Role of black seeds (Nigella sativa) in ameliorating carbon tetrachloride induced haematotoxicity in Swiss albino mice

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The effect of the oral administration of an aqueous suspension of Nigella sativa (50 mg/kg b.wt.) against morphological, cytological and biochemical alterations of blood cells in mice treated with carbon tetrachloride (CCl₄) was studied. CCl₄ was administered at a dose of 1.9 ml/kg b.wt (¼ LD50) every other day for three successive weeks. The results indicated significant changes in the haematologic parameters in animals intoxicated with CCl₄. Morphological and ultrastructural alterations including both nucleus and cytoplasm of peripheral blood cells were also recorded. Treatment of the animals with N. sativa improved both physiological and structural changes induced by CCl₄.

Key words: Carbon tetrachloride, Nigella sativa, mice, blood cells, haematological disorders, cytopathology.

INTRODUCTION

Carbon tetrachloride (CCl₄) is a haloalkane used in a variety of industrial and chemical applications. It has been widely used for its solvent properties, particularly in refrigerator fluids, as a propellant for aerosol cans, as a dry-cleaning agent in industry, as a household spot remover, as grain fumigant and as intermediate in the synthesis of chlorofluorocarbons. As a result of its widespread use, CCl₄ is a common contaminant of ground and surface waters where it persists for years. Therefore CCl₄ is now of greatest concern as an environmental contaminant (ATSDR, 1994; Guo et al., 2000). Nigella sativa L. is a plant of Ranunculaceae family that grows spontaneously and widely in several southern Mediterranean and Middle Eastern countries (Tariq, 2008). N. sativa seed has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. Although it is the oil that is most often used medicinally, the seeds are a bit spicy and are often used whole in cooking curries, pastries and Mediterranean cheeses (Tariq, 2008).

The seeds of N. sativa, also known as black seed or black cumin, are often used as a spice but are also used extensively in the traditional medicine of many countries (Meddah et al., 2009). N. sativa has been used traditionally for the treatment of many diseases owing to the reported antiviral (Salem and Hossain, 2000), antibacterial (Kanter et al., 2003), anti-inflammatory (Hajhashemi et al., 2004), antidiabetic (Kanter et al., 2004), immunomodulatory (Tekeoglu et al., 2007), anti-schistosomiasis (El Shenawy et al., 2008), and hepatoprotective (El-Gharieb et al., 2010) activities. Furthermore, it was found that N. sativa extract has anti-tumor properties (Essawy et al., 1997; Worthen et al., 1998; Khan et al., 2003; Hussein et al., 2005; Mbarek et al., 2007), attenuates toxic side effects caused by several chemotherapeutic agents (Uz et al., 2008) and protects against gentamicin-induced nephrotoxicity (Yaman and Balikci, 2010). In addition, it prevents hippocampal neurodegeneration after chronic toluene exposure in rats (Kanter, 2008). From the experimental and clinical

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studies performed on *N. sativa*, it seems that most of its pharmacological actions are due to its antioxidant activity which is mainly due to its ability to scavenge free radicals and/or inhibit lipid peroxidation (Gupta et al., 2004). In spite of these observations, the protection offered by black seed or its components against toxicity in haematopoietic organs remains incomplete and needs further work to be elucidated. Thus, the objective of this study was designed to determine whether the administration of *N. sativa* into animals intoxicated with CCl₄ would improve the possible induced cellular damage in peripheral blood cells of Swiss albino mice.

**MATERIALS AND METHODS**

**Experimental animals**

Ten weeks old laboratory male Swiss albino mice weighing about 25 g each, were obtained from breeding colony at University of Tanta, Egypt. 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), with a light from 0600 to 1800 h and darkness from 1800 to 0600 h. They were given free access to a commercial pellet diet and tap water, and allowed to acclimatize for two weeks before treatment.

