

The protective effect of black seed (*Nigella sativa*) against carbon tetrachloride-induced chromosomal aberrations and ultrastructural changes of bone marrow cells

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ABSTRACT

The effect of the oral administration of aqueous suspension of Nigella sativa (50 mg/kg b.wt.) against chromosomal aberrations and ultrastructural changes of the bone marrow cells in mice treated with carbon tetrachloride CCl₄ was studied. CCl₄ was administered in two dose levels equivalent to 1/4 (1.9 ml/kg b.wt.) and 1/2 (3.8 ml/kg b.wt.) of the oral LD₅₀ in mice. The data indicated a significant dose-dependent decrease in the mitotic activity of bone marrow cells in animals treated with CCl₄. Also a significant dose-dependent increase in the number of bone marrow cells with different types of chromosomal aberrations was recorded in these animals. Ultrastructural changes were also dose-dependent including both nucleus and cytoplasm of erythroid and myeloid elements of the bone marrow cells. Treatment of the animals with N. sativa improved both genotoxicity and ultrastructural changes induced by the two dose levels of CCl₄.

Key words: Mice, carbon tetrachloride, *Nigella sativa*, chromosomal aberrations, bone marrow cells, ultrastructure, erythroid, myeloid

INTRODUCTION

In recent years, there has been an increasing awareness of the genotoxic potential of a wide variety of drugs and chemicals to which the human population is exposed either environmentally or occupationally (Odeigah, 1997).

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). It was reported that CCl₄ is one of the most commonly used toxins in the experimental study of liver diseases (Wang *et al.*, 2007). Although information is available on the hepatotoxicity of CCl₄, there are only a few studies describing its effect on the morphology and function of haematopoietic system. Blood and bone marrow are of the largest organs and important potential targets in the body for chemical exposure (Lund, 2000). Evaluation of blood has been extensively described by Perkins (1999) and

Ryan (2001); the regenerative capacity of most peripheral lymphoid organs depends on the pluripotent progenitor cells in the bone marrow. Comparison of the cellular changes observed in the bone marrow should always be compared with the complete blood count. The majority of bone marrow changes that are observed in toxicological studies are the physiological responses of the bone marrow to hematological changes or lesions elsewhere in the body (Elmore, 2006).

Herbal medicines derived from plant extracts are increasingly utilized to treat a wide variety of clinical diseases (Lee *et al.*, 2004). *Nigella sativa* is a herbaceous plant that have been used traditionally for centuries in the Middle East, Northern Africa and India for the treatment of various diseases (Brutis and Bucar, 2000 and Gilani *et al.*, 2004). Clinical and animal studies have shown that extracts of the black seeds have many therapeutic effects such as antidiabetic (Kanter *et al.*, 2004), antibacterial (Kanter *et al.*, 2003), hepatoprotective (Nagi *et al.*, 1999), nephroprotective (Ali, 2004), and antitumor (Essawy *et al.*, 1997; Worthen *et al.*, 1998; Khan *et al.*, 2003 and Hussein *et al.*, 2005). From the experimental and clinical 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).

The present study was conducted to evaluate the protective effect of *N. sativa* against the possible chromosomal aberrations and cellular damage induced by CCl₄ in the bone marrow cells of Swiss albino mice.

MATERIALS AND METHODS

Animals

Ten weeks old laboratory males of 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 hr photoperiod (12 hr light/dark cycle), with a light from 0600 to 1800 hr and darkness from 1800 to 0600 hr. They were given free access to a commercial pellet diet and tap water, and allowed to acclimatize for two weeks before treatment

Chemicals used

Carbon tetrachloride (98.8% purity) was purchased from El-Nasr Pharmaceutical Chemical Company (Egypt). *Nigella 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 the tested compound carbon tetrachloride.

Experimental design

The animals were randomly divided into seven experimental groups of 40 mice each.

