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Protective effect of *Curcuma longa* extract on acetaminophen induced nephrotoxicity in mice

*Khorsandi L., Orazizadeh M

Department of Anatomical Sciences, Faculty of Medicine, Jundi-Shapour University of Medical Sciences, Ahwaz, Iran.

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ABSTRACT

Background and purpose of the study: Acetaminophen is a commonly used analgesic and antipyretic agent which, in high doses, causes liver and kidney necrosis in man and animals. *Curcuma longa* has been reported to have anti oxidant and hepato-protective properties. In this study the protective effect of *Curcuma longa* extract on acetaminophen induced nephrotoxicity has been evaluated.

Materials and methods: Sixty NMRI male mause were randomly divided into 6 groups. Control group received normal saline. *Curcuma longa* group received 1000 mg/kg of the extract of the plants, positive control group received 500 mg/kg acetaminophen. Acetaminophen and *Curcuma longa* extract at doses of 400, 800 and 1000 mg/kg were administered to the tested groups (T_1- T_3) at the same time. The jugular arteries of the mice were cut for biochemical testes after 48 hours and the kidney removed in 10% formalin solution for histopathology testes.

Results: BUN, Cr and Uric acid reduced significantly in the T_3 group ($p<0.05$). Necrosis of kidney reduced in test groups especially in T_3 group.

Conclusion: The results of this study indicate that *Curcuma longa* extract may protect kidney against acetaminophen - induced tubular necrosis in mice.

Keywords: Nephrotoxicity, *Curcuma longa*, Acetaminophen, Mice

INTRODUCTION

An acute acetaminophen (paracetamol, *N*-acetyl-*p*-aminophenol; APAP) overdose may results to potentially fatal hepatic and renal necrosis in humans and experimental animals (1). The initial step of its toxicity is formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P450 which at therapeutic doses is removed by conjugation with gluthation solfidryle (GSH). High doses of acetaminophen result in the depletion of cellular GSH which allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury (2-3). Acetaminophen -induced renal injury could also be due to hepatic-derived acetaminophen metabolites, particularly GSH conjugates (4).

Studies have been carried out for agents that would provide maximum protection of the liver, kidney as well as other organs (5). A number of herbs are traditionally used in different countries during drug or toxin induced hepatic and renal disorders (6). *Curcuma longa* (turmeric), a yellow food color and an ingredient in curry powder, for long time has been used in Asian traditional medicine as a stomach tonic and blood purifier, and for the treatment of skin disease and wound healing (7). In recent years, many studies have shown that *Curcuma longa* possesses antioxidant (8), anti-tumor (9), and hepato-protective (10-12) properties. In spite of long use of Curcuma species in traditional medicine, little work has been done on phytomedicinal properties of this plant. In the present study, alcoholic extract of *Curcuma longa* was used to treat mice with an acute toxicity induced by acetaminophen. Results of this study may help to understand the action of *Curcuma longa* on kidney of animals.

MATERIALS AND METHODS

Marerials

The acetaminophen powder was purchased from Darupakhsh Company (Iran). Urea kit was obtained from Sigma (England). *Curcuma longa* rhizome was purchased from the local herbal

Correspondence: layasadat@yahoo.com

market in Ahwaz.Voucher specimens from the plant material was deposited at the Herbal Museum, Faculty of Agriculture, Shahid Chamran University, Ahwas, Iran.

Extraction

The plant were washed with water, dried and powdered in a grinding mill. Five g of the powder was soaked overnight in 150 ml of methanol at room temperature. The solvents were decanted and residues macerated two more days with the same solvent. The pooled solvents were combined and filtered and the filtrates were concentrated under reduced pressure. The yield of the extract was 1.01 and it was then diluted with distilled water.

Animals

Sixty male NMRI mice weighing 25-30 g were obtained from the Animal Care Center, Razi, Karaj, Iran. They were housed under convential laboratory condition at room temperature and maintained at 25±1°C at a relative humidity of 40-75% with a regular 12 h light: 12 h dark cycle. The mice were allowed free access to food and tap water.

Experimental design

Mice were randomly divided into 6 groups, each consisting of 10 animals. All Animals were fasted over night before the experiment. Group1 received normal saline as control negative group. Group2, the *Curcuma longa* group, received 1000 mg/kg of the extract, and group3, the acetaminophen group, received a single dose of acetaminophen (500 mg/kg). Groups 4-6 as test groups (T_1,-T_3), were treated with *Curcuma longa* extract (at doses of 400, 800, and 1000 mg/kg) and a single dose of acetaminophen (500 mg/kg.) at the same time. Acetaminophen and *Curcuma longa* extract were given to the animals by gavage method at the same time. *Curuma longa* extract was diluted with distilled water and acetaminophen suspension was prepared by gum tragacant (0.5%) in normal saline (13). Twenty-four hour after administration of acetaminophen, the mice of each group were anesthesized and the kidneys were removed and kept in %10 formalin solution for histopathology tests.