**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, 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 design**

The animals were randomly divided into five experimental groups of 40 mice each:

- **Group 1**: Each animal had orally received 0.9% isotonic saline solution at a dose level 4 ml/kg b.wt. every other day for three successive weeks and served as a negative control group.

- **Group 2**: Each animal had orally received olive oil at dose level of 4 ml/kg b.wt. every other day for three successive weeks and served as a positive control (vehicle).

- **Group 3**: Each animal had orally received suspension of *N. sativa* at a dose level of 4 ml/kg b.wt. (50 mg/kg b.wt.) every other day for three successive weeks.

- **Group 4**: Each animal had orally received CCl₄ dissolved in olive oil at a dose level of 1.9 ml/kg b.wt. (¼ LD₅₀) every other day for three successive weeks.

- **Group 5**: Each animal had orally received suspension of *N. sativa* at a dose level of 4 ml/kg b.wt. (50 mg/kg b.wt.) every other day alternated with CCl₄ at a dose level 1.9 ml/kg b.wt. (¼ LD₅₀) for three successive weeks. Twenty four hours after the end of experimental period, unanaesthetized mice from both control and experimental groups were sacrificed by slaughtering. Peripheral blood samples were collected from the neck blood vessels.

**Haematological studies**

Anticoagulated blood samples were used for the determination of erythrocytic count, leucocytic count, haemoglobin content and haematocrit value according to Dacie and Lewis (1975). The platelets number was counted and calculated according to Baker and Silverton (1980). Well-spread blood films were stained by Leishman’s stain, examined under oil immersion and the selected sections were photographed.

**Preparation of the blood for transmission electron microscopy (TEM)**

Following centrifugation of blood sample, plasma was removed gently with a pipette to avoid disturbing the buffy coat. About 2 ml of 4 F1G buffered with 0.1 M phosphate buffer was added drop by drop. The buffy coat was allowed to stand for 18 h at 4°C. 1 mm slices of the plug were cut into smaller pieces. Specimens were then postfixed in 2% OsO₄ at 4°C for 2 h, dehydrated in graded series of ethanol and embedded in Epon-araldite mixture in labeled beam capsules. LKB ultramicrotome was used to obtain ultrathin sections (50 nm thick) which were picked upon 200 mesh naked copper grids. Grids were double stained with uranyl acetate for ½ h and lead citrate for 20-30 min. Scoping the grids was achieved by using Jeol 100 CX TEM.

**Statistical analysis**

Data were expressed as means ± SE. The results were computed statistically (SPSS software package, version 10) using one-way analysis of variance (ANOVA). Post-hoc test was performed for inter-group comparison using the LSD. Values of *p*<0.05 were considered statistically significant.

**RESULTS**

**Effect of CCl₄ and/or *N. sativa* on blood picture findings**

The erythrocytic count, haemoglobin content and haematocrit value decreased significantly (*p*<0.05) in CCl₄ intoxicated animals as compared to control (Table 1). In addition, MCV and MCH decreased insignificantly (*p*>0.05) while MCHC increased insignificantly (*p*>0.05) after administration of CCl₄. Obvious thrombocytopenia was found in the same intoxicated animals. Marked leucopaenia in mice treated with ¼ LD₅₀ of CCl₄ was detected. In differential WBC count, CCl₄ treatment affected the neutrophils mainly and resulted in a marked neutropenia (Table 2). Furthermore, significant (*p*<0.05) elevation in the percentages of lymphocytes and monocytes was quite evident. Co-administration of *N. sativa* and CCl₄ significantly prevented the changes recorded in erythrocyte parameters, platelet counts and total leucocyte counts, which showed insignificant
difference from the control values. However, it appeared that the animals treated with CCl₄ plus *N. sativa* still had lymphocytosis and monocytosis accompanied by mild neutropenia.