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

Group IV: Each animal had orally received carbon tetrachloride 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 V: Each animal had orally received carbon tetrachloride dissolved in olive oil at a dose level 3.8 ml/kg b.wt. ($\frac{1}{2}$ LD₅₀) every other day for three successive weeks.

Group VI: Each animal had orally received suspension of *Nigella sativa* at a dose level of 4 ml/kg b.wt. (50 mg/kg b.wt.) every other day alternated with carbon tetrachloride at a dose level 1.9 ml/kg b.wt. ($\frac{1}{4}$ LD₅₀) for three successive weeks.

Group VII: Each animal had orally received suspension of *Nigella sativa* at a dose level of 4 ml/kg b.wt. (50 mg/kg b.wt.) every other day alternated with carbon tetrachloride at a dose level of 3.8 ml/kg b.wt. ($\frac{1}{2}$ LD₅₀) for three successive weeks.

Analysis of mice bone-marrow chromosomes

Before sacrificing, each animal was injected intravenously in tail vein with 20 µg colcemid to arrest chromosomes at metaphases. The bone-marrow cells were collected according to Brusick (1986). Staining was carried out using 10% Giemsa-Gurr (pH 6.8). Screening of slides for mitotic spreads was conveniently accomplished with a 25X magnification objective lens and analysis was done with a 100X objective.

Mitotic index

Animals used for this assay were not injected with colcemid. A mitotic index based on at least 4000 counted cells was recorded. The mitotic activity was estimated as the percentage of dividing cells to the total number of the examined cells (Alder, 1984).

Preparation of the bone marrow cells for transmission electron microscopy (TEM)

Marrow was removed from the femur bones, with care taken so as not to disturb native structure. Samples were immediately

immersed in 2% glutaraldehyde of phosphate buffer (pH 7.4) for about 2 hr at 4°C. The specimens were transported to 4F₁G, and were then fixed in 2% OsO₄ at 4°C for 2 hr. Samples were dehydrated in graded series of ethanol. For transmission electron microscopy, specimens of bone marrow were embedded in Epon-araldite mixture in labeled beam capsules. LKB ultramicrotome was used to obtain semithin sections (1 µm thick). They were mounted on a glass slide and stained with toluidene blue. Ultrathin sections (50 nm thick) were cut from selected areas for TEM. These ultrathin sections were of either pale gold or silver interference color and were picked upon 200 mesh naked copper grids. Grids were double stained with uranyl acetate for $\frac{1}{2}$ hr and lead citrate for 20-30 min. (Reynolds, 1963). Scoping the grids was achieved by using JEOL 100CX transmission electron microscope (TEM).

Statistical analysis

Data are expressed as means±SD. 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 AND DISCUSSION

Analysis of chromosomal aberrations and mitotic index in mice bone marrow cells

Cytological examination revealed that CCl₄ was found to be effective in decreasing cell proliferation (estimated as mitotic index). The results presented in Table (1) show that the analysis of mitotic activity after treatment with carbon tetrachloride alone at both doses made a significant inhibition ($p < 0.05$) in the mitotic index from 12.4% in the control group (GI) to 6.2% in group IV (treated with $\frac{1}{4}$ LD₅₀

of CCl_4) and to 4.6% in group V (treated with $\frac{1}{2}$ LD₅₀ of CCl_4). Treatment with CCl_4 plus *N. sativa* (group VI and VII) was found to lessen the inhibition in mitotic index resulted from the administration of CCl_4 alone. In the meantime, an insignificant decrease in the percentage of the mitotic index was recorded in animals treated with carbon tetrachloride at both dose levels plus *Nigella sativa* as compared to control. It decreased insignificantly ($p < 0.05$) from 12.4% in control to 8.2% and 7.6% in GVI and GVII, respectively.