Biochemical tests

Blood samples collected from the jugular arteries of the mice´s necks. Blood urea nitrogen (BUN), Ceratinine (Cr) and Uric acid concentration was assessed as markers of nephrotoxicity. BUN, Cr and Uric acid were determined spectrophotometrically from serum samples using commercially available kits (Sigma).

Histopathological assessments

The kidneyes were fixed in 10% formalin solution, then dehydrated in graded concentrations of alcohols and embedded in paraffin. Sections of 5μm were prepared and stained with PAS. Light microscopy (Olympus PX 50 F3 model, Japan) was used to evaluate the kidney tissue. Slides were read in a "blind" fashion.

Statistical analysis

The values are presented as means ± SEM. Differences between group means were estimated using a one-way ANOVA followed by Tukey test. Results were considered statistically significant when $p<0.05$.

RESULTS

Biochemical tests

The serum levels of Cr, BUN and uric acid are shown in table 1. Cr, BUN and uric acid levels in the *Curcuma longa* treated mice were similar to the control group ($p>0.05$)**.** In aggreement with previous studies (14-15) a dose of 500 mg/kg of acetaminophen p.o. caused renal injury in mice after 24 h, as indicated by the significant increase in Cr, BUN and uric acid ($p<0.001$)**.**

T_1 and T_2 groups showed no significant reduction in BUN, Cr, and Uric acid when compared to the acetaminophen-treated animals ($p>0.05$). The serum markers were significantly decreased in T_3 group compared to the acetaminophen-treated mice ($p<0.05$).

Histopathological assesment

All parts of kidney showed normal appearance in control group. The kidney of *Curcuma longa* group showed normal architecture. Treatment with acetaminophen caused acute renal damages in glomerulus and proximal tubules. Glomerolus damages were evident by glomerular bleeding and partial endothelial rupture in capsule. Proximal tubules were dilated with loss of cellular boundary. Intraluminal cell debris, karyorrhexis and glassy pink cytoplasm were observed as indicators of the cell death wree observed. The proximal tubule also showed loss of brush border. Debris and granules from epithelial cells leaked into the tubular lumen (Fig. 1).

T_1 group showed a severe tubular necrosis pattern similar to the acetaminophen group. Proximal tubes showed dilatation and loss of brush border and debris and granules from epithelial cells leaked into the tubular lumen. Intraluminal cell debris was also observed (Fig 2). The kidneys of T_2 group showed a tubular necrosis pattern with moderate necrosis and degeneration. Proximal tubules were dilated and brush border were

Table1. Effect of acetaminophen and *Curcuma longa* administration on serum BUN, Cr and Uric acid in mice (Mean ± S.E.M.).

Treatment	BUN/mg L^{-1}	Cr /mg L^{-1}	Uric acid/mg L^{-1}
Control	132± 17	4.18±0.03	9.5 ± 0.05
Curcuma longa	129 ±12	3.96 ± 0.025	9.3 ± 0.04
Acetaminophen 500mg/kg	184 ± 8**	5.43 ± 0.021**	15.67 ± 0.05**
Curcuma longa 400mg/kg + acetaminophen (T_1)	178 ± 7**	5.15 ± 0.01**	15.2 ± 0.11**
Curcuma longa 800 mg/kg + acetaminophen (T_2)	165 ± 13*	4.895 ± 0.022*	14.36 ± 0.07*
Curcuma longa 1000mg/kg + acetaminophen (T_3)	139 ± 14	4.348 ± 0.04	10.15±0.13*

* (P< 0.01), ** (P< 0.001)

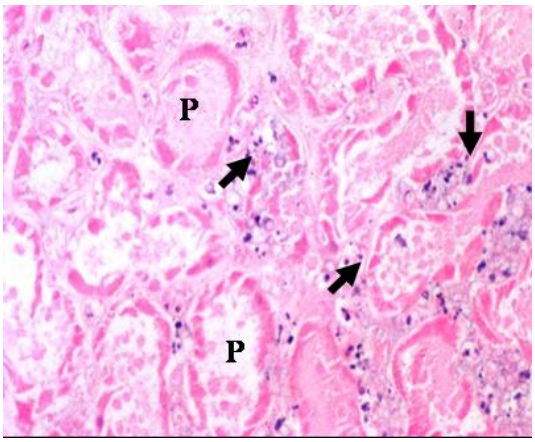

Figure 1. Light microscopy of renal tissue in acetaminophen treated mice. Sever tubular necrosis is observed. The proximal tubules (P) are show dilatation and intraluminal cell debris (arrows). PAS staining, X200.

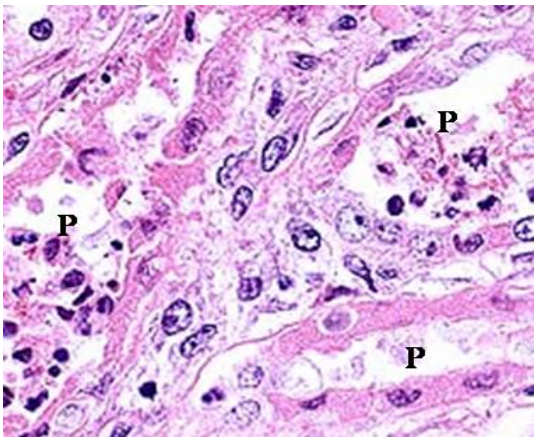

Figure 2. Light microscopy of renal tissue in T_1 group. Severe necrosis and degeneration are shown. Proximal tube show dilatation and brush border in proximal tubules (P) have been disappeared (arrows). PAS staining, X400.