**Effect of CCl₄ and/or *N. sativa* on morphological pattern observed in peripheral blood cells**

Oral administration of CCl₄ induced profound alterations in the morphology of both erythrocytes and leukocytes. The erythrocytes in control mice were normochromic normocytic (Figure 1a). In mice treated with ¼ LD₅₀ of CCl₄ the cells were hypochromic and showed poikilocytosis and anisocytosis. Erythrocytes with marked crenation and cup shaped cells, target cells, sickle cells, spherocytes, stomatocytes and elliptocytes were frequently observed (Figures 1b-d). The previously mentioned alterations were less profound in animals treated with CCl₄ plus *N. sativa*. The cells had nearly normal haemoglobin content and regular contour, only some poikilocytic erythrocytes could be noticed (Figure 1e).

Ultrastructurally, treatment with CCl₄ yielded obvious alterations in all types of leukocytes. The mature neutrophils of control animals appeared with more than one nuclear lobe (Figure 2a). Heterochromatin was located peripherally constituting a tick band subjacent to the nuclear membranes; the central area of the nuclear lobe was occupied chiefly by euchromatin. Treatment with ¼ LD₅₀ of CCl₄ yielded obvious alterations in neutrophils. Neutrophils with extensive segmented nuclei, dilated nuclear envelope, abundant heterochromatin, numerous destructed immature specific and primary cytoplasmic granules and vacuoles were seen (Figures 2b-d). The alterations in neutrophils in mice treated with ¼ LD₅₀ of CCl₄ plus *N. sativa* showed less severity, nuclear lobes were occasionally hyperchromatic (Figure 2e).

### Table 1. Effect of CCl₄ and/or *N. sativa* on erythrocyte values and platelet count in peripheral blood of Swiss albino mice.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>RBC (10⁶/mm³)</th>
<th>Hb (g/dl)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (Pg)</th>
<th>MCHC (%)</th>
<th>PI (10⁹/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>4.72±0.10ab</td>
<td>13.93±0.20a</td>
<td>41.33±2.85ab</td>
<td>88.33±4.48abc</td>
<td>29.83±0.60abc</td>
<td>34.00±2.00abc</td>
<td>270.00±11.55abc</td>
</tr>
<tr>
<td>Olive oil (G2)</td>
<td>4.74±0.35ab</td>
<td>13.57±0.43a</td>
<td>42.67±2.02a</td>
<td>90.00±3.79a</td>
<td>28.80±0.92a</td>
<td>32.00±1.53a</td>
<td>256.67±17.64a</td>
</tr>
<tr>
<td>Suspension of <em>N. sativa</em> (G3)</td>
<td>4.83±0.10b</td>
<td>13.80±0.53a</td>
<td>43.33±3.28a</td>
<td>89.53±5.61a</td>
<td>28.53±0.80a</td>
<td>32.00±1.53a</td>
<td>236.33±8.95a</td>
</tr>
<tr>
<td>¼ LD₅₀ of CCl₄ (G4)</td>
<td>3.57±0.13c</td>
<td>10.87±0.24ab</td>
<td>26.67±0.88c</td>
<td>77.48±3.22bc</td>
<td>26.07±1.85a</td>
<td>37.00±2.31bc</td>
<td>181.67±4.63a</td>
</tr>
<tr>
<td>¼ LD₅₀ of CCl₄ + <em>N. sativa</em> (G5)</td>
<td>4.34±0.14c</td>
<td>12.70±0.38a</td>
<td>35.67±0.88b</td>
<td>82.39±4.29abc</td>
<td>29.27±0.83a</td>
<td>35.67±1.46abc</td>
<td>233.00±78.44a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, for three animals in each group; Values, within columns, with no common superscripts are statistically significant (p<0.05); RBC: red blood cell count, Hb: haemoglobin content, PCV: haematocrit value, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, PI: platelet count.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Total leucocyte count (10⁹/mm³)</th>
<th>Differential leucocyte count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>5.97±0.12ab</td>
<td>Neutrophils 58.67±0.67a</td>
</tr>
<tr>
<td>Olive oil (G2)</td>
<td>4.93±0.18bc</td>
<td>2.67±0.33ab</td>
</tr>
<tr>
<td>Suspension of <em>N. sativa</em> (G3)</td>
<td>5.33±0.35bc</td>
<td>57.33±0.67a</td>
</tr>
<tr>
<td>¼ LD₅₀ of CCl₄ (G4)</td>
<td>4.40±0.50c</td>
<td>56.67±0.33a</td>
</tr>
<tr>
<td>¼ LD₅₀ of CCl₄ + <em>N. sativa</em> (G5)</td>
<td>5.55±0.28bc</td>
<td>41.33±0.67bc</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, for three animals in each group; Values, within columns, with no common superscripts are statistically significant (p<0.05).
Figure 1. Photomicrographs showing peripheral blood smears of mice in different groups: (a) control mice, arrow indicates blood platelets; (b) mice treated with ¼ LD50 of CCl4, note spherocytes (arrowheads), sickle cells (arrows) stomatocytes (double head arrows) and cup shaped cells (double arrowheads); (c) mice treated with CCl4, note crenation and many target cells (arrows); (d) mice treated with CCl4, some target cells (arrows) and cells with crenation (arrowheads) could be seen; (e) mice treated with CCl4 plus N. sativa, erythrocytes have nearly normal haemoglobin content and regular contour. Arrows point at some note hypochromic poikilocytic erythrocytes (arrows). Ne: neutrophil, Eo: Eosinophil, Lc: lymphocyte, Mo: monocyte, Pl: platelets. Leishman's stained preparation of dry smear from EDTA anticoagulated blood. Magnification: 1000x (a-e).
Eosinophils with segmented nuclei and coarse eosinophilic granules with central dense crystals were encountered in the control specimens (Figure 3a). Administration of ¼ LD50 of CCl₄ resulted in drastic abnormalities in the eosinophils which exhibited severe chromatolysis, hyperchromatic nuclear lobes, vacuolated cytoplasm and destructed specific granules without central dense crystals (Figures 3b-d). Treatment with N. sativa attenuated the pathological alterations induced in eosinophils. Normal specific granules with distinct central dense crystals, a few dense primary granules were observed in the cytoplasm (Figure 3e).

