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Nigella sativa seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective effects of aqueous seed extract of *Nigella sativa* (black seed) on carbon tetrachloride (CCl4) induced liver damage in Swiss albino mice. CCl4 (1.9 ml/kg b.w.) was given orally every other day for three weeks. Aqueous suspension of *N. sativa* seeds (50 mg/kg b.w.) was given orally every other day alternated with CCl_4 for three weeks. Blood was collected for the assessment of serum marker enzymes (ALT, AST ,ALP and LDH) and lipid profile(triglycerides, total cholesterol, HDL and total lipids), while liver tissue was used for histopathological assessment. Serum levels of liver enzymes and lipid contents were significantly increased in animals treated with CCL4 as compared to control. Histopathological observations, also revealed severe damage in the structure of liver tissue in animals intoxicated with CCL4. Combined treatment of CCL4-exposed animals with *N.sativa* showed marked improvement in both biochemical and histopathological findings. Only LDH did not affect by *N. sativa* administration.

INTRODUCTION

Liver is an important body organ and is actively involved in different metabolic functions (Meyer and Kulkarni, 2001). Hepatic damage caused by chemicals (or) infectious agents is associated with distortion of these metabolic functions (Wolf, 1999; Cullen, 2005) and may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure (Anand, 1999). However, no effective treatment that delays these complications has yet been found. Several recent studies suggest that traditional herbs and micronutrients may be useful for this purpose (Hinds *et al*., 1997; We *et al*, 2004). Carbon tetrachloride CCl4 is widely used for experimental induction of liver damage (Parola , 1992; Thrall *et al*. 2000). The principle causes of carbon tetrachloride (CCl4) induced hepatic damage are increased lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals (Poli, 1993; Ohta *et al*., 2000).

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Rajesh and Latha, (2004) showed that various herbal extracts could protect organs against CCl4 induced oxidative stress by altering the levels of increased lipid peroxidation and enhancing the decreased activities of antioxidant enzymes. *N. sativa* is an annual herbaceous plant that belongs to the family (Ranunculaceae) . It contains both fixed and essential oils, proteins, alkaloids and saponin. Thymoquinone, the major component of the essential oil, is the biologically active gradient of this plant (Ali and Blunden, 2003) . It was reported that, thymoquinone(TQ) has many medicinal properties like; anti-oxidant (Meral *et al*., 2001; Shalaby and khater, 2007; Zegarac *et al*., 2009), anti-inflammatory (Hajhashemi *et al*., 2004), anti-tumor (Banerjee *et al*., 2010), analgesic and other properties (Kaleem *et al*., 2006; Bhatti *et al*., 2009). On the other side, Kapoor (2009) proved that TQ protects hepatic tissue from deleterious effects of toxic metals such as lead, and attenuates hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride. However, in spite of the large number of the antioxidant and hepatoprotective studies carried out worldwide on *N. sativa* oil and TQ, scrutiny of published articles (Al-Ghamdi, 2003) showed that there is a need to investigate the

effect of whole *N. sativa* seeds, especially since traditionally the whole seeds are used for treatment in folk medicine rather than the oil extract or TQ. Therefore, the main objective of this study is to find out if aqueous extract of whole *N. sativa* seeds possesses protective effect against CCL4-induced hepatotoxicity in experimental animals.

MATERIALS AND METHODS

Chemicals used

CCl4 (98.8% purity) was purchased from El-Nasr Pharmaceutical Chemical Company (Egypt). *N. sativa* seeds (black seed) were purchased from a local herb grocery (Egypt). Seeds were cleaned, air-dried and were then powdered mechanically to prepare a suspension in isotonic saline solution. The suspension (1.25 g powder of *N. sativa* + 100 ml isotonic saline) was freshly prepared and left a few minutes before administration. Olive oil (Laboratory grade) was obtained from Sigma Chemical Co. (St. Louis, MO). It had been used as a vehicle for carbon tetrachloride.

Experimental animals

Ten weeks old laboratory male Swiss albino mice weighing about25 g each, were used. Animals were housed in plastic cages in an animal room under controlled temperature (23±2°C), and 12 h photoperiod (12 h light/dark cycle). They were given free access to a commercial pellet diet and tap water, and allowed to acclimatize for two weeks before treatment.

Experimental design

The animals were randomly divided into three groups of 10 mice each:

- **Group 1:** Each animal had orally received 0.9% isotonic saline solution at a dose level 4 ml/kg b.w. every other day for three successive weeks and served as a control group.
- **Group 2:** Each animal had orally received CCl_4 at a dose level of 1.9 ml/kg b.w. (¼LD50) mixed in olive oil every other day for three successive weeks.
- **Group 3:** Each animal had orally received suspension of *N. sativa* at a dose level of 4 ml/kg b.w. (50 mg/kg b.w.) every other day alternated with CCl_4 for three successive weeks.