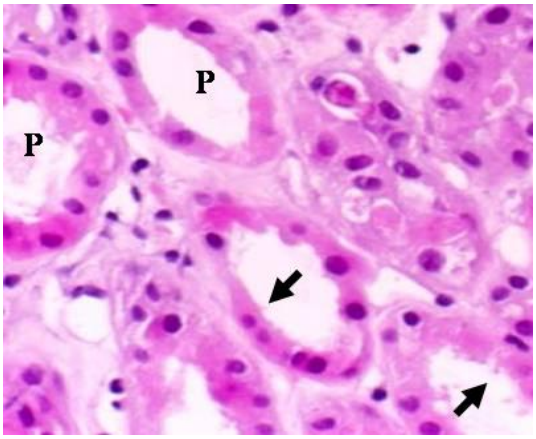

Figure 3. Light microscopy of renal tissue in T_2 group. Moderate necrosis and degeneration are shown. Proximal tubules (P) show dilatation and there is brush border (arrows) in some of them. PAS staining, X400

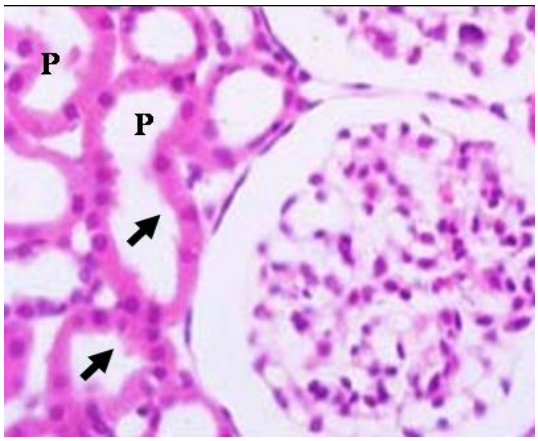

Figure 4. Light microscopy of renal tissue in T_3 group. Normal architecture of renal tissue is observed. Arrows indicated brush border in proximal tubules (P). PAS staining, X400

observed in some animals, but intraluminal cell debris was less than T_1 group (Fig. 3). T_3 group showed mild tubular necrosis and architecture of kidney was normal. In this group brush border were observed in the majority of proximal tubules and intraluminal cell debris were absent (Fig. 4).

DISCUSSION

It has been suggested that N-acetylcystein (NAC), which is used to treat acetaminophen-induced hepatotoxicity, may be harmful to the kidneys (14). The protective effects of melatonin, Vit E and NAC (N-acetylcystein) against acetamino-

phen toxicity in mice have been evaluated in a comparative study. BUN and serum Creatinine, ALT and AST levels, which increased significantly following acetaminophen treatment, decreased significantly after pretreatment with either Vit E or melatonin. NAC did not reduce BUN and creatinine, but reduced ALT and AST levels. Melatonin was the most effective agent in reversing acetaminophen toxicity, which may be due to its higher efficacy in scavenging various free radicals and also its ability in stimulating the antioxidant enzymes (16).

It has been reported that Curcumin, the yellow pigment isolated from *Curcuma longa,* has a strong antioxidant actvity and possesses palliative action on gentamicin-induced nephrotoxicity and ameliorates the histopathological and biochemical indices of nephrotoxicity in rats. While gentamicin treatment reduced cortical GSH concentration to about 31%, Curcumin significantly mitigated these effects and Curcumin-treated rats showed apparently normal proximal tubule (17). It has also been demonst-rated that Curcumin has protective effect against adriamycin-induced renal injury by suppressing oxidative stress, increasing kidney GSH content and gluthation peroxidase activity (18).

The results of this study demonstrate that *Curcuma longa* extract is effective in protecting against the nephrotoxic effects of acetaminophen. The kidney of acetaminophen-intoxicated mice showed acute damages in proximal tubule. The histological pattern of mice kidney treated with 400 and 800 mg/kg of *Curcuma longa* extract and acetaminophen (T_1 and T_2 groups) showed a tubular necrosis pattern with a sever or moderate necrosis and degeneration (Figures 3 and 4) , while in T_3 group, which received 1000 mg/kg *Curcuma longa* extract and acetaminophen, mild necrosis was observed. The acute elevation of BUN, Cr and uric acid was reduced in the test groups which was statistically significant in T_3 group ($p<0.05$).

All results show that the *Curcuma longa* is beneficial to the kidney. The protective mechanism may be due to direct binding with acetaminophen toxic metabolites and decreasing the attraction of acetaminophen metabolites for other cellular GSH. Additionally it has been reported that Curcuma *longa* treatment increased the concentration of hepatic GSH and maintained a high level activity of GSTase (glutathione–S-transferase) which led to increase in the excretion of toxic acetaminophen metabolites (19).

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