Control animals displayed lymphocytes with typically spherical nuclei and scanty cytoplasmic organelles (Figure 4a). In animals intoxicated with CCl₄, Lymphocytes showed various structural abnormalities including irregular shaped hyperchromatic nuclei with dilated or invaginated nuclear envelope, multiple nuclear vacuoles, highly vacuolated cytoplasm, destructed mitochondria and hypertrophied Golgi body (Figures 4b-d). Treatment with N. sativa lessened the most drastic abnormalities in blood lymphocytes; only few cells showed degenerated areas of heterochromatin and cytoplasm containing few altered mitochondria (Figure 4e). Monocytes of the control animals were found to be large cells with large kidney shaped nuclei and cytoplasm containing numerous ribosomes, polysomes and small dense mitochondria (Figure 5a). After oral administration of ¼ LD50 of CCl₄, monocytes showed several dramatic changes including both the nucleus and cytoplasm (Figures 5b-c). The nuclei had dilated nuclear envelope with irregular contour, obvious nucleoli, abnormal heterochromatin distribution and intranuclear inclusions. The cells also appeared with vacuolated cytoplasm containing vacuolated mitochondria or mitochondria with less distinct cristae and many destructed granules. In
addition, the cell surfaces of monocytes had long filopodia. An improvement was noted in animals treated with CCl₄ + N. sativa where monocytes showed normal kidney shaped eccentric nuclei and cytoplasm containing mitochondria with distinct membranes and multiple groups of well developed Golgi apparatus (Figures 5d-e). A few dense granules and vacuoles could also be noticed. Worth mentioning is that no alterations could be detected in the shape of different cell types of peripheral blood in mice that received only N. sativa.

