ORIGINAL ARTICLE

Protective effect of *Nigella sativa* against carbon tetrachlorideinduced acute liver injury in experimental rabbit models

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Acute liver injury is a serious state of extensive damage of liver tissue caused by various reasons. In traditional medicine, certain medicinal plants have been used to cure and prevent some liver diseases. The objective of this study was to evaluate the hepatoprotective activity of oil extract of *Nigella sativa* seeds in rabbit models with induced acute liver injury. Carbon tetrachloride (CCl_4) was used to induce hepatotoxicity at a dose of 1.25 ml/kg as a mixture with olive oil. *N. sativa* oil extract was administered at a dose of 0.2 ml/kg/day orally for 7 days. The hepatoprotective effect was assessed by liver function tests and histopathological sections of the liver. Significant reduction in the liver damage was found in animals treated with the extract, as indicated by low levels of serum enzymes, serum bilirubin and improvement of serum protein. Besides, restoration of hepatocellular architecture was evident, as indicated by the presence of normal hepatic vessels, absence of necrosis, and fatty infiltration. The oil extract of *N. sativa* seeds showed significant hepatoprotective activity.

Key words: Black cumin, hepatocellular architecture, nigellone, thymoquinone

INTRODUCTION

Acute liver injury (ALI) is a clinical condition that results from severe and extensive damage of the hepatocellular tissue, with reduced cell mass and blood flow. It is associated with increase in serum alanine aminotansferase (SALT), serum aspartate aminotansferase (SAST), total serum bilirubin (TSB), and serum alkalanine phosphatase (SALP).[1] In the absence of effective hepatoprotective drugs in modern medicine, a number of medicinal plants in traditional medicine, like *Nigella sativa*, have been used to cure and prevent some liver diseases.[2]

N. sativa (black cumin) is an annual flowering plant, native to Southwest Asia, which grows to a height of 20–30 cm with linear leaves. Its capsule is composed of 3–7 united follicles, each containing numerous seeds. The black seeds are extensively used as a spice to flavor food.[3] *N. sativa* oil contains[4] nigellone (protects from histamine-induced bronchial spasms), nigelline (a paralytic agent in large doses), beta-sitosterol (antitumour sterol), and thymoquinone (blocks pancreatic cancer cell growth).

Therefore, it is interesting to evaluate the potential hepatoprotective effects of this plant in experimental rabbit model of acute liver injury induced by a hepatotoxic agent (carbon tetrachloride (CCl_4)).[5]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.91227

MATERIALS AND METHODS

Chemicals

All chemicals used in the study were of analytical grade. CCl_4 was procured from Merck India Ltd., India. The kits for the estimation of SALT, SAST and total serum protein (TSP) were from Dialab, Austria. Kits for TSB and SALP were from Biolabo SA, Maizy, France.

Plant Extraction

The test medicinal plant was purchased from a well-known herbal shop (Al-Medina) in Baghdad and was identified and authenticated by Iraqi National Institute for Herbs. The seeds of black cumin were cleaned, dried, then squeezed with an electrical squeezer.

The squeezed out oil was passed through sieve no. 40 to remove the debris. The sieved oil was stored in an airtight and black container at room temperature. Then,

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Received: 02-08-2011; **Accepted:** 19-08-2011

it was allowed to stand to be used for laboratory purposes. The oil extract should be used within 12 hours.[6]

Animals

Eighteen local domestic rabbits (850–1050 g) were supplied by animal house of Baghdad College of Medicine. They were housed in separate cages provided with a wide wire mesh floor and and were maintained at a controlled temperature of 28°C with a 12-hour light/dark cycle. They were fed standard oxoid pellets and water *ad libitum*. The study was conducted according to the guidelines of the Animal Ethics Committee of the College of Pharmacy, Al-Yarmouk University (Approval No. AEC/322/10/CPAYU).

