl_4 -induced suppressive effect on antioxidant enzyme and decrease the CCl_4 -induced increase in nitric oxide. This ability of curcumin to protect the liver from inflammatory condition especially if given before the exposure of the liver to such oxidant agent may be due to its anti-inflammatory effect through inhibition of expression of cyclo-oxygenase-2 [39]. The protective effect of curcumin against CCl_4-induced hepatotoxicity may be related to its ability to elevate the antioxidant agents in the body. It was stated that many activities of curcumin can be explained by its ability to suppress acute and chronic inflammation by scavenging reactive oxygen species and nitrogen oxide and enhancing antioxidant defense by increasing reduced glutathione level [40]. In present study, curcumin administration prior to CCl_4 prevented the CCl_4-suppressive effect on the levels of antioxidant agents SOD and GSH and normalized the CCl_4-induced levels of NO, γGT, AST and ALT. The protective effect of curcumin is also proved by histopathological examination of livers of rats administered curcumin prior to CCl_4 adminstration which were almost normal in structure with slight changes. This protection may be due to effective blocking of oxidative stress and cytokines production. It was reported that pretreatment of curcumin protected against lipopoly saccharide (LPS) induced liver damage through decreasing the level of TNF-α and IL6 and prevented cytotoxic effect of oxygen free radicals and cytokines [41]. The protective effect of curcumin may be through its ability to attenuate the CCl_4-induced oxidative stress in accordance with a previous report by Fu *et al* [42]. that curcumin attenuated oxidative stress and suppressed inflammation.

To investigate the ability of curcumin to treat CCl_4 toxicity and to help the regeneration of damaged liver cells a group of rates, were given daily oral administration of curcumin for 4 weeks after CCl_4 injection. The results showed that curcumin tended to alleviate the CCl_4-induced suppressive effect on the antioxidant enzymes in addition to promoting the regeneration of hepatic cells, as histological examination showed that liver of some rats showed evidence of regeneration. The results of the biochemical analysis were in the same direction as the curcumin oral administration kept the antioxidant enzymes and the liver enzymes ALT and AST near their normal levels. This may be attributed to its ability to stabilize the plasma membranes.

In this study, there was a significant decrease in the mean values of total protein, immunoglobulins (IgG, IgM and IgA) in CCl_4 treated groups, while in the groups treated with curcumin the values returned nearly to the normal. This is in accordance with what reported by Prakash *et al.* [43] about the ability of curcumine for inducing choleretic hepato protection. This explained by Jagetia, and Aggarwal, [44] stated that curcumin has been shown in the last two decades to be a potent immunomodulatory agent The significant decrease in erythrocytic count (RBCs), hemoglobin, packed cell volume and WBCS could be attributed to CCl_4 toxicity. These features of anemia disappeared in the treated groups with curcumin and the blood parameter values returned nearly to the normal levels this in accordance with Vachharajani *et al.* [45] that curcumin, an active ingredient of turmeric and an anti-inflammatory agent, could disrupt interactions between circulating blood cells and endothelium and improvement its survival.

In conclusion, the results presented above showed that dietary administration of curcumin to rats can counteract the hepatic injury induced by CCl_4 injection. It seems that curcumin directly affects major targets, just as ROS scavenging and induction of antioxidant mechanism in the body in addition to its hepatoprotective effect. As the pathogenesis of many diseases is believed to be related to reactive oxygen species and nitrogen oxide overproduction in the body, curcumin may be recommended to be added to human food being especially for those whom are at risk of development of some ROS which induced diseases as cancer, Alzheimer, parkinsonism and aging.

REFERENCES

1. Commandeur, J.N. and N.P. Vermeulen, 1996. Cytotoxicity and cytoprotective activities of natural compounds. The case of curcumin. Xenobiotica, 26: 667-680.
2. Ammon, H.P. and M.A. wath, 1991. Pharmacology of curcuma longa. Planta Medica, 57: 1-7.
3. Joe, B. and B.R. Lokesh, 1997. Effect of curcumine and capsaicin on Arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages. Lipids, 32: 1173-1180.
4. Taher, M.M., G. Lammering, C. Hershey and K. Valerie, 2003. Curcumine inhibits ultraviolet light induced human immunodeficiency virus gene expression. Molecular and Cellular Biochemistry, 254: 289-297.

