Prophylactic effects of thymoquinone against carbon tetrachloride-induced hepatic damage in Sprague-Dawley rats

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ABSTRACT

This study is conducted to investigate the prophylactic effect of thymoquinone (TQ), the major active ingredient of Nigella sativa seeds, against carbon tetrachloride (CCl4)-induced hepatic damage in Sprague-Dawley (SD) rats. Forty rats were divided into four even groups. The first group served as control. The second, third and fourth groups received CCl4, CCl4 and TQ, and TQ only, respectively for 5 weeks. CCl4 (2 ml/kg b.w.) was given orally by gastric tube twice a week on Sunday and Thursday. TQ (20 mg/kg b.w.) was given daily in corn oil by gastric tube. At the end of the experiment, rats were sacrificed, and blood samples and liver specimens were obtained for biochemical analysis and morphological examination, respectively. Control and TQ-treated rats showed normal serum activity for alanine (ALT) and aspartate (AST) aminotransferases, and normal liver histology. Treatment of rats with CCl4 significantly increased serum activity of ALT and AST aminotransferases compared to control rats. Histopathologically, livers from CCl4-treated rats showed dilatation of blood sinusoids and portal blood vessels, Kupffer cell activation, vacuolar degeneration of hepatocytes, focal areas of necrosis and mild hepatic fibrosis. Using transmission electron microscopy (TEM), CCl4 caused clear lesions in the liver including dilatation of endoplasmic reticula, increased extracellular matrix and formation of abundant fatty globules and numerous autophagosomes in hepatocytes. On the other hand, co-administration of TQ with CCl4 significantly decreased serum ALT and AST activities, and attenuated most of CCl4-induced hepatic pathological changes. The present study indicates that TQ has the potential to attenuate CCl4-induced hepatic damage in SD rats.

INTRODUCTION

Liver as a vital organ in the body playing a central role in metabolic homeostasis and detoxification of a variety of drugs and xenobiotics is vulnerable to a wide range of toxic, microbial, metabolic, circulatory and neoplastic insults (Taub, 2004; Shamsi-Bagbahan et al., 2014). Carbon tetrachloride (CCl4), an industrial solvent, is a hepatotoxic agent and its administration is widely used as an animal model of toxin-induced liver injury that allows the evaluation of both necrosis and subsequent inflammation (Huh et al., 2004). CCl4-induced hepatic damage is widely used for hepatoprotective drug screening. Hepatotoxicity of CCl4 involves its biotransformation into free radicals such as trichloromethyl free radical (CCl3) and trichloroperoxy radical (CCl3O2·), and increased lipid peroxidation (Feng et al., 2010). Herbal products have been used in traditional folk medicine for centuries to maintain health or to treat various human diseases (Myagmar et al., 2004). Currently, much attention has been paid to traditional herbal medicine for treating liver diseases. This is due to: (i) conventional and/or synthetic drugs can cause serious side effects especially when used for prolonged periods of time and (ii) discovery and development of modern technology which led to isolation and purification of many active ingredients from medicinal herbs (Sahrawat et al., 2006). TQ, the major active compound derived from the medicinal plant Nigella sativa, had anticancer effects against several cell lines and animal models (AbuKhader, 2013). The seeds of N. sativa have long been used in traditional medicine for a wide range of illnesses, including headache, dysentery, infections, obesity, back pain, bronchial asthma, hypertension and gastrointestinal problems (Al-Rawai, 2002). TQ was shown to protect against hepatic damage (Mansour et al., 2001). For instance, Ogus et al., (2012) demonstrated that oral administration of TQ relieved bile duct proliferation, periductular fibrosis and hepatic damage in bile duct-ligated rats. Lebda et al., (2011) reported that TQ prevented liver enzyme leakage and lipid peroxidation induced by D-galactosamine in rats. Nagi et al., (2010) observed that TQ protected mice against acetaminophen-induced hepatotoxicity.

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Helal (2010) attributed these effects of TQ to its anti-inflammatory, anti-oxidant and anti-apoptotic properties. Nowadays, accidental hepatotoxicity that results from the exposure to toxic doses of hepatotoxins is significantly decreased due to increasing public awareness. On the other hand, mild hepatotoxicity results from some drugs, herbs or diets are popular and can chronically lead to apparent hepatic impairment. Accordingly, our current study tried to investigate the prophylactic effect of TQ against mild hepatic damage induced by oral administration of CCl₄ in SD rats.