Animals were randomly allocated to three groups of six animals each. Group I served as control and received normal saline 3 ml p.o. as a single daily dose. Group II received distilled water 3 ml p.o. as a single dose, 2 hours before the administration of CCl_4. Group III was given oil extract of black cumin 0.2 ml/kg p.o. as a single daily dose, 2 hours before CCl_4 administration and continued receiving it for 7 days. At 11.00 a.m., Groups II and III were given CCl_4 as 1:1 (v/v) mixture of CCl_4 in olive oil at a dose of 1.25 ml/kg p.o. for induction of ALI. On day 8, blood samples were collected from marginal ear vein of the animals of all the groups for biochemical analysis of SALT, SAST, SALP, TSB and TSP using spectrophotometer method,[7] for comparison with the values of the samples collected before induction. Then, all the animals were sacrificed under light ether anaesthesia to have liver specimens. Histopathological examination was carried to check for hepatocellular changes using polarised microscope after fixating the sections in 10% formalin for 48 hours and staining with haematoxylin and eosin dye.[8]

Statistical Analysis

All the results were expressed as mean ± SEM. The difference among means was analysed by student's *t* test.[9] A probability value of $P<0.05$ was considered to be statistically significant.

RESULTS

Administration of CCl_4 to the animals resulted in a marked increase in TSB, SAST, SALT, and SALP, while TSP decreased when compared with the control group. Group III which was given the oil extract of *N. sativa* showed a reduction in the SALT, SAST, SALP, and TSB levels. It also reversed the depletion of total protein significantly when compared with Group II (group administered CCl_4) [Table 1].

Black cumin had a strong effect in lowering SALT and SAST levels faster than its effect on the other parameters, especially on 4th post-induction day, with levels equal to 33.66±5.2 and 34.5±5.98, respectively, compared to 38.31±1.71 and 41.09±4.15, respectively, of the control rats.

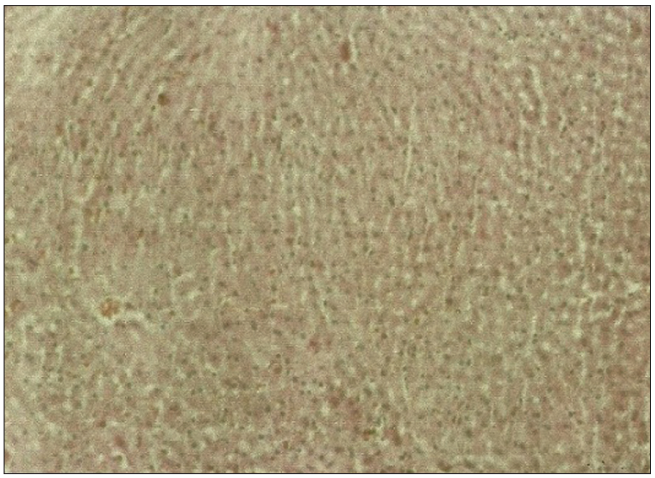

Figure 1: Normal rabbit liver section shows hepatocyte architecture with normal lobular appearance (H and E stain, ×10)

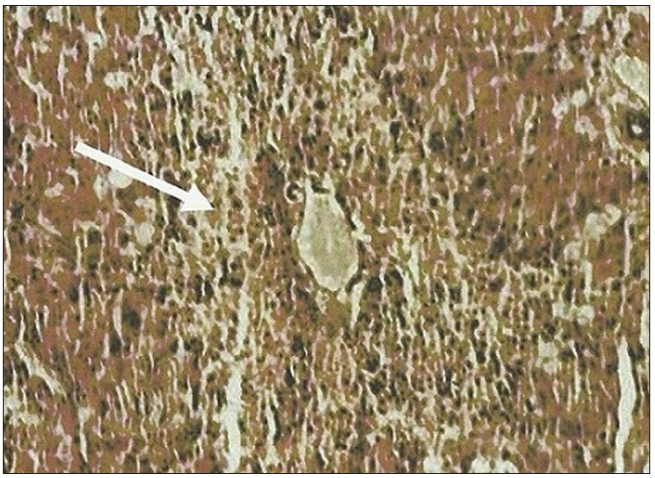

Figure 2: Rabbit liver section after administration of $CC1_4$ as a single oral dose, showing massive necrosis, fatty change, lymphocyte infiltration and congestion (H and E, ×10)

