International Journal of Surgery 13 (2015) 33–37

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

The use of thymoquinone in nephrotoxicity related to acetaminophen

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HIGHLIGHTS

- Acetaminophen overdose is one of the most commonly reported types of toxic ingestion.
- Acetaminophen induced renal failure is a consequence of acute tubular necrosis.
- Thymoquinone treatment has therapeutical effect on Acetaminophen induced nephrotoxicity in rats.

ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form
11 November 2014
Accepted 17 November 2014
Available online 22 November 2014

Keywords:
Thymoquinone
Acetaminophen
Nephrotoxicity

ABSTRACT

Aim: We aimed to investigate efficacy of intraperitoneally administered Thymoquinone (TQ) in acetaminophen (APAP) induced renal toxicity. **Material and method**: Forty Wistar Albino rats were randomly divided into 4 groups of ten rats each. Control group was untreated group while rats in TQ group were treated with single dose TQ. In APAP group rats were treated with single dose acetaminophen. In TQ + APAP group TQ and APAP were administered respectively. Rats were sacrificed at 24th hour; urea, creatinine and nitric oxide levels were measured also malondialdehyde activity were assessed in renal tissue specimens. Tissue damage scores were recorded in histopathological assessment. **Results**: Urea and creatinine levels were found significantly higher in APAP group than control group ($p < 0.003$). Urea and creatinine levels in APAP + TQ treated group were significantly lower than APAP treated group ($p < 0.01$). Serum NO activity and tissue MDA levels were higher in APAP group than control group ($p \leq 0.002$). In contrast to APAP treated group serum NO activity and tissue MDA levels were found significantly lower in TQ + APAP group ($p \leq 0.03$). In APAP group significant histopathological change was found compared with control group ($p \leq 0.001$) where there was no significant change between control and TQ treated groups ($p > 0.05$). In APAP group we found significantly higher tissue damage scores than control group ($p \leq 0.001$). In APAP + TQ group lower tissue damage scores were found compared with APAP group ($p \leq 0.001$) while higher scores were found compared with control group ($p \leq 0.001$). **Conclusion**: We can conclude that TQ treatment has therapeutical effect in APAP induced nephrotoxicity in rats.

1. Introduction

Acetaminophen (APAP) overdose is one of the most commonly reported types of toxic ingestion, worldwide; in 2005 over 165,000 cases of acetaminophen overdose were reported in the United States alone [1]. When ingested, high doses of APAP and its metabolites cause toxicity in both the liver and extra hepatic tissues [2,3]. APAP hepatotoxicity is a well-known and extensively studied clinical phenomenon, although renal insufficiency occurs in approximately 1–2% of patients with APAP overdose [4]. APAP-induced tissue and organ injuries have similar mechanisms, and only slight differences emerge between hepatic and extra hepatic manifestations. Although rarely observed compared to hepatotoxicity, renal toxicity may lead isolated organ damage or fatal multisystem organ failure.

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http://dx.doi.org/10.1016/j.ijsu.2014.11.020
1743-9191/

There are several potential mechanisms of renal toxicity, based on data obtained from both animal and human studies. These include the metabolic pathways in the cytochrome P-450 enzyme system, as well as prostaglandin synthetase and N-deacetylase enzymes [5].

Thymoquinone (TQ) is obtained from *Nigella sativa* seeds and has been investigated in many pharmacological and experimental investigations in recent years. Several studies have been reported protective effects of orally administered TQ on several organs [6–10] that had been exposed to various free-radical-generating agents, including doxorubicin [7], carbon tetrachloride [8], APAP [9] and cisplatin [10]. In addition, TQ has also shown anti-inflammatory [11] and anti-microbial [12] effects. Badary et al. showed inhibition of the 5-lipoxygenase enzyme in human neutrophils and the superoxide radical scavenging activity of TQ, via inhibition of lipid peroxidation induced by Fe^{+3} ascorbate [13].

In our study, we investigated the effect of orally administered single dose of TQ on renal tissues that had been exposed to a toxic dose of an intraperitoneally administered APAP. We measured urea, creatinine levels and nitric oxide (NO) activities, as well as tissue malondialdehyde (MDA) levels. In addition, we conducted a histopathological investigation of renal tissue specimens.