**MATERIALS AND METHODS**

All experimental procedures in the present study were done in accordance with the guidelines of the European Union Council (86/609/EU) and the manuscript was approved by the ethical committee (Assiut University, 7/2015). In which, 40 adult male SD rats (180 – 230g) were obtained from the Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Rats were maintained under standard conditions with 12–h light/dark cycles and 22°C, and 60% humidity. The food in the form of dry chow pellets and water were available at libitum. CCl₄ (≥99.9%) and TQ were obtained from Sigma-Aldrich (Germany).

**Study design and treatment protocol**

After acclimatization for one week, rats were randomly divided into 4 groups (10 rats each). The first group served as control and received daily normal saline (0.5 ml/rat) by gastric tube. The second group received CCl₄ (2 ml/kg b.w.) twice a week on Sunday and Thursday by gastric tube (Singh et al., 2015). The third group was co-administered with CCl₄ (2 ml/kg b.w.) twice a week on Sunday and Thursday, and daily TQ (20 mg/kg b.w. in corn oil) by gastric tube.

The fourth group received daily TQ (20 mg/kg b.w. in corn oil) by gastric tube. After 5 weeks of treatment, rats were sacrificed, and blood samples and liver specimens were obtained for biochemical analysis and morphological examination, respectively.

**Measurement of serum ALT and AST activities**

Blood samples were allowed to clot and serum was obtained after centrifugation at 3000 rpm for 15 min. Serum ALT and AST activities were measured colorimetrically using ALT and AST assay kits according to the manufacturer (Sigma-Aldrich, Germany). Briefly, ALT and AST activity assays depend on the transfer of amino group from alanine and aspartate to α-ketoglutarate resulting in generation of pyruvate and glutamate, respectively.

Generated pyruvate and glutamate are proportional to the activities of ALT and AST in the serum and can be colorimetrically measured using automatic biochemical analyzer (Hitachi 902, Roche Diagnostics, Germany) at wavelengths of 570 and 450 nm for ALT and AST, respectively.

**Histopathological examination**

Five different specimens were obtained from the liver of each rat. Specimens were fixed in 10% neutral buffered formalin for 24 - 48 h, dehydrated in graded alcohol series, cleared in xylene and embedded in paraffin wax. Paraffinized specimens were cut at 4 µm and tissue sections (one section/each specimen) were stained with hematoxylin and eosin (HE) (Bancroft and Stevens, 1990).

The stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia-5060, Japan). The most common pathological findings were recorded.

**Transmission electron microscopy examination**

Also, five different specimens were obtained from the liver of each rat and immediately immersed in 2.5% gluteraldehyde solution for TEM. Specimens were then trimmed, fixed in gluteraldehyde solution in 0.1M sodium cacodylate buffer, pH 7.2, and placed in a thermal box cooled to 4°C for 2 h. They were post-fixed in 1% osmium tetroxide in a sodium cacodylate buffer and then dehydrated in ascending series of ethyl alcohol and embedded in Spurr’s resin.

Ultrathin sections stained with uranyl acetate and lead citrate were examined by TEM (JEOL100 CXII, Japan) operated at 80 KV in the Electron Microscopy Unit, Pathology Department, College of Medicine, King Khalid University (Bancroft and Stevens, 1990). The most common ultrastructural findings were recorded as present or present.

**Statistical analysis**

Data were presented as means ± standard error of means (SEM). When one-way ANOVA showed significant differences among groups, Duncan’s post hoc test was used to determine the specific pairs of groups that were statistically different. Analysis was performed using statistical program SAS 1998. p≤0.05 was considered as statistically significant.

**RESULTS AND DISCUSSIONS**

**Serum ALT and AST activities**

Treatment of rats with CCl₄ significantly increased serum ALT and AST activities by 14% and 19%, respectively, compared to control rats (Fig. 1). On the other hand, concomitant treatment of rats with TQ and CCl₄ significantly decreased serum ALT and AST activities by 12% and 14%, respectively, compared to CCl₄-treated rats (Fig. 1). TQ alone did not affect the activities of the serum enzymes (Fig. 1). In consistent, Cai et al., (2015) reported that elevation of serum ALT and AST activities is a constant finding following CCl₄ treatment in different experimental animal models. ALT and AST are cytoplasmic aminotransferases that release extracellularly and go into the circulation upon hepatocytes damage. They are commonly used biomarkers for measuring hepatic injury in both experimental and clinical studies (Goorden et al., 2013).
Histopathology

Light microscopic examination of HE-stained liver sections from control rats showed hepatic cells with a well-preserved cytoplasm and well-defined nuclei (Fig. 2A). The most common histopathological findings as the result of CCl₄ treatment were dilatation of blood sinusoids and portal blood vessels (Fig. 2B,C), activation of Kupffer cells (Fig. 2C), vacuolar degeneration of hepatocytes (Fig. 2D), focal areas of necrosis surrounded by a number of Kupffer cells (Fig. 3A) and mild portal fibrosis (Fig. 3B).

