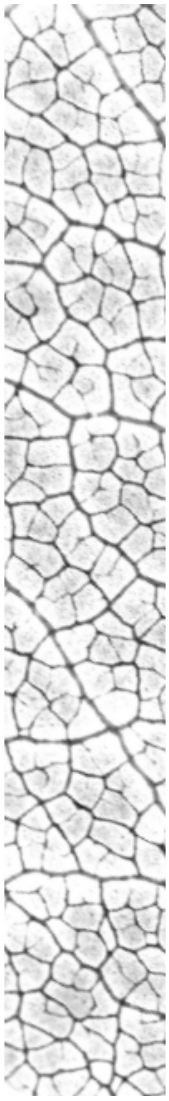

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Curcumin pretreated hepatoprotectivity against antimalarial drug chloroquine induced hepatotoxicity in albino rats

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Abstract
The present study was aimed to find out the protective effect of curcumin on hepatotoxicity resulting by commonly used antimalarial drug chloroquine (CQ). Albino rats were administered with CQ 200mg/Kg body wt. We observed statistically significant hepatotoxicity following CQ administration. We further observed a significant alterations in biochemical parameters such as total protein, aspartate transferase, alanine transaminase, superoxide dismutase and catalase on tested curcumin (300mg/Kg b.wt) against CQ-induced hepatotoxicity and also found encouraging results with histopathological examination of liver section when compared with normal group rats. It is evidenced that curcumin exerts significant protection against CQ induced toxicity due to its antioxidant activity. In conclusion, thus our study strongly suggest that curcumin along with CQ should be recommended for treating malaria, so as to avoid the toxic influences of the above mentioned drug.

Keywords: chloroquine, curcumin, hepatotoxicity, malaria

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INTRODUCTION

Liver is a major organ, plays a central role in transforming and clearing the chemical agents (Romanelli *et al*. 2004) and also found to be susceptible to injury to some agents (Ahmed *et al*. 2006) when exposed to overdoses (Yakubu *et al*. 2018). Those chemicals that cause liver injury are termed as hepatotoxins. About 900 more drugs have been implicated in causing liver injury (Friedman *et al.* 2003). Most drugs enters the body via gastrointestinal tract and by absorption they reaches the liver through hepatic portal vein. Thus liver gets exposed to a highest concentration of toxicants (Reed 1994). Drugs encountered through other routes also reaches the liver through hepatic artery as well as portal vein (Kulkarni and Byczkowski 1994, Stacey *et al*. 1993).

Chloroquine can be used to cure malaria (Gasasira *et al*. 2003, Medilinks 2002, Noston and Brasseur 2002, Olajide *et al*. 2016, Olanrewaju and Johnson 2001, Schwartz 2003, Staedke *et al*. 2001, Wellem *et al*. 2001); auto immune disorders such as rheumatoid arthritis and systamic lupus erzytheromatosis (Augustijus 1993, Borba *et al*. 2004, Ippolito *et al*. 2018, Lee 2011, Romanelli *et al*. 2004); inflammation, extra intestinal amebiasis (or) gout (Issacson *et al*. 1982), liver abscess, Polymorphous light eruption, solar uticaria, chronic cutaneous vasculitis and also exhibit anti-HIV effect (Savarino *et al.* 2001).

Behind its uses chloroquine causes serious side effects such as gastrointestinal upset, head ache, visual disturbances, decreased red blood cells, hair loss, muscle weakness and hearing loss and cardiotoxic action. (Ekpechi and Okara 1964). Chloroquine gets accumulated in liver (Firat *et al.* 2012) and reported to cause severe life threatening toxic hepatitis (Pari 2004) (Dass *et al*. 2000).

Curcuma longa (Turmeric in Tamil) is an herbaceous perennial of about 60 -90 cm height. Leaves are large and long, measures up to one meter. Flowers are yellow white in colour, appears on a spike like stalk and do not produce any viable seeds.

