Journal of Applied Pharmaceutical Science Vol. 6 (02), pp. 167-171, February, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.60226
ISSN 2231-3354

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

Khaled M. A. Hassanein[1*], Ahmed Al-Emam[2], Khaled Radad[1]

[1]Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt. [2]Department of Forensic Medicine and Toxicology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

ARTICLE INFO

Article history:
Received on: 09/12/2015
Revised on: 27/12/2015
Accepted on: 12/01/2016
Available online: 27/02/2016

Key words:
Carbon tetrachloride, hepatotoxicity, histopathology, thymoquinone, ultrastructure.

ABSTRACT

This study is conducted to investigate the prophylactic effect of thymoquinone (TQ), the major active ingredient of *Nigella sativa* seeds, against carbon tetrachloride (CCl_4)-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 CCl_4, CCl_4 and TQ, and TQ only, respectively for 5 weeks. CCl_4 (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 CCl_4 significantly increased serum activity of ALT and AST aminotransferases compared to control rats. Histopathologically, livers from CCl_4-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), CCl_4 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 CCl_4 significantly decreased serum ALT and AST activities, and attenuated most of CCl_4-induced hepatic pathological changes. The present study indicates that TQ has the potential to attenuate CCl_4-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-Baghbanan *et al.*, 2014). Carbon tetrachloride (CCl_4), 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). CCl_4-induced hepatic damage is widely used for hepatoprotective drug screening. Hepatotoxicity of CCl_4 involves its biotransformation into free radicals such as trichloromethyl free radical (CCl_3) and trichloroperoxyl radical (CCl_3O_2-), 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 (Sehrawat *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-Rowais, 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, peri-ductular 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.

* *Corresponding Author*
Khaled M. A. Hassanein, Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.
Email: kh-hassanien@hotmail.com

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 CCl_4 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. CCl_4 (≥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 CCl_4 (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 CCl_4 (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 tetraoxide 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 signficant.

RESULTS AND DISCUSSIONS

Serum ALT and AST activities

Treatment of rats with CCl_4 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 CCl_4 significantly decreased serum ALT and AST activities by 12% and 14%, respectively, compared to CCl_4-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 CCl_4 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).

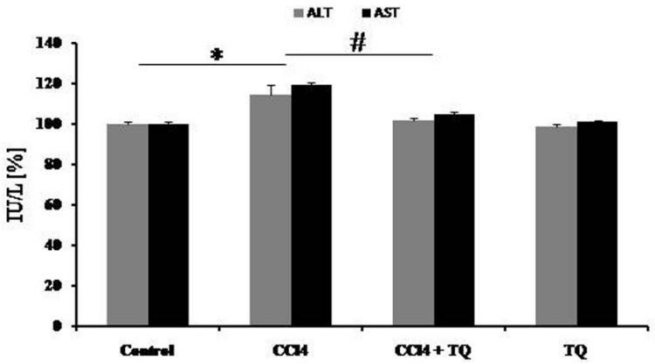

Fig. 1: Measurement of serum ALT and AST activities in treated rats. 100% corresponds to the activities of ALT and AST in the serum of control rats. Values represent the mean±SEM of the serum ALT and AST activities in treated rats. (∗p=0.01, #p=0.05)

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 CCl_4 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).

