Jundishapur Journal of Natural Pharmaceutical Products 2007; 2(1): 7-12

Jundishapur Journal of Natural Pharmaceutical Products

THE PROTECTIVE EFFECT OF THE *CURCUMA LONGA* EXTRACT ON ACETAMINOPHEN –INDUCED HEPATOTOXICITY IN MICE

Kalantari H[1*], Khorsandi LS,[2] Taherimobarakeh M[2]

[1]*School of pharmacy, Jundishapur University of Medical Sciences, Ahwaz, Iran*

[2]*School of Medicine, Jundishapur University of Medical Sciences, Ahwaz, Iran*

Received: *6 April 2006* ***Accepted:*** *18 April 2007*

Abstract

Acetaminophen is widely used as an analgesic and antipyretic drug, which can cause hepatic injury when given in high doses. The purpose of this study was to find out the hepatoprotective effect of *Curcuma longa* extract against acetaminophen-induced hepatotoxicity in mice. In this study six groups of mice (each group consisted of 10 mice) were used. The first group(C) received only normal saline as the negative control group and the second group (A) had been fed by 750 mg/kg acetaminophen as positive control group. The test groups (T_1-T_4) treated by *Curcuma longa* rhizoma extract in doses 200,400,800 and 1000 mg/kg respectively and after 24 hours blood was taken from the jugular arteries of the mice´s necks for biochemical tests and at the same time liver was removed and kept in 10% formalin solution for histopathology tests. The results showed that the acute elevation of serum transaminases (ALT ,AST) significiently reduced in the groups receiving *Curcuma longa* and the difference with the positive group (A) was significant ($p<0.01$). Necrosis of liver showed appropriate decrease according to histopathologic observation. It is concluded that *Curcuma longa* extract seems to have protective effect on hepatotoxicity induced by acetaminophen.

Keywords:

Curcuma longa, Acetaminophen, Hepatotoxicity, Mice.

Introduction

Acetaminophen is widely used as analgesic-antipyretic agent. Acetaminophen is remarkably safe drug when used at usual therapeutic doses. It is metabolized primarily by sulfation and glucuronidation of the parahydroxyl group. Neither unchanged acetaminophen nor its glucuronide and sulfate conjugates are toxic. A small fraction of an administered dose of acetaminophen is converted to a reactive metabolite by the cytochrome P-450-dependent, mixed-function oxidase enzymes present in hepatic cells. If taken in large overdoses, it becomes a potent hepatotoxin, producing fulminant hepatic and renal tubular necrosis, which can be lethal in human and animal (1). Most instances of acetaminophen-related hepatic injury have resulted from large, single overdose taken in an attempt at suicide. Mice has been shown to be very sensitive to the hepatic effects of acetaminophen, developing fulminant centrizonal necrosis similar to that observed in the human (2). Protection against acetaminophen induced toxicity has been performed by several investigators (3,4). C*urcuma longa* (turmeric), a yellow food additive powder is one of the ingredient in curry powder. It has been used in Asian traditional medicine as a stomach tonic, blood purifier, in the treatment of skin disease and wound healing (5).

[*]E-mail: kalantarih@yahoo.com

In recent years, many studies have shown that C*urcuma longa* possesses antioxidant, antitumor, and hepatoprotective effects (6,7,8,9,10). The present study was undertaken to test whether oral administration of C*urcuma longa* could protect mice from acetaminophen-induced hepatotoxicity.

Materials and methods

The acetaminophen powder was supplied by Daropakhsh Co. Iran. The standard SGOT and SGPT kits were purchased from local market. The plant rhizome was purchased from an authorized shop and identified as *Curcuma longa* by the experts of School of Agriculture of Shahid Chamran University, Ahwaz, Iran. The other necessary materials were obtained from our toxicology laboratory.