**DISCUSSION**

In the present study, oral administration of CCl₄ (¼ LD50) greatly affected all hematological parameters. It led to decrease in RBCs count, Hb, PCV and MCV values. Depletion in the number of RBCs count along with the Hb concentration was detected in mice treated with CCl₄ at a dose of 0.05 ml per mouse for five weeks (Mandal et al., 1998). According to Ballinger (2007), depletion in RBCs count and Hb content leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture. The depression in RBCs count and Hb content recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation. Tung et al. (1975) mentioned that, the reduction in the values of blood parameters (PCV, RBC and Hb) may be attributed to the hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation.

Improvement of blood picture in mice treated with CCl₄ plus N. sativa may be considered as an expression of the role of N. sativa against CCl₄ induced microcytic hypochromic anemia. These results support that of Zaoui et al. (2002) who found that Hb and PCV levels were...
significantly increased by 6-17%, respectively in rats treated with fixed oil of *N. sativa* (1 ml kg⁻¹ day⁻¹ for 12 weeks). Also, Abdel-Wahhab and Aly (2005) reported that treatment with *N. sativa* oil (5 mg kg⁻¹ b.w. for 30 consecutive days) showed an antioxidant activity and succeeded in the improvement of all haematological parameters in rats treated with aflatoxin-contaminated diet. Moreover, Kanter et al. (2005) and Demir et al. (2006) stated that treatment with *N. sativa* increased the lowered RBC, Hb, PCV, WBC count in cadmium treated rats.

In the current work, administration of CCl₄ induced significant decrease in total WBC (marked leucopenia). This result supports the finding of Jirova et al. (1996) and Mandal et al. (1998) who stated that exposure to CCl₄ induced significant decrease in leukocyte count in peripheral blood of mice. Treatment with CCl₄ plus *N. sativa* lessened CCl₄-induced changes in total and differential WBC count. In agreement, *N. sativa* improved the WBC count in rats treated with aflatoxin (Abdel-Wahhab and Aly, 2005) and cadmium (Demir et al., 2006). In addition, studies in mice and rats have shown that *N. sativa* extract significantly protects from cisplatin-induced falls in leukocytes count (Nair et al., 1991; El-Daly, 1998) and influences leukocytes activities (Haq et al., 1995; Houghton et al., 1995).

In the present study, treatment with CCl₄ induced severe abnormalities in peripheral blood cells. Erythrocytes showed marked anisocytosis, hypochromasia and severe poikilocytosis including crenation, target cells, spherocytes, stomatocytes, cup shaped cells, sickle shaped cells, elliptocytes, rouleaux formation and fragment, suggesting the occurrence of hemolytic and microcytic anemia. Similar abnormalities in blood cells were observed in the peripheral blood of rats treated orally with CCl₄ (2 ml kg⁻¹ b.w. twice a week for up to 12 week) (Doi et al., 1991). The destruction of red cells reflects the failure in hepatocellular functions and the