Twenty four hours after the end of experimental period, unanesthetized mice from both control and experimental groups were sacrificed by slaughtering. Peripheral blood samples were collected from the neck blood vessels. Serum was separated out by centrifugation at 3000 xg for 10 min. Serum samples were used to determine ALT, AST, ALP and LDH activities as well as lipid profile. On the other hand, the liver of each mice was promptly removed for further histopathological study.

Assessment of liver enzymes and lipid contents in serum

Liver enzymes AST, ALT, ALP, LDH and lipid profile - triglycerides, total cholesterol, HDL and total lipids, were determined using commercially available diagnostic kits (Biomerieux SA, France).

Histopathological studies

Each sample of liver obtained was washed in saline and fixed in 10% formalin for the routine histological technique. Fixed tissues were embedded in paraffin wax, sectioned in rotary microtome (5 µm thick) and then stained with haematoxylin and eosin (H&E dye). At least three different sections were examined per each sample of liver. Score system was used for histopathological examinations. Vacuolar degeneration, inflammatory cell infiltration, congestion and necrosis was used as criteria. The parameters were graded as follow: 0 = no abnormality, + = mild injury, ++ = moderate injury and +++ = severe injury (Murat Bilgin *et al*., 2011).

Statistical Analysis

The data (expressed as mean ± SE) were analyzed by one way ANOVA and LSD post hoc test using SPSS software. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Biochemical measurements

Table 1 represents the effects of CCl4 and *N. sativa* on liver function tests. Compared with the control group, the ALT, AST, ALP and LDH activities in serum of mice treated with CCl4 every other day at an oral dose of 1.9 ml/kg b.w. were significantly elevated after three weeks. The orally administrated *N. sativa* at a dose of 50 mg/kg b.w could significantly decreased the ALT, AST and ALP activities in contrast to CCl4 treated mice, but *N. sativa* did not show effect on LDH activity in CCl4 treated mice.

Effect of CCl4 and *N. sativa* on blood lipid profile are presented in Table 2. CCl4 administration increased triglyceride, cholesterol, HDL and total lipids contents in serum ($p<0.05$). While treatment with *N. sativa* could significantly reduce hyperlipidemic values in CCl4 treated mice and restored them to control levels.

Histological examinations

Liver sections from control mice showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus, central vein and compact arrangement of hepatocytes (Fig. 1). In contrast to this, CCl4 caused hydropic changes and necrosis in centrilobular hepatocytes (Fig. 2). Congestion of the central vein and sinusoids were seen with acute inflammatory cells infiltrating mainly in the central zone. The midzonal and peripheral hepatocytes showed vacuolization and fatty change (steatosis) which included the intracellular accumulation of neutral fats (Fig. 3). Derangement of hepatocyte cords were also determined. In animals treated with CCl4 plus *N.* sativa, tissue damage and necrosis were of less extent (Fig. 4) than the CCl4 group. No derangement was observed at hepatocyte cords (Fig.5). The scoring of histological damage is displayed in Table 3.

Table. 1: Effect of *N. sativa* on CCl_4 induced liver damage (mean±SE, n=5)

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (U/L)
Control	46.33±2.60	88.33±18.93	91.67±8.09	624.33±57.89
CCl_4	198.67±11.20*	526.67±49.78*	175.33±6.89*	2826.67±142.52*
CCl_4 + *N. sativa*	85.33±20.57$^{*,\#}$	170.33±26.14$^{*,\#}$	138.00±17.52$^{*,\#}$	2304.66±250.30*

* p < 0.05 compared with control
# p < 0.05 compared with CCl_4

Table 2: Effect of *N. sativa* on CCl_4 induced deviation of blood lipid contents (mean±SE, n=5)

Group	Triglyceride (mg/L)	Cholesterol (mg/L)	HDL (mg/L)	LDL (mg/L)	Total lipid (mg/L)
Control	73.33±5.24	87.33±7.75	34.33±1.76	28.67±8.66	337.66±16.71
CCl_4	157.67±10.47*	130.00±5.13*	67.34±2.60*	40.67±7.62*	600.33±27.14*
CCl_4 + *N. sativa*	88.34±12.86$^{\#}$	102.22±2.84$^{\#}$	42.66±2.60$^{\#}$	31.33±2.18	420.00±17.61$^{\#}$

* p < 0.05 compared with control
# p < 0.05 compared with CCl_4

Table . 3: Semiquantitive score of histopathological findings

Group	Hydropic degeneration	Liver steatosis	Inflammatory cell infiltration	Congestion	Necrosis
Control	0	0	0	0	0
CCl_4	+++	++	+++	+++	+++
CCl_4 + *N. sativa*	+	0	+	++	+

Damage grade are as follow: 0 (absent), + (mild), ++ (moderate) and +++ (severe).
0 = no abnormality, + = mild injury, ++ = moderate injury and +++ = severe injury.
The livers of ten animals in each group were examined.