Curcumin [(1E, 6E)-1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadine-3, 5 dione], a β- diketone compound contains the molecular formula of $C_{21}H_{20}O_6$ and molecular weight of 368.39 daltons with a structural formula of

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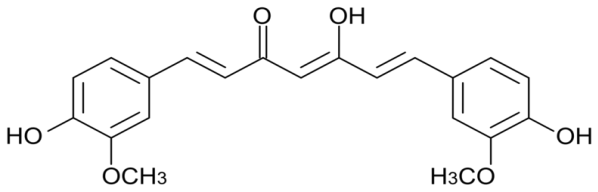
Table 1. Activity of enzymes AST and ALT in serum

S.No	Parameter	Group I (Normal)	Group II (CQ induced)	Group III (CUR only)	Group IV (CUR+CQ)
1.	AST (IU/L)	71.86±7.25	106.76±6.92	87.29±7.05[b]	88.93±0.06[a]
2.	ALT (IU/L)	22.11±6.01	39. 61±0.51	31.29±0.51[b]	34.26±0.65[a]

Mean ± S.D.(six values). [a] p<0.001; [b] p<0.005 vs normal



The study was carried out to investigate the possible role of curcumin in protection against chloroquine – induced liver damage.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this study were analytical grade and purchased from a local firm (India) and were of highest purity grade.

Plant Extract

50 gm of curcumin was extracted from turmeric using soxhlet apparatus and 95%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 4^0 C until a constant weight was obtained (Deters *et al*. 2000).

Animals

Albino male rats (150-200g) were purchased from Prasanth Aquarium, Marthadom. They were maintained at 25 ± 1^0 C at a relative humidity of 40-75% with regular 12h light, 12h dark cycle. The rats were allowed to free access the feed ad libitum and tap water.

Experimental Design

Animals were randomized and divided into 4 groups (n=6 each groups).

Group 1: Control rats, fed with normal diet and water (Jassabi *et al*., 2011)

Group 2: Rats received Chloroquine (CQ) (200 mg/Kg/day) for three days orally. (Jassabi *et al*. 2011)

Group 3: Rats received only Curcumin (CUR) (300 mg/Kg/day) daily for 14 days, orally**.** (Jassabi *et al*. 2011)

Group 4: Rats administered with (CUR+CQ). Rats received Curcumin (300 mg/Kg/day) for 14 days orally before the oral administration of chloroquine (200 mg/Kg/ day) for 14 more days. (Jassabi *et al*. 2011).

Scarification, Collection and Separation of Serum

After the experiment period (3, 14, 28 days) rats in the different groups were sacrificed by cervical dislocation and blood was collected in tubes without anticoagulant. Allow the tubes to stand for 30 minutes in slanting position at room temperature for serum separation. The separated serum was used for various biochemical estimations.

Biochemical Determination

Total protein was determined by Bradford (1976), aspartate transferase (SGOT) by Retiman and Frankel (1957) and Alanine transaminase (SGPT) by Retiman and Frankel (1957).

Liver Homogenate

Liver were removed, washed with ice-cold saline and homogenized in 0.1 M Tris-HCL buffer (pH 7.4) using ultra homogenizer. Homogenate was centrifuged at 10,000rpm for 15 min and supernatants were then centrifuged at 10,000rpm for 40 min. Supernatants was obtained and superoxide dismutase estimation was done by Misra and Fridovich 1978 and catalase by Sinha, 1972.

Histopathological Analysis

Small pieces of liver tissues from each group were collected in 10% formalin solution, processed and embedded in paraffin wax. Sections of 5-6µm in thickness were prepared and stained with hematoxylin and eosin (Drury *et al*. 1967).

Statistical Analysis

All results were expressed as the mean ± S.D for six rats per group. One way analysis of variance (ANOVA) followed by Bonforrni's analysis were used to determine the significance between the control and other groups. Statistical significance declared when P values was <0.001 and <0.05.

RESULT

Table 1 and **Fig.1** depicts the changes in the activity of hepatic enzymes Aspartate transaminase and Alanine aminotransferase. The level of these enzymes gets increased after chloroquine induction in (Group-II) animals when compared to the normal rats (Group-I). It was found that the activity of these enzymes were significantly (P<0.001) brought back near to the normal in curcumin and chloroquine treated rats (Group-IV).