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**Figure 4.** Electron micrographs showing blood lymphocytes of mice; (a) control group, arrowhead indicates dense granules, arrows point to small cytoplasmic projections; (b) after treatment with ¼ LD50 of CCl₄, lymphocyte with dilated nuclear envelope (arrow), arrowheads point to less dense granules; (c) after treatment with CCl₄, note dilated nuclear envelope (double arrow) and invaginated nuclear envelope (thick arrow), arrowhead points to fragmented part of nucleus and arrows point to filopodia; (d) after treatment with CCl₄, note the cell surface appears with microvilli (arrows); (e) after treatment with CCl₄ plus N. sativa, lymphocyte with large spherical nucleus (N) showing slightly dilated nuclear envelope and lysis of heterochromatin at certain areas (arrowheads). Lc: lymphocyte, N: nucleus, Nu: nucleolus, M: mitochondria, G: Golgi apparatus, R: ribosomes, V: cytoplasmic vacuoles. Magnification: 10000x (a, d), 15000x (b), 13000x (c,e).
Figure 5. Electron micrographs showing blood monocytes of mice; (a) control group, arrowhead points to dense granules; (b) after treatment with 1/4 LD50 of CCl₄, arrowhead destructed mitochondria demonstrates destructed mitochondria, arrows point to filopodia; (c) after treatment with CCl₄, note intranuclear inclusions (arrows) and vacuolated mitochondria (double arrow); (d & e) after treatment with CCl₄ plus N. sativa, note mitochondria with distinct mitochondrial membrane and vesicular cristae (arrowhead), and small dense granules (arrows). Mo: Monocyte, N: nucleus (N), Nu: nucleolus, G: Golgi apparatus, M: mitochondria, R: ribosomes, rER: rough endoplasmic reticulum, gr: cytoplasmic granules, V: vacuoles. Magnification: 13000x (a), 10000x (b, d), 7500x (c), 15000x (e).

various morphological abnormalities in RBCs are probably due to the changes in the membrane cholesterol and phospholipid content and/or ratio (Sherlock and Dooley, 1993). According to Travlos et al. (1996), the presence of altered red cell morphology is consistent with erythrocyte damage and is presumed to be related to direct oxidative injury to the red cells by the chemicals or to the pitting function of the spleen. It may be assumed that the free radicals resulting from CCl₄ metabolism caused liver injury and a proportion of these free radicals liberated from the liver into the blood and may affect the membranes of circulating red cells and induced a significant increase in the number of target cells (Doi et al., 1991). Improvement in erythrocytes in animals treated with N. sativa could be due to the important role of N. sativa in decreasing the oxidative damage in cell membrane. This is in agreement with Kanter et al. (2005) who reported that treatment with N. sativa oil (interperitoneal injection of 0.2 ml kg⁻¹ b.w. for 30 days) reduced membrane destruction and haemolytic changes in erythrocytes of cadmium-treated rats.

Electron microscopy revealed various ultrastructural abnormalities in the leukocytes in the blood of mice treated with CCl₄. These abnormalities included both the nucleus and cytoplasm. Neutrophils frequently appeared with irregular hypersegmented nuclear lobes and destructed/vacuolated cytoplasm with indistinct contour. Hypersegmented neutrophils were frequently noticed in patients with megaloblastic anemias (Sinha et al., 2006). Destructed specific granules with less electron density and less distinct central crystals were pronounced in eosinophils of mice treated with CCl₄. Abnormalities in the size, shape and cytochemistry of eosinophilic granules have been reported in patients with myelocytic leukaemia and lymphoma (Mnathorpe et al., 1977; Anteunis et al., 1978). Moreover, some lymphocytes displayed less electron dense cytoplasm with dense destructed mitochondria. Most of these abnormalities were described by Pandolfi et al. (1982) in peripheral blood lymphocytes of patients with chronic lymphocytic
leukemia. Ghadially (1985) suggested that these changes can be used as tentative markers for leukemias and lymphomas in man. Monocytes had highly irregular U-shaped nuclei with abnormal heterochromatin content and also had a few disintegrated mitochondria. Similar alterations in leucocytes have been previously described by El-Mofty et al. (2000a) and observed also in peripheral blood of mice treated orally with Nizoral at a dose level of 200 mg kg⁻¹ b.w. for 12 months (El-Mofty et al., 2000b). Oral administration of *N. sativa* improved most of the ultrastructural changes induced in leucocytes after CCl₄ administration. This means that the black seed was able to constitute a live of defense, protecting the blood cells from the severe damaging effect of the toxins.

This investigation shows that *N. sativa* has the ability to protect the haematopoietic cells from the damaging effects of exposure to CCl₄ and this protection might be attributed to the antioxidative power of *N. sativa*.

**REFERENCES**