Various types of structural chromosomal aberrations were observed after the administration of carbon tetrachloride alone or plus *Nigella sativa* suspension compared with the negative control group (Fig.1). The chromosomal damages observed in this study were in the form of stickiness (St), chromosome fragments (F) and chromatid gaps (Ga) - (Fig.1a, b). Centric fission (CF) and polyploidy were also observed but not counted (Fig.1c, d). Ring chromosomes (Ri) and chromatid deletions (Cd) were presented in (Fig.1d, f). Robertsonian Centric Fusion (RCF) (Fig.1, a, e, g, h) and centric fission (Fig.1a-d,g) were scored. Generally, the cytogenetic findings presented in Table (1) revealed that the chromosome aberrations were infrequent in the control animals or those treated with olive oil alone as well as those treated with *Nigella sativa*, while they were more frequent in the animals treated with carbon tetrachloride alone. These results are in agreement with those obtained by Rossi *et al.* (1988) who recorded an increase in the frequency of chromosomal aberrations in both bone marrow and liver cells of male mice treated with CCl_4 at dose level of 1 ml/kg/b.wt. (the animals treated 48 hr. before cell collection). In contrast, Sasaki *et al.* (1998) and Suzuki *et al.* (1997) concluded that the CCl_4 suspended in olive oil on mice (BDF₁ male mice, 8 weeks

old) bone marrow after a single or twice treatment at a dose of 500, 1000 and 2000 $\mu\text{g/kg}$ b.wt. and in peripheral blood after a single intraperitoneal injection at a dose of 1000, 2000 and 3000 $\mu\text{g/kg}$ b.wt., dose not induce chromosomal aberrations in the mouse bone marrow under these experimental conditions.

Treatment with $\frac{1}{4}$ LD₅₀ of CCl_4 plus *N. sativa* suspension (GVI) yielded a decrease in RCF, chromatid gaps and polyploidy from 4%, 3% and 2%, respectively in GIV (treated with $\frac{1}{4}$ LD₅₀ of CCl_4 alone) to 2%, 2%, and 1% respectively in GVI (treated with $\frac{1}{4}$ LD₅₀ of CCl_4 plus *N. sativa*), but failed to decrease the stickiness. Treatment with $\frac{1}{4}$ LD₅₀ of carbon tetrachloride plus *N. sativa* suspension (50 mg/kg b.wt.) yielded an increase in all kinds of aberrations compared with that of control (GI). Generally, stickiness, breaks and centric fission were the commonest types of aberrations detected in group VI, while RCF, chromatid gaps and polyploidy were the least common. Breaks and stickiness in group VI were 3% and 7%, respectively. Moreover, the centric fission increased to 3% compared with 0% of the control. Statistical analysis revealed that the treatment with $\frac{1}{4}$ LD₅₀ of CCl_4 plus *Nigella sativa* (GVI) showed an increase in the percent of total chromosomal damages from 0.50 ± 0.84 in control case to 3.0 ± 2.10 in treated animals (GVI). This difference was found to be statistically significant. In addition, it was found that the percent of chromosomal damage increased insignificantly in GVI compared with that of GIV.