The plant materials were washed with water, dried and powdered in a grinding mill and then ethanolic extraction of *Curcuma longa* was prepared by stirring the powder in 80% alcohol for 3 h at room temperature. The mixture was centrifuged and supernatant obtained was filtered and then concentrated to dryness under vacuum. NMRI Male mice weighting 20 ±5 g were obtained from Hesarak Institute, Karaj, Iran. They were randomly divided in 6 groups and housed in poly carbonate cages with 22-25˚C, 12 h light and dark cycles. They were fed with ready compact food provided from Shoshtar animal food company and drinking from tap water. After over night fasting, normal saline was given to the negative control group and the positive control group was administrated by 750 mg/kg acetaminophen. Then *Curcuma longa* extract were given to the test groups (T_1, T_2, T_3 and T_4) in doses of 200 mg/kg, 400 mg/kg, 800 mg/kg and 1000mg/kg respectively. Acetaminophen and *Curcuma longa* extract were given to the animals by gavage method at the same time. As acetaminophen does not dissolved in distilled water, an acetaminophen suspension was prepared by gum tragacant (0.5%) in normal saline (11).Then after 24 h blood was taken from the jugular arteries of the mice´s necks for biochemical tests and at the same time liver were removed and kept in 10% formalin solution for histopathology tests. The liver enzyme levels were measured by applying standard method. ANOVA and Tukey tests were used to compare the results and P values less than 0.01 were considered significant. The histopathological examination also carried out accordingly.

Results

In agreement with previous studies (3,11,12) an oral dose of 750 mg/kg acetaminophen caused significant liver injury in mice as indicated by the substantial increase in serum ALT and AST activities. Treatment of mice with 200 mg/kg, 400 mg/kg, 800 mg/kg and 1000 mg/kg of crude extract of *Curcuma longa* exhibited a significant ($p< 0.05$) liver protection as the level of ALT and AST activities were reduced as compared to the positive and negative control groups (Table 1).

Histopathological studies showed that treatment with acetaminophen caused liver damage including severe centrizonal necrosis (zone 3), which was also accompanied by congestion and accumulation of inflammatory cells. In contrast, hepatic necrosis and congestion appeared less marked in *Curcuma longa* extract-treated mice (except T_1).

The extent of hepatic necrosis in test groups (T_2, T_3, T_4) were significantly lower than that in the positive and negative control groups (Fig. 1,2,3,4). In group received 200 mg/kg of the crude extract necrosis of the liver and ALT and AST levels were reduced, but the differences were not statistically significant.

The differences between T_2 with T_3 and T_4 were statistically significant ($p<0.01$), but the differences between T_3 and T_4 were not significant ($p>0.05$). Therefore, we suggest

that the best dose of *Curcuma longa* is 800 mg/kg.

Discussion

Previous studies demonstrated some natural product such as garlic oil (12) and arabic gum (13) may protect partial liver damage induced by acetaminophen. Several investigators have demonstrated beneficial effects of *Curcuma longa* in liver damage. Lin showed that *Curcuma longa* significantly reduce the elevation of ALT and AST induced by D-galactoseamine in rat. In addition lipid peroxidation and necrosis in the liver of the rats were reduced (8).

Table 1: Effect of acetaminophen and *Curcuma longa* administration on serum ALT and AST in mice (Results expressed as mean ± S.E.M. of 10 mice)

Treatment	ALT(IU)	AST(IU)
Control (C)	163±13	231± 8
Acetaminophen (A)	3693 ± 321*	2593± 123*
Curcuma longa 200 mg/kg + acetaminophen (T_1)	3275 ± 311*	2286 ± 141*
Curcuma longa 400 mg/kg + acetaminophen (T_2)	1685 ± 231**	976 ± 43**
Curcuma longa 800 mg/kg + acetaminophen (T_3)	383 ± 12**	431 ± 21**
Curcuma longa 1000 mg/kg + acetaminophen (T_4)	295 ± 5**	394 ± 15**

*Significant decrease ($P < 0.01$) compared to control group.
**Significant decrease ($P < 0.01$) compared to acetaminophen group.