5. Pal, A. and A.K. Pal, 2000. Studies on the genotoxicity of turmeric extracts in bacterial system. International J. Antimicrobial Agents, 16: 415-417.
6. Biswas, S.K., D. Mcclure, L.A. Megson and I. Rahman, 2005. Curcumine induces glutathione biosynthesis inhibits NF- kappa B activation and interleukin 8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxide Redox Signal, 7: 32-41.
7. Naik, R.S., A.M. Mujumdor and S. Ghaskadbi, 2004. Protection of liver cells from ethanol cytotoxicity by curcumine in liver slice culture in vitro. J. Ethnopharmacol., 95: 31-37.
8. Xia, X., Y. Pan, W. Zhang, G. Chang and L. Kong, 2006. Ethanolic extracts from curcuma longa attenuates behavioral, immune and neuroendocrine alterations in a rat chronic mild stress modle. Biological and Pharmaceutical Bulletin, 29: 938-944.
9. Halliwell, B., 1993. Oxygen species in pathology with special reference to the skin. In: oxidative stress in dermatology. Marcel Dekker Inc. New York, pp: 3-11.
10. Ramadori, G. and T.S. Armbrust, 2001. Cytokines in the liver. European journal of gastroenterology and Hepatol., 13: 777-784.
11. Sunilson, J.A.J., M. Muthappan, A. Das and R. Suraj, 2009. Hepatoprotective activity of Coccinia grandis leaves against carbon tetrachloride induced hepatic injury in rats. International J. Pharmacol., 5: 222 - 227.
12. Guntupalli, M., V. Chandana, P. Pushpangadan and I.A. Shirwaikar, 2006. Hepatoprotective effect of rubiadin, a major constituent of Rubia cordifolia Linn. J. Ethnopharmacol.,103: 484-490.
13. Jaeschke, H., C.D. Williams, M.R. McGill and A. Farhood, 2010. Herbal extracts as hepatoprotectants against acetaminophen hepatotoxicity. World J. Gastroenterl., 16: 2448-2450.
14. Nishikimi, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications, 46: 849-854.
15. Beutler, E., O. Duron and M.B. Kelly, 1963. Colorimetric method for determination of reduced glutathione. J. laboratory and Clinical Med., 61: 882-890.
16. Szasz, G., 1969. A kinetic photometric method for serum γ glutamyl transferase. Clinical Chemistry, 15: 124-136.
17. Reitman, S. and S. Frankel, 1957. A colorimetric method for determination of serum glutamic oxaloacetate and glutamic pyruvate transaminase. American J. Clinical Pathol., 28: 56-63.
18. Montgomery, H.A.C. and J.F. Dymock, 1961. Determination of nitric oxide. Analyst, 86: 41-41.
19. Zielke, K., S. Okada and JS. O'Brien, 1972. Fucosidosis: diagnosis by serum assay of alpha-L-fucosidase. J . Laboratory and Clinical Med., 79: 164-169
20. Erhard, M.H., I. Von Quistorp, I. Schranner, A. Jüngling, B. K aspers, P. Schmidt and R. Kühlmann 1992. Development of specific enzyme-linked immunosorbent antibody assay systems for the detection of chicken immunoglobulins G, M and A using monoclonal antibodies. Poultry Sci., 71: 302-310.
21. Miller, S.E. and J.M. Weller, 1971. Text book of clinical pathology 8th edition the Williams and Wilking Company Boltimore. pp: 45. Zimmerman HJ.
22. Drabkin, D.L., 1949. Standardization of haemoglobin measurements. American J. the Medical Sci., 217: 710-711.
23. Culling, C.F.A., 1983. Handbook of histopathologic and histochemical technique, 3rd edition. Butter Worth London, Boston: 214.
24. Senedecor, G.W. and P.W. Cochran, 1989. Statistical methods, 8th ed. Iowa State University, Press, Ames, pp: 158-160.
25. Halliwell, B., J.M. Gutteridge and C.E. Cross, 1992. Free radicals, antioxidants and human disease: Where are we now?. J. Laboratory and Clinical Med., 119: 598-620.
26. Noguchi, T., K.L. Fong, E.K. Lai, S.S. Alexander, M.M. King, L. Olson, J.L. Poyer and P.B. McCay, 1982. Specificity of a phenobarbital-induced cytochrome P-450 for metabolism of carbon tetrachloride to the trichloromethyl radical. Biochemical Pharmacol., 31: 615-624.
27. Packer, J.E., T.F. Slater and R.L. Willson, 1978. Reactions of the carbon tetrachlonderelated peroxy free radical ($CC1_3O_2$) with amino acids: pulse radiolysis evidence. Life Sci., 23: 2617-2620.
28. Tom, W.M., L.Y. Fong, D.Y. Woo, V. Prasongwatana and T.R. Boyde, 1984. Microsomal lipid peroxidation and oxidative metabolism in rat liver: influence of vitamin A intake. Chemico- Biological Interactions, 50: 361-366.