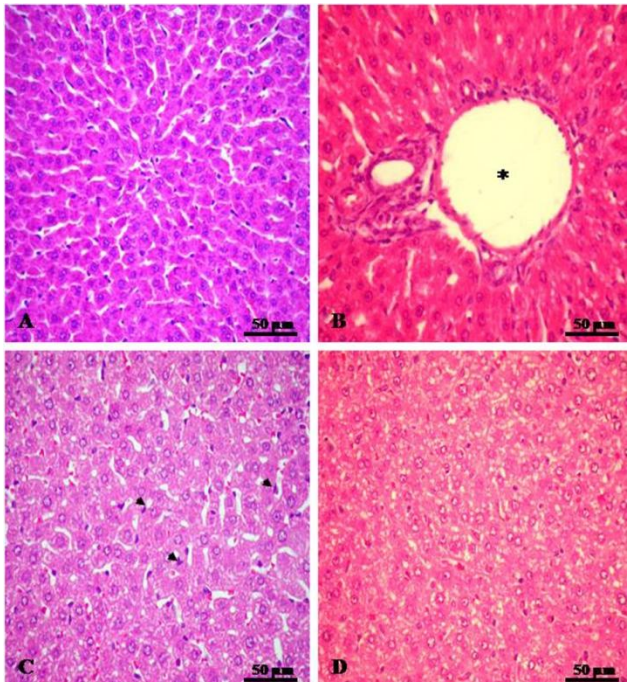

Fig. 2: Representative micrographs for histopathological changes in treated rats. **A)** Control rats showing normal hepatocytes with intact cytoplasm and centrally located vesicular nuclei. **B)** CCl_4-treated rats showing dilatation of a portal vein (asterisk). **C)** CCl_4-treated rats showing activation of Kupffer cells (arrow heads). **D)** CCl_4-treated rats showing vacuolar degeneration of hepatocytes. HE.

Co-administration of rats with TQ and CCl_4 attenuated CCl_4-induced histopathological changes (Fig. 3C,D) and decreased their incidence compared to rats treated with CCl_4 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 CCl_4 (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 CCl_4 treatment.

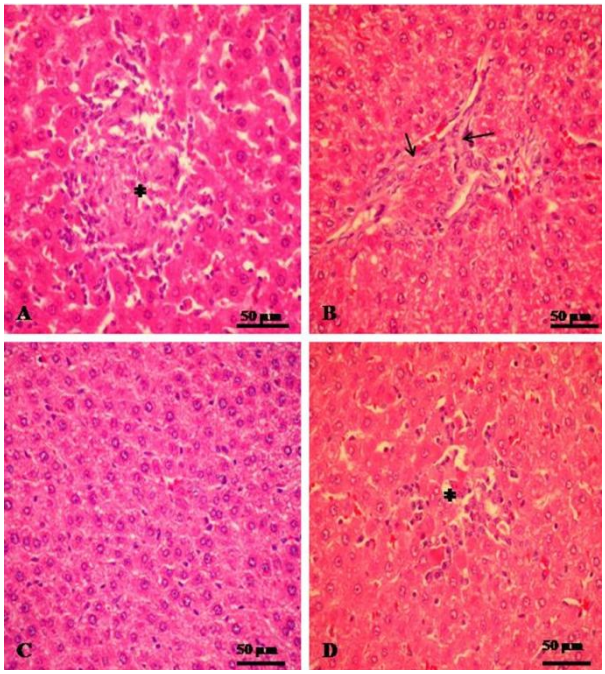

Fig. 3: Representative micrographs for histopathological changes in treated rats. **A)** CCl_4-treated rats showing a focal area of necrosis surrounded by a number of Kuppfer cells (asterisk). **B)** CCl_4-treated rats showing mild fibrosis in a portal area (arrows). **C,D)** Concomitant treatment of rats with TQ and CCl_4 showing marked decrease of hepatic vacuolation and Kupffer cell activation, and attenuation of focal areas of necrosis (asterisk) HE.

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

Lesions	Figures	Control and TQ-treated rats	CCl_4-treated rats	CCl_4+TQ
Light microscopy:				
Portal vascular dilatation	Fig. 2B	-	+++	+
Kupffer cell activation	Fig. 2C	-	+++	+
Hepatic vacuolation	Fig.2D	-	+++	+
Focal areas of necrosis	Fig. 3A	-	+++	++
Hepatic fibrosis	Fig. 3B	-	+++	++
Electron microscopy:				
Dilatation of endoplasmic reticulum	Fig. 4B	-	+++	+
	Fig. 4C	-	+++	+
Extracellular matrix	Fig.4D	-	+++	+
Fatty globules	Fig.4E	-	+++	+
Autophagosomes				

-	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 peri-nuclear 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).