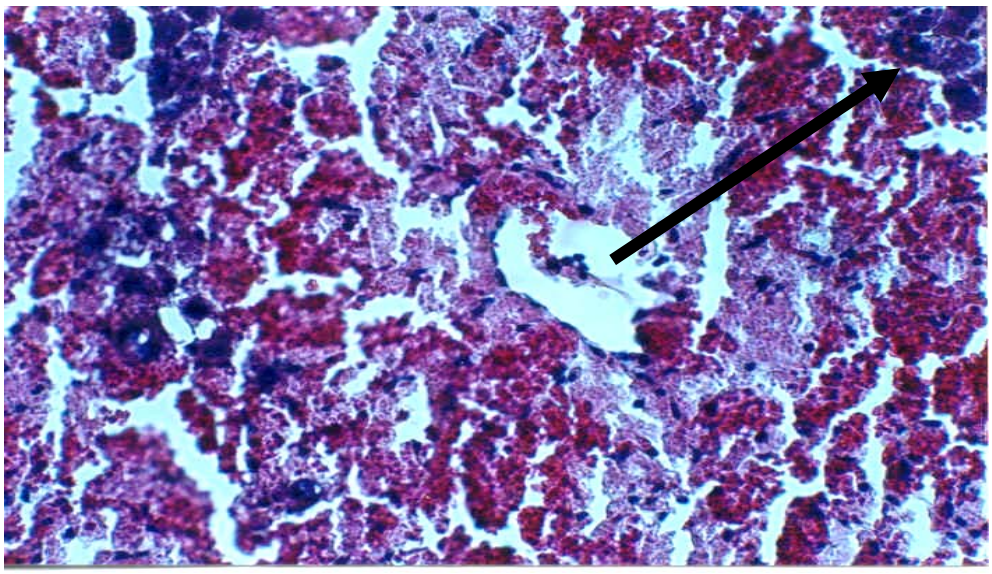

Fig. 1: Section of mouse liver tissue treated by 500 mg/kg acetaminophen (positive control group). Massive centrilobular necrosis (arrows), accumulation of inflammatory cells and congestion have been seen. (H&E. X200).

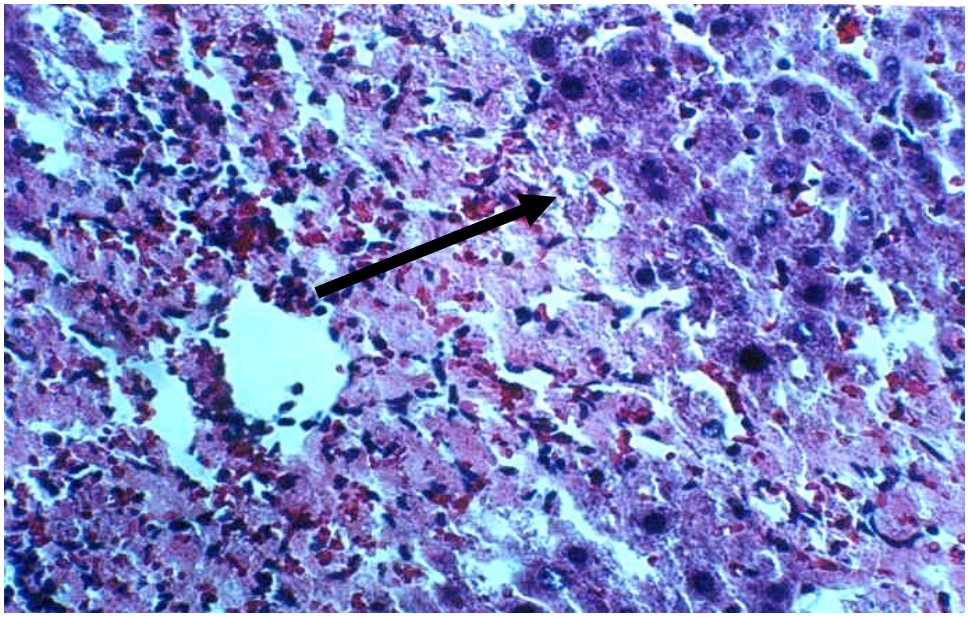

Fig. 2: Section of mouse liver tissue treated by 500 mg/kg acetaminophen + 400 mg/kg *Curcuma longa* extract (T_2). Centrizonal necrosis (arrows), accumulation of inflammatory cells and congestion are less than positive control group. (H&E. X200).