29. Srilaxmi, P., G.R. Sareddy, P.B. Kishor, O.H. Setty and P.P. Babu, 2010. Protective efficacy of natansnin, a dibenzoyl glycoside from Salvinia natans against CCl4 induced oxidative stress and cellular degeneration in rat liver. BMC Pharmacol.,10: 1-13.
30. Burk, R.F., J.M. Lane and K. Patal, 2008. Relationship of oxygen and glutathione in protection against CCl_4 induced hepatic microsomal lipid peroxidation and covalent binding in the rat. J. Clinical Investigation, 74: 1996- 2001.
31. Gordillo, K.R., S. Jose, S. Mineko, T. Victor, V. Paula, G. M. Mario and M. Pablo, 2007. Curcumin prevents and reverses cirrhosis induced by bile duct obstruction or CCl_4 in rats: role of TGF-b modulation and oxidative stress. Fundamental and Clinical Pharmacol., 22: 417-427.
32. Hsu, Y.W., C.F. Tsai, W.K. Chen and F.J. Lu, 2009. Protective effect of seabuckthorn (Hippophaerhamnoidesl.) seed oil against CCl4 induced hepato toxicity in mice. Food and chemical Toxicol., 47: 2281-2288.
33. Zein, S., Ibrahim, Ishizuka Mayumi, Soliman Mohamed, Elbohi Khlood, Sobhy Wageh, Muzandu Kaampwe, Azza M. Elkattawy, Q. Sakamoto Kentaro and Fujita Shoichi, 2008. Protection by Nigella sativa against carbon tetrachloride-induced downregulation of hepatic cytochrome P450 isozymes in rats. Japanese Journal of Veterinary Res., 56: 119-128.
34. Harbrecht, B.G., B. Wu, S.C. Watkins, H.P. Jr. Marshall, A.B. Peitzman and T.R. Billiar, 1995. Inhibition of nitric oxide synthase during hemorrhagic shock increases hepatic injury. Shock, 4: 332-337.
35. Chan, M.M., H.I. Huang, M.R Fenton and D. Fong, 1998. In vivo inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. Biochemical Pharmacolol., 55: 1955-1962.
36. Hewawasam, R.P., K.A.P.W. Jayatilaka, C. Pthinrana and L.K.B. Mudduwa, 2004. Hepatoprotective effect of Epaltes divaricatta extract on tetrachloride induced hepatotoxicity in mice. Indian J. Medical Res., 120: 30-34.
37. Chao, L.i. J.i.e. Qian and Lin J.u-Sheng, 2006. Purification and characterization of a-L-fucosidase from human primary hepatocarcinoma tissue. World J. Gastroenterol., 12: 3770-3775.
38. Prakash, O., G. Singh and M. athur, 2008. Protective effect of herbal formula against CCl_4 -induced hepatotoxicity. International J. Pharmaceutics, 4: 282-286.
39. Shakibaei, M., T. John, G. Schulze-Tanzil, I. Lehmann and A. Mobasheri, 2007. Suppression of NF-kappaB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes: Implications for the treatment of osteoarthritis. Biochemical Pharmacol., 73: 1434-1445.
40. Sikora, E., G. Scapagnini and M. Barbagallo, 2010. Curcumin, inflammation, ageing and age-related diseases. Immunity and Ageing, 7: 1-4.
41. Kaur, G., N. Tirkey, S. Bharrhan, V. Chanans and K. Chopra, 2006. Inhibition of oxidative stress and cytokine activity by curcumin in amelioration endotoxin induced experimental hepatotoxicity in rodent. Clinical and experimental Immunol., 145: 313-321.
42. Fu, Y., S. Zheng, J. Lin, J. Ryerse and A. Chen, 2008. Curcumin protects the rat liver from CCl_4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Molecular Pharmacol., 73: 399-409.
43. Prakash, V.B. and A. Mukherjee, 2010. Hepato-protective Effect of an Ayurvedic Formulation Prak-20 in CCl4 Induced Toxicity in Rats: Results of Three Studies. International J. Pharmaceutical and Clinical Res., 2: 23-27.
44. Jagetia, G.C. and B.B. Aggarwal, 2007. "Spicing up" of the immune system by curcumin. J. Clinical Immunol., 27: 9-35.
45. Vachharajani, V., S.W. Wang, N. Mishra, M. El Gazzar, B. Yoza and C. McCall, 2010. Curcumin modulates leukocyte and platelet adhesion in murine sepsis. Microcirculation, 17: 407-416.