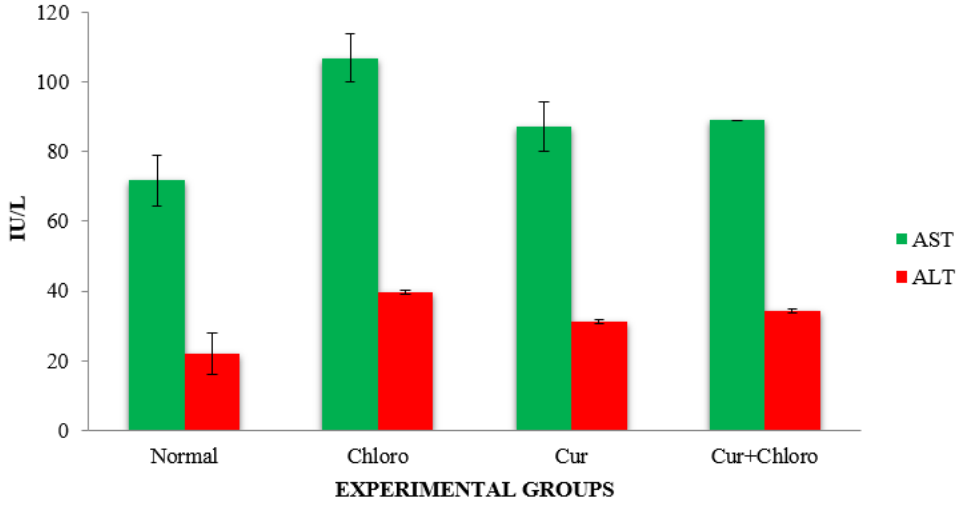


Fig. 1. Graphical representation of activity of serum enzymes AST and ALT in Normal , CQ induced, CUR treated, CUR pretreated rats

Table 2. Level of serum Protein in experimental animals

S.No	Parameter	GroupI (Normal)	GroupII (CQ induced)	GroupIII (CUR only)	GroupIV (CUR +CQ)
1	Protein	7.33 ± 0.27	3.47 ± 0.10	6.62 ± 0.62[a]	5.98± 0.10[a]

Mean ± S.D. (six values). [a] p<0.001; [b] p<0.005 vs normal.
Protein is measured in **g/dl**

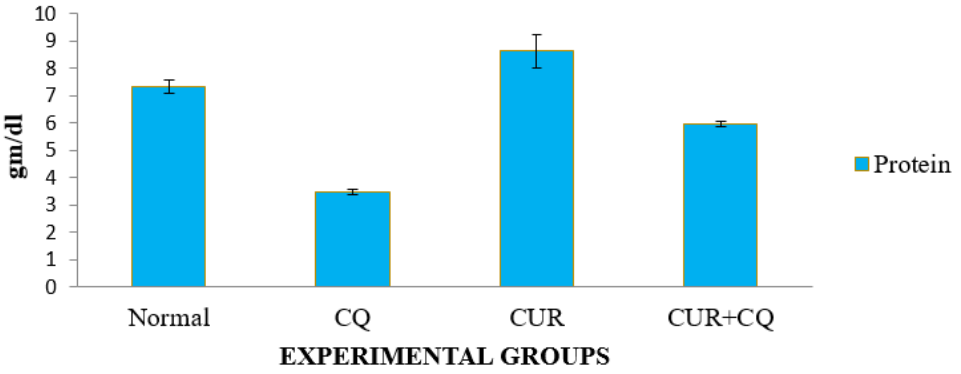


Fig. 2. Graphical representation of serum Protein level in Normal, CQ induced, CUR treated and CUR pretreated rats

Table 3. Activity of tissue enzyme SOD and CAT in liver

S.No	Parameter	GroupI (Normal)	GroupII (CQ induced)	GroupIII (CUR only)	GroupIV (CUR+CQ)
1	SOD (In Liver)	5.54 ± 0.31	3.07 ± 0.11	4.97± 0.04[b]	4.79 ± 0.17[a]
2	CAT (In Liver)	163.78 ± 2.45	148.05 ± 1.44	163.88 ± 3.01	156.10 ± 1.23[a]