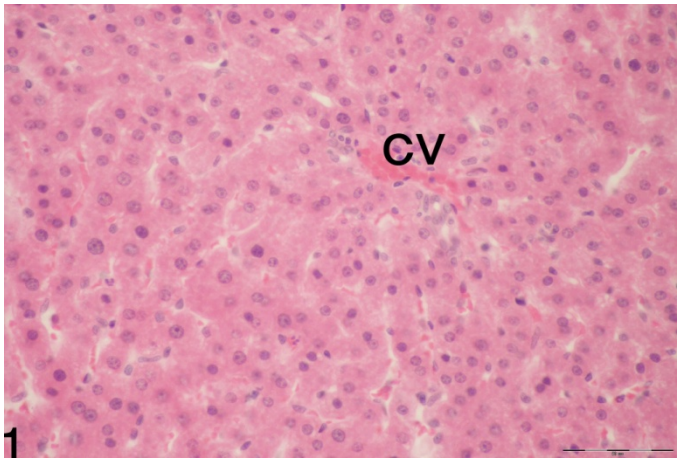

Fig. 1: Control mouse liver showing no pathological changes in which the normal hepatic cells are arranged in cords separated by widened sinusoids. CV = central vein. Scale bar: 50 µm.

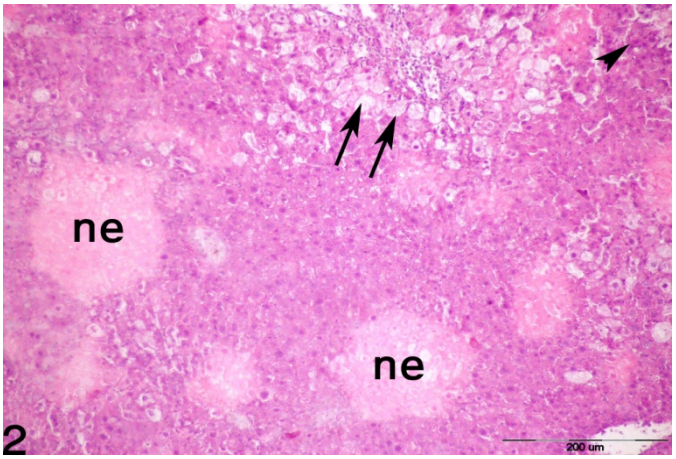

Fig. 2: Section of intoxicated mouse liver with CCl_4 showing hydropic degeneration (arrows), lymphocyte infiltration (arrowhead), and necrosis (ne). Congestion of central vein can be noted. Scale bar: 200 µm.

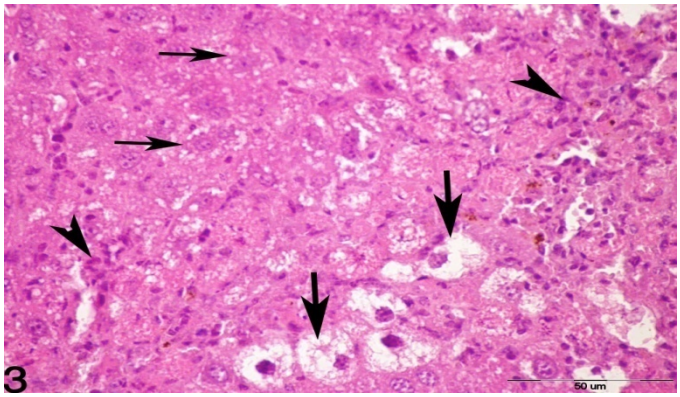

Fig. 3: Section of intoxicated mouse liver with CCl_4 showing hepatocellular damage (thick arrows), aggregations of inflammatory cells (arrowheads), and liver steatosis (thin arrows). Scale bar: 50 µm.

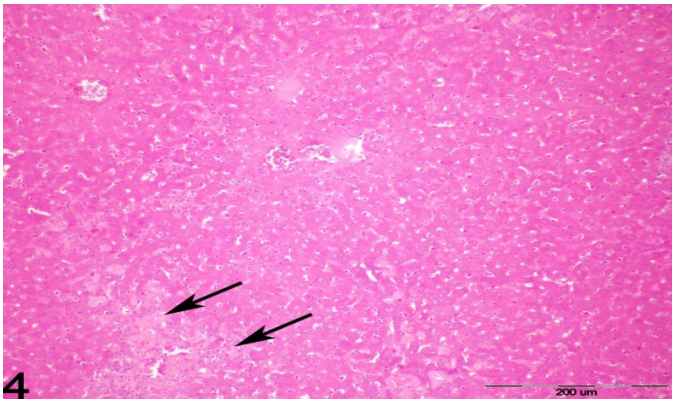

Fig. 4: Section of CCl_4 + *N. sativa* treated mouse showing improvement in the liver tissue. Arrows indicate focal hepatocellular necrosis. Scale bar: 200 µm.