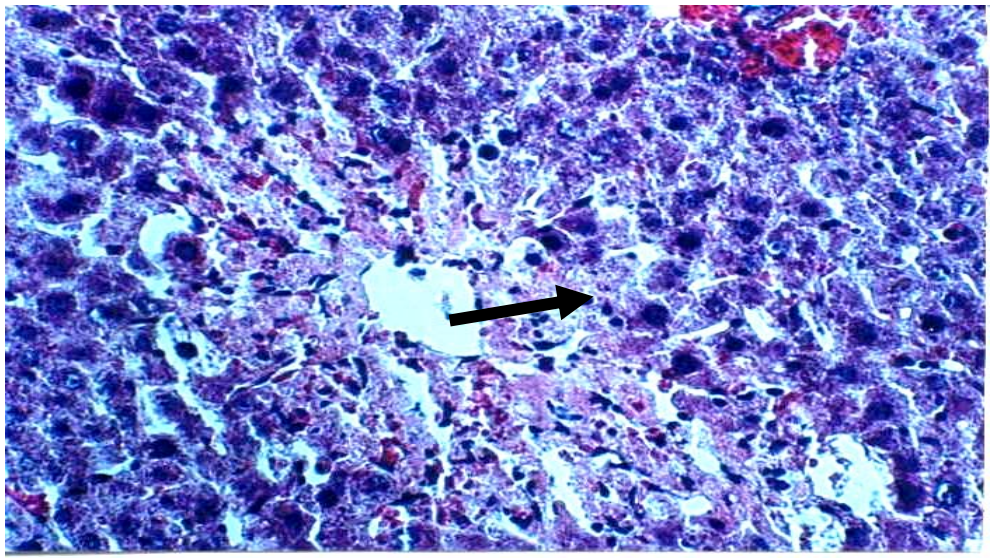

Fig. 3: Section of mouse liver tissue treated by 500 mg/kg acetaminophen + 800 mg/kg *Curcuma longa* extract (T_3). The extent of lobular necrosis (arrows), accumulation of inflammatory cells and congestion are lower than previous groups. (H&E. X200).

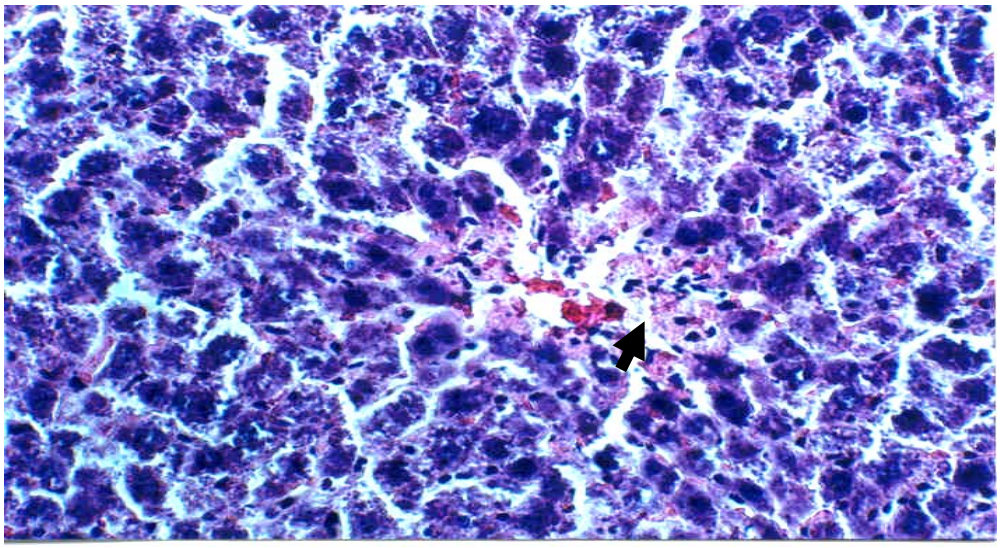

Fig. 4: Section of mouse liver tissue treated by 500 mg/kg acetaminophen + 1000 mg/kg *Curcuma longa* extract (T_4). The extent of lobular necrosis (arrows), accumulation of inflammatory cells and congestion are significantly reduced. (H&E. X200).

