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Biochemical Investigations on the Protective Role of Curcumin in Liver Damage by Chloroquine

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ABSTRACT

The present study was undertaken to evaluate the protective effect of curcumin on hepatic biochemical status of CQ-induced Wistar rat. It has been shown that CQ administration brought about a significant changes in antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHpx) activities, whereas alanine aminotransferase (ALT) activity was found to be significantly increased following CQ treatment. Lipid Per Oxidation (LPO) was found to be elevated in a significant manner in the CQ treated group as compared to control. Serum values of ALT were utilized to evaluate liver injury. The CQ administration increased serum values of ALT about 3 fold compared to those in CON rats, while pretreatment with curcumin significantly inhibit the increase of this enzyme induced by CQ. Significant decrease in activities of Antioxidant enzymes (CAT, SOD and GSHpx) was observed in liver homogenates treated with CQ only after getting pretreatment with curcumin these activities were normal. Results also showed that liver homogenates of rat treated with CQ contained higher level of MDA as well as protein carbonyl contents when compared with control values. These concentration levels decreased significantly in the group that received CQ with curcumin.

Key words: Chloroquine phosphate, curcumin, hepatic toxicity, protective effect

INTRODUCTION

Chloroquine (4-aminoquinoline) (CQ) is used to treat malaria and a variety of inflammatory diseases including systemic lupus erythematosus and rheumatoid arthritis (Borba *et al.*, 2004). Despite much studies during the last 40 years, the exact mechanism by which chloroquine kills the malaria parasite remains controversial (Foley and Tilly, 1997; Foote and Cowman, 1994; Peters, 1998). The drug chloroquine inhibits RNA and DNA biosynthesis and produces rapid degradation of ribosomes and dissimilation of ribosomal RNA. Inhibition of protein synthesis is also observed, evidently as a secondary effect. Inhibition of DNA replication is proposed as a general mechanism of the antimicrobial action of chloroquine. Chloroquine accumulates in very high concentrations in the parasite food vacuole.

The liver regulates many important metabolic oxidative stresses and causes oxidative disorders in the cells function (Romanelli *et al.*, 2004). Hepatic injury is associated with distortion of these metabolic functions specially these xenobiotics (Nasreddine and Beydoun, 2007; Santos *et al.*, 2008).

The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailment. Drug/chemical-mediated hepatic injury is the most common manifestation of drug toxicity (Ahmed and Siddiqi, 2006). Curcumin was proved by others that changes and pathogenesis that may potentially produce exhibits a protective effects against oxidative damage (Eybl *et al.*, 2008). In addition to its wide range of pharmacological properties that include antioxidant, anti-inflammatory and anti-cancer effects (Ramsewak *et al.*, 2000; Miquel *et al.*, 2002), curcumin is

known to protect liver against the toxic effects of agents like galactosamine, CCl₄, acetaminophen and pentobarbitone (Donatus *et al.*, 1990). There is evidence that curcumin enhances liver detoxification by increasing the activity of glutathione-S-transferase, an enzyme which conjugates glutathione with a wide variety of toxins to facilitate their removal from body (Piper *et al.*, 1998). The aim of present study was to investigate the extent of hepatotoxicity of chloroquine and protective role of curcumin.

MATERIALS AND METHODS

This study was conducted in Parasitology lab of Department of Zoology, College of Science, King Saud University, during October, 2014. Chloroquine phosphate (99.3% Pure) and other chemicals were obtained from Sigma Aldrich (UK). Curcumin was purchased from local chemical supplier. The experimental protocol was approved by the local animal experiment ethics committee

Toxicological studies

Animals: Twenty adult male wistar rats weighing between 100 and 150 g and 7-8 weeks old were selected for this study. The animals were kept in well-ventilated wire mesh cages measuring 35×25×18 cm exposed to a 12 h light cycle in an air-conditioned atmosphere at a temperature of 26±2°C and provided with food and water.

Experimental protocol: Four groups of animals were made. Group I marked as the vehicle control (CON), Group II marked as the chloroquine group (CQ) supplemented with standard dose of chloroquine, 200 mg kg⁻¹ body weight per day orally for 14 days and then sacrificed. The third group marked as curcumin control group (CUR) for which curcumin was given orally with 300 mg kg⁻¹ body weight per day for 14 days then killed. The fourth group was marked as (CURCQ) was given curcumin 300 mg kg⁻¹ body weight per day for 14 days then supplied with chloroquine 200 mg kg⁻¹ body weight per day orally for further 14 days and then sacrificed. The drugs and curcumin was given by oral gavage.

Sacrification schedule and collection of blood: Rats were sacrificed by cervical dislocation; livers were removed washed with in Ice-cold saline and blood were collected by cardiac puncture. Blood was centrifuged (10 min, 4500 rpm) and serum was separated. Livers were homogenized in chilled

0.1 M Tris- HCl buffer (pH 7.4) using ultra homogenizer. Homogenate was centrifuged at 10,000 rpm for 15 min and supernatants were then centrifuged at 10,000 rpm for 40 min. Supernatants was obtained and protein estimation was done by using micro biuret method described by Itzaki and Gill (1964) and aliquoted were for the determination of enzymatic activities and lipid peroxidation as malondialdehyde (MDA) from thiobarbituric acid reaction in livers homogenates (Al-Jassabi and Khalil, 2006).

Heparinized blood samples centrifuged and plasma removed. Blood Urea Nitrogen (BUN) determined by Chang and Abbott (2006), creatinine by Amacher (2002), Gamma-glutamyltransferase by Kaplowitz (2005), catalase (CAT) by Scott and Harrington (1990) and superoxide dismutase were determined (Stief, 2003). The GSH was determined in 10,000 rpm supernatant fraction of homogenate by Flohe and Gunzler (1984). The extent of DNA-protein cross-links were assayed by the method of Carmichael (Collins *et al.*, 1996).