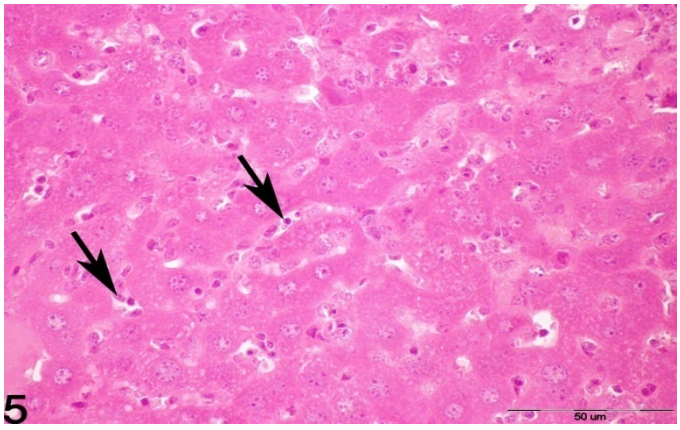

Fig 5: Section of CCl_4 + *N. sativa* treated mouse showing significant liver protection as evident by the presence of normal hepatic cords with moderated sinusoidal congestion (arrows), few inflammatory cells and absence of liver steatosis. Scale bar: 50 µm.

DISCUSSION

This study was undertaken to demonstrate the protective ability of aqueous suspension of *N. sativa* seeds against liver damage induced by CCl4 in Swiss albino mice.

In the current work, damage of the liver caused by CCl4 was evident by the alteration in serum marker enzymes and lipid profile concentrations beside the histopathological changes in liver tissue. Administration of CCL4 significantly increased the serum levels of liver enzymes (AST, ALT, ALP) and LDH, which are indices of liver cell damage and leakage of enzymes from cells (Rajesh & Latha, 2004 ; Bashandy & Al Wasel, 2011). Ravikumar *et al*., (2005) reported that, rise in ALT activity is almost always due to hepatocellular damage and is usually accompanied by rise in AST and ALP.

CCL4 is found to produce free radicals, which affect the cellular permeability of hepatocytes leading to elevated levels of liver enzymes (Kumar *et al*., 2009). On the other hand, the activity of serum lipid profile such triglycerides, total cholesterol, HDL and total lipids was elevated, and this indicates deterioration in hepatic function due to the damage caused by CCl4 administration.

Histopathological studies also support the biochemical analysis. Examination of liver sections of mice received CCL4 revealed disruption of the normal structural organization of the hepatic lobules and loss of the characteristic cord-like arrangement of the normal liver cells.. Many hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization. The nuclei of these cells were pyknotic. Leukocyte infiltration and fatty deposition were also evident. Previous results showed that, CCL4 induced centrilobular hepatocellular vacuolar degeneration and necrosis (Trivedi & Mowat, 1983; Berman *et al*., 1992 ; Brandao *et al*. 2000). The CCL4 induced hepatotoxicity has been referred to the excessive formation of free radicals formed during its detoxification in the hepatocytes smooth endoplasmic reticulum by the cytochrome P450 (Wang *et al*., 1997). Balahoroglu *et al*. (2008) reported that production of lipid peroxidation induced by CCL4 may lead to changes in biological membranes which result in serious cellular injury in liver.

Treatment with *N. sativa* seeds significantly declined the effects of CCl4 induced damage and it was evidenced by the decreased level of liver enzymes and lipid profile and restoration of hepatocellular architecture.

Similarly, Al-Razzuqi *et al.* (2011) had reported protective effect of oil extract of *N. sativa* seeds against carbon tetrachloride induced acute liver injury in experimental rabbit models. Also, protective effect of black seed oil against lead acetate-induced hepatic tissue damage in mice was investigated (Alarifi*et al*., 2o12). Thymoquinone, the active constituent of *N. sativa*, has been well documented as a potent antioxidant, particularly against the CCl4-induced free radical species (Al-Ghamdi , 2003). Thymoquinone prevents the formation of toxic stable complex by a combination of CCl 3 O 2 free radical and the glycolipid component of cell membrane, and therefore restores cellular architecture and prevents the leakage of its enzymes. (Al-Ali *et al*., 2008).

The present bio-chemical and histological results proved that *Nigella sativa* seeds possess potential to protect the liver tissue against oxidative damages and could be used as an effective protector against CCl4 induced liver damages.

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