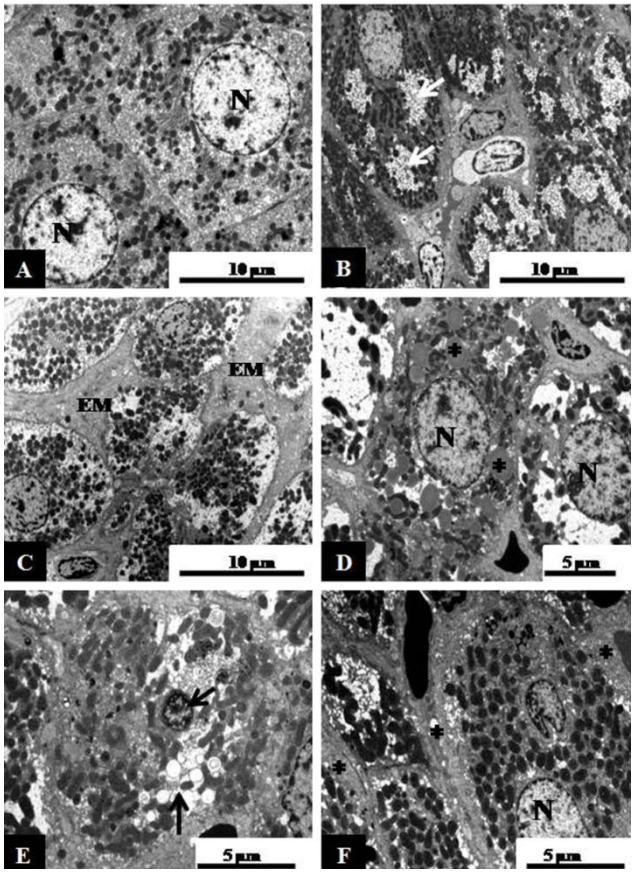

Fig. 4: Representative ultrastructural micrographs for hepatocytes from control and CCl_4-treated rats. **A)** Normal hepatocytes showing intact cell membrane, centrally located nucleus (N) and many spherical mitochondria. **B)** Hepatocytes from CCl_4-treated rats showing dilatation of endoplasmic reticula (asterisks). **C)** Hepatocytes from CCl_4-treated rats showing increase extracellular matrix (EM). **D)** A hepatocyte showing presence of numerous perinuclear fatty globules (asterisks). **E)** A hepatocyte showing presence of numerous autophagosomes (small arrows). **F)** Concomitant treatment of rats with TQ and CCl_4 showing milder ultrastructural changes compared to rats treated with CCl_4 alone. See mild dilatation of endoplasmic reticula and decreasing deposition of extracellular matrix (asterisks).

TQ-treated rats had no apparent changes by TEM. In consistent, Hsu (1998) and Knockaert *et al.,* (2012) reported that vesicular changes of the endoplasmic reticulum and large lipid droplets were prominent ultrastructural changes as the result of CCl_4 treatment in mice and rats, respectively. Increasing number of autophagosomes in the livers from CCl_4-treated rats seemed to result from sequestration of damaged intracellular organelles.

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

Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol, 2004; 5: 836-847.

Shamsi-Baghbanan H, Sharifian A, Esmaeili S, Minaei B. Hepatoprotective herbs, avicenna viewpoint. Iran Red Crescent Med J, 2014; 16: e12313.

Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/cmet signaling pathway is required for efficient liver regeneration and repair. Proc Natl Acad Sci USA, 2004; 101: 4477-4482.

Feng Y, Siu KY, Ye X, Wang N, Yuen MF, Leung CH, Tong Y, Kobayashi S. Hepatoprotective effects of berberine on carbon tetrachloride-induced acute hepatotoxicity in rats. Chin Med, 2010; 18: 33.