Similarly curcumin, a phenolic compound from the rhizome of the *Curcuma longa,* suppresses liver damage induced by CCl_4 (9).The results of this study demonstrate that *Curcuma longa* extract is effective in protecting against the hepatotoxic effects of acetaminophen in mice. The biochemical tests showed that the acute elevation of serum transaminases (ALT, AST) induced by acetaminophen significantly reduced in the groups received *Curcuma longa* extract ($p<0.01$). Liver necrosis decreased according to histological observation as shown in Fig. 2, 3 and 4. The characteristic zone 3 necrosis of acetaminophen appears to be produced by an electrophilic metabolite of drug N-acetyl-p-benzoquinonimine, (NAPQI) that binds covalently to liver protein macromolecules and probably also oxidizes lipid, or the critical sulphydryl groups (protein thiols) and alters the hemostasis of calcium (14) . The zone 3 location of the necrosis is a consequence of the location in that zone of the enzyme system (cytochrome P-450) responsible for converting acetaminophen to its active metabolite (NAPQI). The amount of the metabolite formed normally is low, since the therapeutic dose of acetaminophen taken is not large and its metabolic fate is largely in the direction of conjugation with glucuronate and sulfate. The small amounts of active metabolite formed are readily detoxified by reaction with glutathione (GSH) to form mercapturic acid. Hepatic necrosis occurs only when the amount of active metabolite (NAPQI) produced exceeds the binding capacity of GSH. This occurs when the dose of drug taken is large. The adverse effects of a large dose or even smaller dose are enhanced by factors that increase the fraction of drug converted to an active NAPQI or decrease the availability of GSH. Thus hepatic injury depends on the quantity of acetaminophen ingested, the activity of the cytochrome P-450 system, and the adequacy of GSH stores (15).

The protective effect of *Curcuma longa* against acetaminophen - induced liver injury can be achieved by various mechanisms. The hepatoprotective mechanism may function through direct binding with acetaminophen toxic metabolites, decreasing the acetaminophen metabolites for other cellular GSH.

Curcuma longa treatment increased the concentration of hepatic GSH and maintained a high level activity of GSTase which led to acceleration of the excretion of toxic acetaminophen metabolites (16).

In conclusion it is clear from the above results and data obtained that *Curcuma longa* extract seems to have protective effect on hepatotoxicity induced by acetaminophen but to prove this statement it is better to perform further study such as ultra structural and molecular studies.

References

1. Laura P, Philip R, Jack A. Acetaminophen-induced hepatotoxicity. Toxicol. 2003; 3: 1499-1506.
2. Zimerman HJ, Maddrey WC. Disease of the liver. 7 th ed., Philadelphia, JB Lippincot Company, 1996.
3. Pablo M, Tania G, Alvarez VP, Mourelle M. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J. Appl. Toxicol. 1992; 12: 439-42.
4. Liu CC, Liu Y, Madhu C, Klassen CD. Protective effects of oleanolic acid on acetaminophen-induced hepatotoxicity in mice. J. Pharmacol. Exp. Ther. 1993; 266: 1607-13.
5. Pizorrono G, Emon M, Tabato M. Textbook of Natural Medicine, 2nd ed. Churchill Livingstone, London, 1999: 689-693.
6. Sewan R, Subrana L. The antioxidant activity of *Curcuma longa*. J. Ethnopharmacol. 1995; 45(2): 59-67.
7. Aggrawal BB, Kumar A, Phatric AC. Anticancer potential of curcumin. Anticancer Res. 2003; 23(1): 63-76.
8. Lin SC. Protective and therapeutic effect of *Curcuma longa* on B-D-Galactosamin induced liver damage. Pharm. Res. 1996; 10(2):131-135.
9. Park EJ, Jeon CH, Kong G. Protective effect of curcumin in mice liver injury induced by carbon tetrachloride. J. pharmacol. 2000; 437:44-52.
10. Ready AC, Lokesh BR. Effect of curcumin on iron-induced hepatotoxicity in rats. J. Toxicol. 1999; 107(1):39-45.
11. Kalantari H, Valizadeh M. Nifedipine in the treatment of liver toxicity induced by acetaminophen overdose in mice. Acta Medica Iranica 2000; 4: 240-244.
12. Kalantari H, Salehi M. The protective effect of garlic oil on hepatotoxicity induced by acetaminophen in mice and comparison with N-acetylcysteine. J. Saudi Med. 2001; 22(12): 1080-1084.
13. Ayman M, Adel M, Othman A, Abdullah M, Mahmoud N. Protective effect of *Arabic gum* against acetaminophen-induced hepatotoxicity in mice. Pharm. Res. 2003; 48: 631-635.
14. Rowden AK, Novell J, Krik MA. Update on acetaminophen toxicity. Med. Clin. North Am. 2005; 89(6): 1145-59.
15. Haetmut J, Gregory J, Arthur I, Cederbaum A. Mechanism of hepatotoxicity induced by paracetamol. Toxicol. Sci. 2002; 65: 166-176.
16. Susan M, Rao MN. Induction of glutathione-s-transferase activity by *Curcuma longa* in mice. Ethnopharmacol. 1992; 42: 262-4.