2. Materials and methods

2.1. Experimental animals

We used 40 male Wistar rats (3 months; 200–300 g b.wt.), which we obtained from the Health Sciences Application and Research Center, Dicle University, Turkey. The study protocol was approved (2012-04) by the Committee of Experimental Animals of Dicle University, and all experimental procedures complied with the committee's Guide for the Care and Use of Laboratory Animals. We housed the rats in cages in special rooms with controlled temperature (21–23 °C), humidity and regular light cycles (12 h), and maintained them on a standard diet and water *ad libitum*. We allowed them free access to standard rat chow and water before the experiments, and although we fasted them overnight the day before the study, they had access to water *ad libitum*.

2.2. Experimental protocol

We purchased APAP from Merck (Germany), and the experiment was conducted according to the modified procedures described previously. APAP was dissolved in 40% polyethylene glycerol 400 for intraperitoneal administration [14].

We purchased TQ from Sigma–Aldrich (Catalog no.274666), and dissolved it in warm drinking water (65 °C) then cooled it to room temperature before oral administration.

We randomly created several experimental study groups in a single-blind manner using coded:

- Group I: Animals that received neither APAP nor TQ (sham group, $n = 10$);
- Group II: Animals that we treated with 500 mg/kg i.p. APAP (APAP group, $n = 10$);
- Group III: Animals that we treated with 500 mg/kg i.p. APAP and 1 h later 10 mg/kg TQ was given at a single dose via the oral route (APAP + TQ group, $n = 10$);
- Group IV: Animals that received only 10 mg/kg orally administration TQ (TQ group, $n = 10$).

At hour 24 of intervention, we anaesthetized the rats with 50 mg/kg ketamine hydrochloride (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun; Bayer AG, Leverkusen, Germany), via intramuscular injection, and sacrificed them by taking blood from the heart via a 5 ml syringe for biochemical analysis. Likewise, we removed kidney tissues for histopathological examinations.

2.3. Biochemical analysis

We immediately centrifuged each collected blood sample at 4000 rpm for 10 min, and then transferred these into Eppendorf tubes on ice. We kept the samples in a −80 °C deep freeze until the completion of the study.

Biochemist was blind manner using coded Eppendorf tubes. We carried out blood biochemical analyses of urea, creatinine by an autoanalyzer Architect c16000 (Abbott Diagnostics, Abbott Park, IL, USA). We determined the NO level in the blood and the tissue by measuring nitrite concentrations of a stable metabolic product of NO with oxygen. We converted NO_3 into NO_2 with elementary zinc, and determined NO_2 concentration in serum and tissue by classic colorimetric Griess reaction [15]. In addition, we measured tissue MDA activity in rat kidney tissue and spectrophotometrically measured MDA, as described previously [16].

2.4. Histopathological evaluation

We took the kidney tissues for histopathological evaluation. We removed foreign tissue residues and blood, washed with saline, and put into plastic containers holding 10% formaldehyde solution.

Pathologist was blinder manner using coded plastic containers. We fixed the tissue specimens in 10% formalin for 48 h, then embedded them in paraffin and cut them into 5 μm sections. We used hematoxylin–eosin staining method for slides, and a pathologist blinded for groups examined the sections under a light microscope (Nikon ECLIPSE 80i), using 200× magnification to assess the degree of renal injury.

Renal tissue damage was scored as shown in Table 1 [17].

2.5. Statistical analysis

We performed statistical analysis using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA) and presented the data as mean ± standard deviation values. We compared the groups using the nonparametric Kruskal–Wallis test, and used the Mann–Whitney *U* test for binary comparisons. We compared the groups for histopathological evaluation scores using the nonparametric chi-squared test. We considered a p value of <0.05 to be significant.

3. Results

We found higher urea and creatinine levels in the APAP group than in the control group ($p < 0.003$) (Table 2). The mean urea and

Table 1
Renal tissue damage was scored as shown in Table.

Renal tissue damage	Histopathological changes
Grade 0	No diagnostically change
Grade 1	Swallowing of tubular cells, loss of brush borders, changes that range between cellular condensation – which shows loss of nucleus– and elongation in 1/3 of tubular structures
Grade 2	Additionally to changes in Grade 1; loss of nuclear structures that include 2/3 of tubular structures
Grade 3	Loss of nuclear structures that include more than 2/3 of tubular structures