Myagmar BE, Shinno E, Ichiba T, Aniya Y. Antioxidant activity of medicinal herb rhodococcum vitis-idaea on galactosamine-induced liver injury in rats Phytomedicine, 2004; 11: 416-423.

Sehrawat A, Sharma S, Sultana S. Preventive effect of tannic acid on 2-acetylaminofluorene induced antioxidant level. tumor promotion and hepatotoxicity: a chemopreventive study. Redox Rep, 2006; 11: 85-95.

AbuKhader MM. Thymoquinone in the clinical treatment of cancer: Fact or fiction?. Pharmacogn Rev, 2013; 7(14): 117–120.

Al-Rowais NA. Herbal medicine in the treatment of diabetes mellitus. Saudi Med J, 2002; 23: 1327–1331.

Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol, 2001; 110: 239-251.

Ogus S, Kanter M, Erboga M, Erenoglu C. Protective effects of thymoquinone against cholestatic oxidative stress and hepatic damage after biliary obstruction in rats, J Mol Histol, 2012; 43: 151-159.

Lebda FM, Ahmed MA, Abd El Samad AA, Shawky MK. Protective effect of thymoquinone against D-galactosamine-induced liver injury in rats. Australian Journal of Basic and Applied Sciences, 2011; 5: 49-58.

Nagi M., Almakki HA, Sayed-Ahmed MM, Al-Bekairi AM. Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. Food Chemical Toxicology, 2010; 48: 2361-2365.

Helal GK. Thymoquinone supplementation ameliorates acute endotoxemia-induced liver dysfunction in rats. Pak J Pharm Sci, 2010; 23: 131-137.

Singh N, Khullar N, Kakkar V, Khaur IP. Sesamol loaded solid lipid nanoparticles: a promising intervention for control of carbon tetrachloride induced hepatotoxicity. BMC Complement Altern Med, 2015; 3: 142.

Bancroft JD, Stevens A. Theory and Practice of Histological Technique. Edinburg: Churchil Livingstone, 1990: 113-305.

Cai Z, Lou Q, Wang F, Li E, Fang H, Xi J, Ju L. N-acetylcysteine protects against liver injure induced by carbon tetrachloride via activation of the Nrf2/HO-1 pathway. Int J Clin Exp Pathol, 2015; 8: 8655-8662.

Goorden SM, Buffart TE, Bakker A, Buijs MM. Liver disorders in adults: ALT and AST. Ned Tijdschr Geneeskd,. 2013; 157: A6443.

Tasci I, Mas N, Mas MR, Tuncer M, Comert B. Ultrastructural changes in hepatocytes after taurine treatment in CCl_4 induced liver injury. World J Gastroenterol, 2008; 21: 4897-4902.

Hsu CT. Ultrastructural changes in liver damage induced by carbon tetrachloride in spontaneously hypertensive rats and Wistar-Kyoto rats. J Auton Nerv Syst, 1998; 28: 79-83.

Knockaert L, Berson A, Ribault C, Prost PE, Fautrel A, Pajaud J. Carbon tetrachloride-mediated lipid peroxidation induces early mitochondrial alterations in mouse. liver Lab Invest, 2012; 92: 396-410.

El-Sayed WM. Upregulation of chemoprotective enzymes and glutathione by *Nigella sativa* (black seed) and thymoquinone in CCl_4-intoxicated rats. Int J Toxicol, 2011; 30: 707-714.

Essawy AE, Abdel-Moneim AM, Khayyat LI, Elzergy AA. *Nigella sativa* seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice. J Applied Pharmaceutical Science, 2012; 2: 21-25.

Al-Ghamdi M. Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage. The American Journal of Chinese Medicine, 2003; 31: 721-728.

How to cite this article:

Hassanein KMA, Al-Emam A, Radad K. Prophylactic effects of thymoquinone against carbon tetrachloride-induced hepatic damage in Sprague-Dawley rats. J App Pharm Sci, 2016; 6 (02): 167-171.