Statistical analysis: All results were expressed as the Mean±SE. One way analysis of variance (ANOVA) was used to determine the significance of differences between the groups. Statistical significance was declared when p-value was equal or less than 0.05.

RESULTS

Serum biochemistry: Serum values of ALT were utilized to evaluate liver injury. CQ administration increased serum values of ALT about 2 fold compared to those in CON rats, while pretreatment with curcumin significantly inhibit the increase of this enzyme induced by CQ (Table 1).

Liver ROS and antioxidant enzymes: Significant decrease in activities of Antioxidant enzymes (CAT, SOD and GSHpx) was observed in liver homogenates treated with CQ only, after getting pretreatment with curcumin these activities were normal (Table 2). Results also showed that liver homogenates of rat treated with CQ contained higher level of MDA as well as protein carbonyl contents when compared with control values. The MDA is a product of oxidative damaged to lipids and in this study the concentration of MDA in liver is considered as biomarker of CQ toxicity. These concentration levels decreased significantly (p≤0.05) in the group that received CQ with curcumin.

Table 1: Serum biochemistry in control and following treatment with curcumin, chloroquine and chloroquine+curcumin (200 mg kg⁻¹ body weight/day) in rats

Serum biochemistry	Control	Curcumin	Chloroquine	Chloroquine+Curcumin
ALT (U L ⁻¹)	90.11±2.14	83.71±2.49	161.10±1.39	118.61±5.39
GGT (U L ⁻¹)	69.11±3.14	67.42±3.11	151.10±1.11	85.11±2.24
Creatinine (mg dL ⁻¹)	0.91±0.001	0.89±0.002	2.44±0.07	1.11±0.22
BUN (mg dL ⁻¹)	32.40±2.24	31.90±2.71	91.22±5.51	77.33±4.14

Data is expressed as Mean±SE

Table 2: Liver ROS, antioxidant and other parameters in control, curcumin, chloroquine and chloroquine+curcumin treated wistar rats

Liver ROS,	Control	Curcumin	Chloroquine	Chloroquine+ Curcumin
GSH ($\mu\text{M g}^{-1}$)	6.800 \pm 0.40	6.840 \pm 0.50	2.14 \pm 0.16	5.92 \pm 0.39
GSHpx ($\mu\text{M min}^{-1} \text{mg}^{-1}$)	0.880 \pm 0.003	0.900 \pm 0.005	0.19 \pm 0.001	0.77 \pm 0.002
LPO (μM)	0.101 \pm 0.11	0.095 \pm 0.06	5.52 \pm 1.34	0.94 \pm 0.12
PCC (μM)	2.800 \pm 0.10	2.090 \pm 0.09	39.43 \pm 2.96	5.54 \pm 0.12
DNA Protein cross link (%)	0.91	0.87	9.63	1.94
CAT ($\text{U mg}^{-1} \text{protein}$)	365.200 \pm 29.2	379.100 \pm 32.4	71.00 \pm 4.6	221.92 \pm 19.3
SOD ($\text{U mg}^{-1} \text{protein}$)	17.630 \pm 3.14	19.650 \pm 2.46	8.52 \pm 1.62	16.20 \pm 2.16

Data is expressed as Mean \pm SE

The percentage of DNA-protein cross link increased significantly in group treated with CQ by several folds and these value were comes to normal in CQ curcumin treated group.

DISCUSSION

Malaria is a disease that was once on the verge of eradication but has recently returned with greater vigor. This return calls for better preventive and curative treatments and for improved disease control methods. The widespread use of antimalarial drugs further demands the critical evaluation of drug toxicity and damage to tissues. Chloroquine was first synthesized in Germany but it was not recognized as a potent antimalarial drug until the 1940s during the US World War II military effort. By 1946, it was found to be far superior to other contemporary synthetic antimalarials (Coggeshall and Craige, 1949). Chloroquine became the cornerstone of antimalarial chemotherapy for the next 40 years. It quickly became the drug of choice globally to treat uncomplicated *P. falciparum* infections and it was used as part of the Global Malaria Eradication campaign. Chloroquine is one of the least expensive antimalarials available and is still in wide spread use. This drug can be taken both as a prophylactic and as a treatment. It has received much more attention on its toxicity (Soga *et al.*, 2004).

In the present study CQ induced hepatotoxicity was evidenced by measurements of biochemical parameter that coincide by other workers (Ding *et al.*, 2000; Zwanzger *et al.*, 2007; Reddy *et al.*, 2007; Nitti *et al.*, 2008). Now there is tendency to limit its clinical use due to adverse effects mainly hepatotoxicity (Abdel-Zaher *et al.*, 2008). The increased level of ALT reflects damage to hepatocytes (Komatsu *et al.*, 2002). In the present observations administration of hepatotoxic doses of CQ to rats resulted in the development of oxidative stress damage in hepatic tissues this was evident by increasing the degree of lipid peroxidation, inhibiting of enzymatic antioxidants and increasing the level of methylglyoxal in liver.

Salient feature of curcumin is that exhibits strong antioxidant activity, comparable to vitamin C and E and it was shown to be a potent scavenger of a variety of reactive oxygen species (Eybl *et al.*, 2008). In its capacity it also assists generating endogenous antioxidants such as vitamin C, vitamin E and GSH (Suresh and Srinivasan, 2007; Roberts *et al.*, 2008). Our results demonstrate that pretreatment

of rats with curcumin lower the hepatic damages. Reduction of GSH level induced by CQ in liver was inhibited by the treatment of rats with curcumin.

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