Mean ± S.D.(six values). [a] p<0.001; [b] p<0.005 vs normal.
SOD is expressed in **Units/mg of protein**
CAT is expressed in **Units/mg of protein**

Table 2 and **Fig. 2** represents the change in the level of serum protein. The level of protein found to be decreased after chloroquine induction in Group-II animals when compared to normal Group - I animals. It was found that the level was significantly (p<0.001; 0.005) brought back near to normal level in curcumin only and treated rats (Group -III & IV).

The activity of antioxidant enzymes, such as superoxide dismutase and catalase in tissues of normal and experimental groups of rats are shown in **Table 3**, **Fig. 3** and **4**. The activities of superoxide dismutase and catalase in liver were significantly lower in chloroquine injected rats (Group-II) compared to normal group (Group-I). The effect were pronounced in pretreated groups. The SOD in liver (P<0.001), catalase in liver (P<0.001) were reverse back near to the normal when compared to normal group (Group-I).

Histopathological studies of normal, chloroquine, curcumin only and curcumin and chloroquine treated groups are shown in **Fig. 5**. Chloroquine treated liver shown by cytoplasmic vacuolation, nuclear enlargement and vesiculation of the hepatocytes (**Fig. 5b**) as

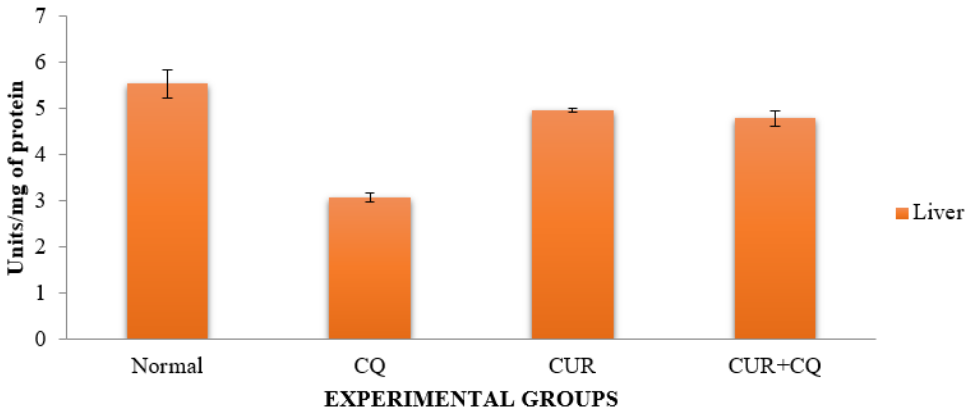


Fig. 3. Graphical representation of activity of tissue enzyme SOD in Liver

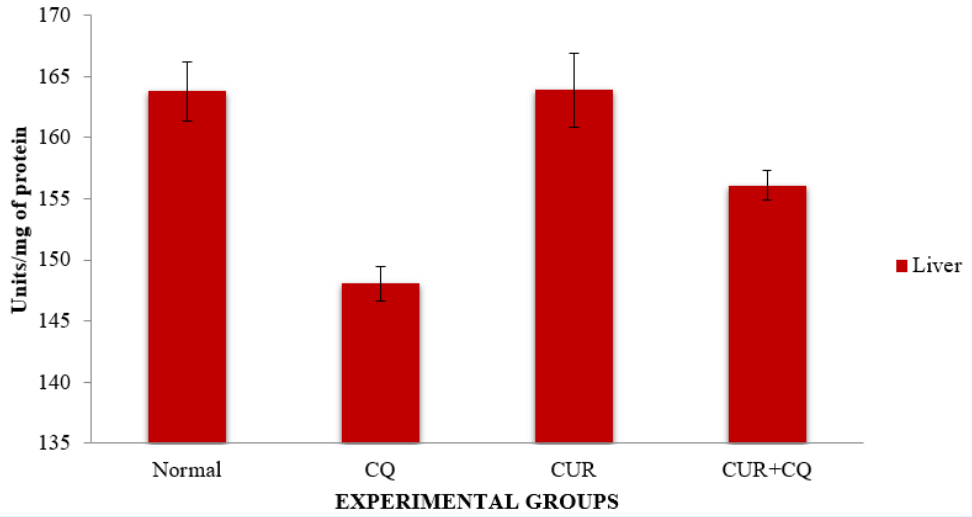
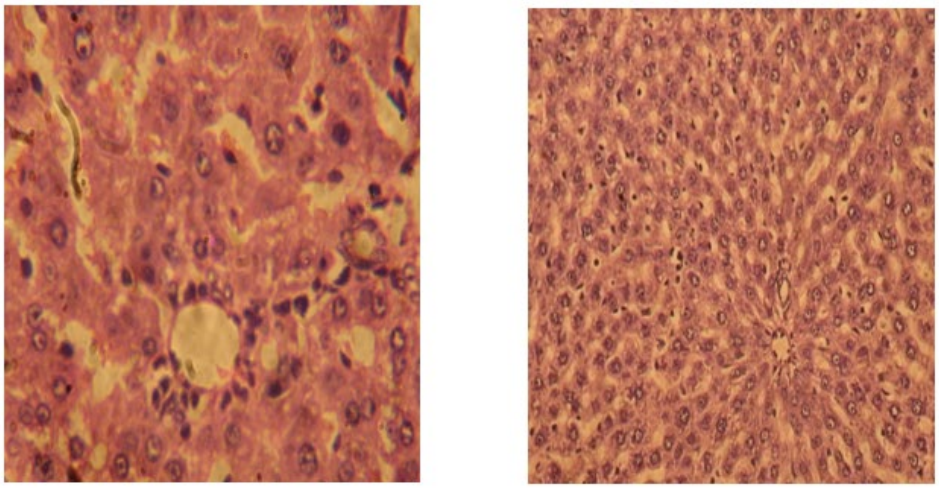


Fig. 4. Graphical representation of activity of tissue enzyme CAT in Liver



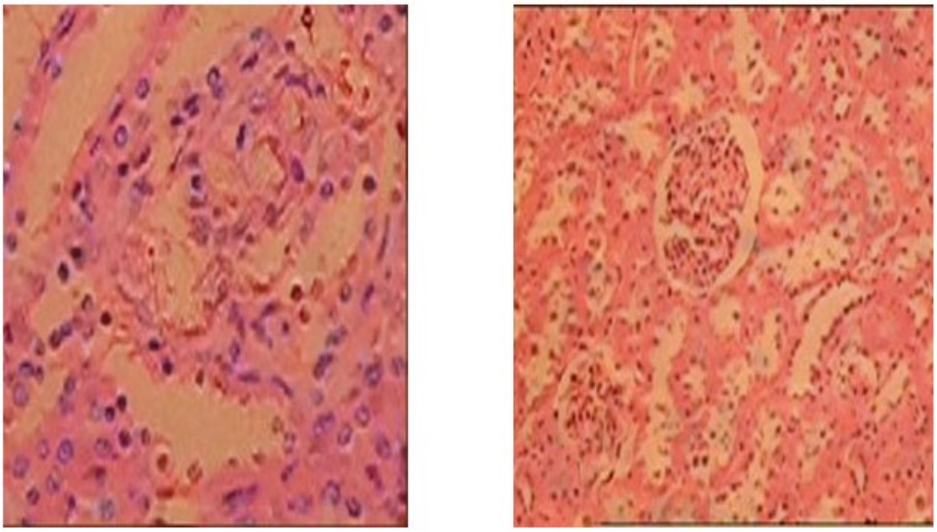
(a) Normal liver (b) CQ induced liver

Fig. 5. Histopathological studies of Normal, CQ induced, CUR treated and CUR pretreated rats liver

compared with normal liver (**Fig. 5a**). The above change were reduced in liver of rats treated with curcumin and chloroquine (**Fig. 5d**) as well as curcumin only (**Fig. 5c**).