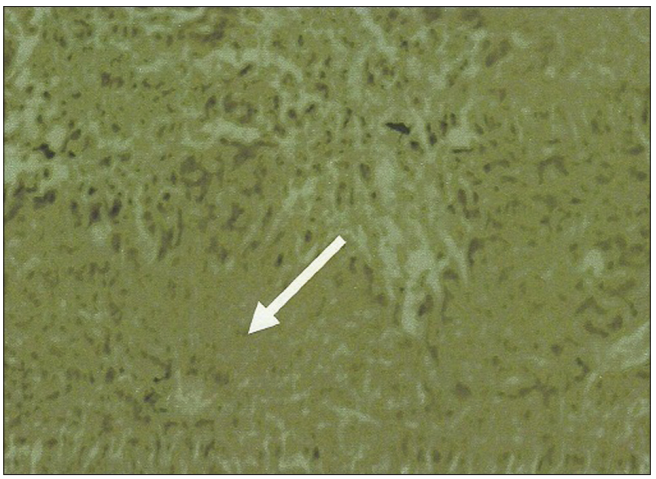

Figure 3: Rabbit liver section treated with black cumin oil showing mild necrosis, mild fatty change, mild inflammatory infiltration and no congestion (H and E stain, ×10)

The histological studies of liver sections support the results obtained from serum enzyme assays which showed normal

Table 1: Effect of *N. sativa* extract on CCl_4-induced hepatotoxicity in rabbits

Groups	SALT (U/L)	SAST (U/L)	SALP (U/L)	TSB (µmol/L)	TSP (g/dL)
I (control)	38.31±1.71	41.09±4.15	49.66±2.53	55.91±0.36	09.7±0.36
II (CCl_4 only)	140.3±1.80*	173.8±1.99*	291.73±7.99*	63.26±0.49	07.3±0.13*
III (*N. sativa* + CCl_4)	33.66±5.2*	34.5±5.98**	49.06±0.13*	54.27±0.95**	8.95±0.07**

Values are mean ± SEM, n=6. *P<0.01 compared with Group I; **P<0.05 compared with Group II. CCl_4 – Carbon tetrachloride; SALT – Serum alanine aminotansferase; SAST – Serum aspartate aminotansferase; SALP – Serum alkalanine phosphatase; TSB – Total serum bilirubin; TSP – Total serum protein

hepatic architecture [Figure 1] in Group I, whereas liver sections of Group II showed total loss of hepatic architecture with massive fatty changes, congestion of sinusoids, intense necrosis, and infiltration of the lymphocytes around the central vein [Figure 2]. The histological architecture of liver sections of group showed a more or less normal lobular pattern with a mild degree of fatty change, cell necrosis, and lymphocyte infiltration, indicating the protective effect of the plant extracts [Figure 3 and Table 1].

DISCUSSION

Hepatotoxic compounds like CCl_4 are known to be used for induction of hepatotoxicity in experimental animal models. It is biotransformed in the cytochrome P450 system to its metabolite "trichloromethyl free radical (CCl_3)", which in the presence of oxygen forms trichloromethyl peroxyl free radical (CCl_3O_2) that attacks lipids of endoplasmic reticulum, eliciting lipid peroxidation with the leakage of hepatocellular enzymes like SALT, SAST, and SALP in the serum, and causing an increase in serum TB levels and decrease in serum total protein.[10] Administration of oil extract of *N. sativa* to CCl_4-intoxicated animals showed significant hepatoprotective activity by restoring the hepatocellular activity. It has been reported that thymoquinone (the active component of black cumin), presents in high levels in *N. sativa*, possesses antioxidant properties.[11] Thymoquinone prevents the formation of toxic stable complex by a combination of CCl_3O_2 free radical and the glycolipid component of cell membrane, and therefore restores cellular architecture and prevents the leakage of its enzymes.[4] Also, it is found that beta-sitosterol, a component of black cumin, blocks the bioactivity of CCl_4 by inhibiting the activity of P4502E1 (the enzyme responsible for CCl_4 metabolism), thereby preventing the hepatoperoxidation.[12]

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How to cite this article: Al-Razzuqi RA, Al-Hussaini JA, Al-Jeboori AA. Protective effect of Nigella sativa against carbon tetrachlorideinduced acute liver injury in experimental rabbit models. Int J Green Pharm 2011;5:198-200.

Source of Support: Nil, **Conflict of Interest:** None declared.