Co-administration of rats with TQ and CCl₄ attenuated CCl₄-induced histopathological changes (Fig. 3C,D) and decreased their incidence compared to rats treated with CCl₄ alone (Table 1). No apparent histopathological changes were seen in the liver of TQ-treated rats. In contrast to these mild hepatic changes, subcutaneous injection of CCl₄ (2 ml/kg b.w.) produced clear hepatic necrosis, inflammation, fatty accumulation and fibrosis after 12 weeks in rats (Tasci et al., 2008). Absence of severe histopathological changes including centrilobular necrosis and apparent fatty changes in our study was attributed to the oral route and short duration of CCl₄ treatment.

Table 1: Incidence of main hepatic lesions in treated rats (5 tissue sections/each treated rat).

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Figures</th>
<th>Control and TQ-treated rats</th>
<th>CCl₄-treated rats</th>
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<td><strong>Light microscopy:</strong></td>
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<tr>
<td>Portal vascular dilatation</td>
<td>Fig. 2B</td>
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<tr>
<td>Kupffer cell activation</td>
<td>Fig. 2C</td>
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<tr>
<td>Hepatic vacuolation</td>
<td>Fig. 2D</td>
<td>-</td>
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<td>Focal areas of necrosis</td>
<td>Fig. 3A</td>
<td>-</td>
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<tr>
<td>Hepatic fibrosis</td>
<td>Fig. 3B</td>
<td>-</td>
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<td><strong>Electron microscopy:</strong></td>
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<tr>
<td>Dilatation of endoplasmic reticulum</td>
<td>Fig. 4B</td>
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<td>Extracellular matrix</td>
<td>Fig. 4C</td>
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<td>Fatty globules</td>
<td>Fig. 4D</td>
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<td>Autophagosomes</td>
<td>Fig. 4E</td>
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- No lesions found in examined tissue sections
+ Lesions found clearly in 5 – 15 tissue sections/10 treated rats
++ Lesions found clearly in 16 – 25 tissue sections/10 treated rats
+++ Lesions found clearly in 35 – 50 tissue sections/10 treated rats
Electron microscopy

Normal hepatocytes appeared with intact cell membrane and many spherical mitochondria (Fig. 4A). The most common ultrastructural changes as the result of CCl_4 treatment were in the form of dilatation of endoplasmic reticula (Fig. 4B), increased extracellular matrix (Fig. 4C), and formation of numerous perinuclear fatty globules (Fig. 4D) and autophagosomes (Fig. 4E). Co-administration of TQ and CCl_4 relieved most of these changes and decreased their incidence compared to rats treated with CCl_4 alone (Table 1). In which, TQ relieved dilatation of endoplasmic reticula and deposition of extracellular matrix (Fig. 4F).

Effect of TQ against CCl_4-induced liver damage

Co-administration of TQ with CCl_4 significantly lowered the activities of ALT and AST, and decreased the incidence of histopathological and ultrastructural changes compared to CCl_4-treated rats. In consistent, Mansour et al., (2001) and El-Sayed (2011) reported that TQ significantly reduced CCl_4-induced ALT and AST elevation in mice and rats, respectively. Essawy et al., (2012) found that aqueous extract of *Nigella sativa* ameliorated most histopathological changes induced by CCl_4 in mice. This cytoprotective effect of TQ against CCl_4-induced hepatic damage was reported to be attributed to the antioxidant effects of TQ. In this context, El-Sayed (2011) reported that TQ increased the activities and mRNAs levels of some antioxidant enzymes such as glutathione S-transferase, NAD (P) H-quinoneoxidoreductase and microsomal epoxide hydrolase. Al-Ghamdi (2003) found that TQ produced potent antioxidant effect against CCl_4-induced free radical species.

In conclusion, the present study indicates that TQ has the potential to attenuate CCl_4-induced hepatic damage in SD rats. Moreover, the results support the role of TQ as a nutritional supplement to prevent liver maladies.

COMPETING INTERESTS

The authors declared no potential conflicts of interest.

REFERENCES


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