DISCUSSION

Chloroquine is an antimalarial drug (Olajide et al., 2016) that treats propylaxis of malaria caused by species of plasmodium like *P.faciparum*, *P.ovale*,



(c) Cur treated liver (d) Cur pretreated liver

Fig. 5 (continued). Histopathological studies of Normal, CQ induced, CUR treated and CUR pretreated rats liver

P.vivax and *P.malariae*, which can be used widely in areas where malaria is endemic (Borba *et al*.,2004; Romanelli *et al*. 2004). Often this drug is also used to treat rheumatoid arthritis and systemic lupus erythromatosis (Ippolito *et al*. 2018). In this study, attention is made on the toxic nature of chloroquine on liver of rats. The hepatotoxicity of chloroquine is induced by biochemical measurement changes, antioxidant assays and histopathological studies. Our result coincide with the results of other investigators (Ding *et al*. 2000, Jassabi *et al*. 2011, Nitti *et al*. 2008, Reddy *et al*. 2007, Zwanzger *et al*. 2007).

Liver is the largest organ in the body and is the center of all metabolic activities in the body. Liver is mainly involved in the metabolism and inactivation of many drugs and other foreign substance. Hence therefore liver is more susceptible to the toxicity of drugs than other organs. Certain drugs when taken in over doses above the recommended value causes severe injury to the liver.

Serum aspartate transaminase and alanine aminotransferase are the marker enzymes of liver, used to diagnose the hepatic damage. Because these enzymes are cytoplasmic in location and gets released into the circulation. Among these two enzymes alanine aminotransferase is more specific to liver for detecting liver injury than the enzyme aspartate transaminase.

The increased serum enzymes aspartate transaminase and alanine aminotransferase in chloroquine induced rats reflex the hepatic damage. Pretreatment of curcumin protects the liver damage from chloroquine, which is evidenced by a significant reduction in the level of aspartate transaminase and alanine aminotransferase. These increased level of AST and ALT on administration of hepatotoxic doses of chloroquine to rats were similar to findings of Komatsu *et al*. (2002).

On chloroquine induction, liver gets damaged hence, the level of protein gets decreased. This is because the liver is the major site of protein synthesis Pretreatment of chloroquine with curcumin the activity of liver organ restores and increase the level of serum protein.

Superoxide dismutase, metalloproteinase catalysis the conversion of superoxide radical to H_2O_2 and lowers the level of O2-, (Shanmugam *et al*. 2018). Catalase, a heme protein catalyses the decomposition of hydroperoxide to water (Arokoyo *et al*. 2018, Dumlu *et al*. 2018) and oxygen and thus protects the cell from oxidative damage caused by hydroperoxide and OH- (Yakubu *et al*. 2018). In our study, in chloroquine-administered rats (Group II) there is a significant decline in the activities of these enzymes (SOD and CAT) which reveals that lipid peroxidation and oxidative stress were provoked by intoxication of chloroquine. Similarly, Jenkins and Goldfarb 1993, also reported that decreased SOD activity reflects oxidative stress. Moreover, an increased activity of superoxide dismutase and catalase in curcumin-treated rats might result from the scavenging activity of curcumin on those free radicals generated by chloroquine-induced lipid peroxidation, thereby decreasing the utilization of these antioxidant enzymes to reduce the chloroquine-induced oxidative stress.

Histopathological results reveals that the incidence of degeneration of liver cells of albino rats upon oral administration of chloroquine. The results shown that cytoplasm in vacuolation, nuclear enlargement and vasiculation of the hepatocytes, as compare to the normal histology. Curcumin treatment causes regeneration of liver cells.

Our results demonstrate that pretreatment of rats with curcumin reduced the hepatic damage by inhibiting the action of chloroquine in liver by increased AST, ALT, protein content and antioxidant enzymes.

In conclusion, the results strongly suggest that curcumin has protective effect on chloroquine-induced liver damage. From the results, we can infer that curcumin has antioxidant activity, responsible for protection against the chloroquine induced damage. Therefore, curcumin has been suggested to be helpful in the protection of chloroquine – induced damage in hepatic tissue.

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