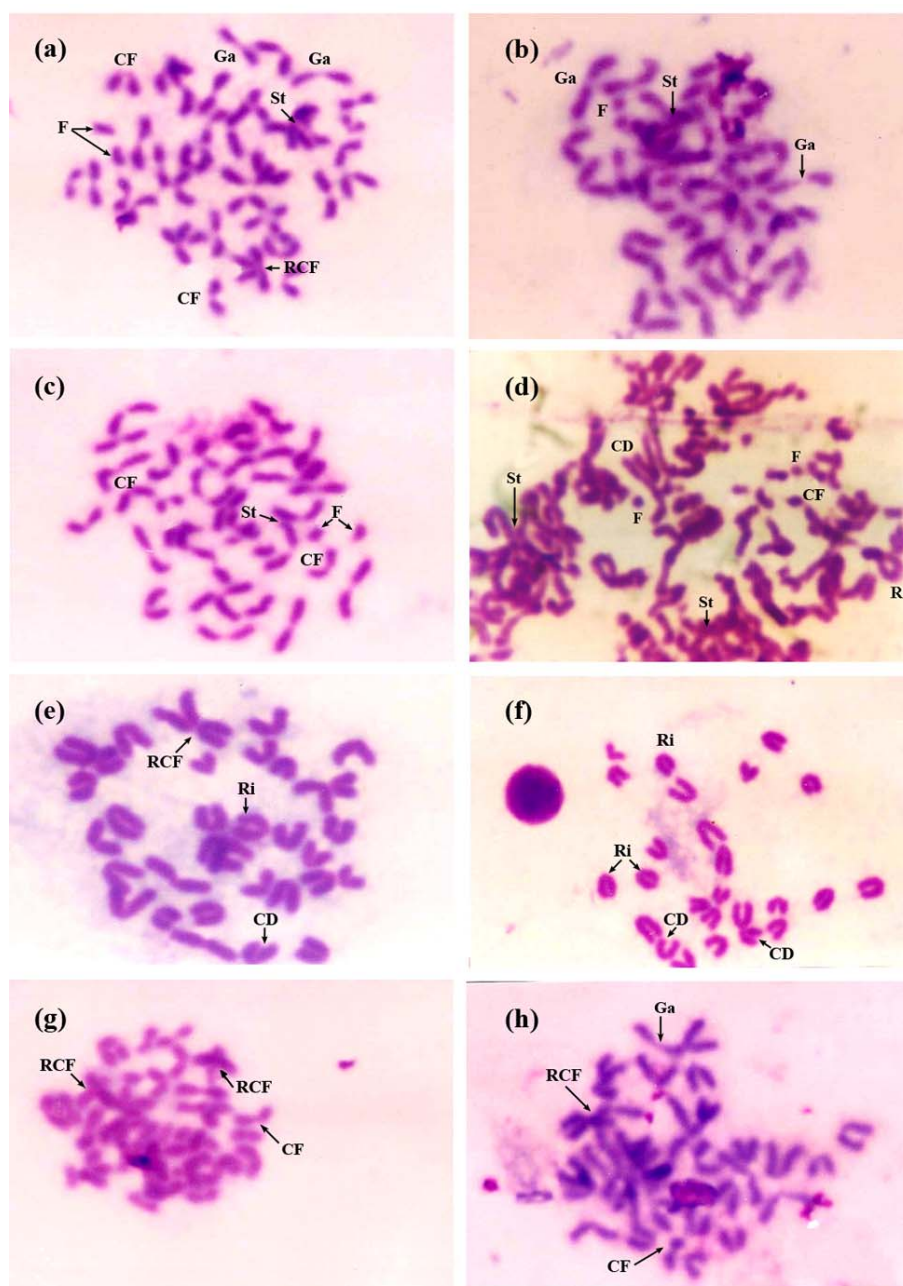


Fig.(1): Photomicrographs of Giemsa stained preparations showing the different chromosomal aberrations from bone marrow cells from different experimental groups. (a) stickiness (St), chromosome fragments (F), Robertsonian Centric Fusion (RCF), centric fission (CF) and chromatid gap (Ga). (b) stickiness (St), chromosome fragment (F), and chromatid gaps (Ga). (c) stickiness (St), chromosome fragments (F) and centric fissions (CF). (d) polyploidy, note stickiness (St), ring chromosome (Ri), chromosomes fragments (F), chromatid deletion (CD) and centric fission (CF). (e) ring-chromosome (Ri), chromatid deletion (CD) and Robertsonian Centric Fusion (RCF). (f) ring-chromosomes (Ri), chromatid deletions (CD). (g) Robertsonian Centric Fusion (RCF) and centric fission (CF). (h) Robertsonian Centric Fusion (RCF), centric fission (CF) and chromatid gap.

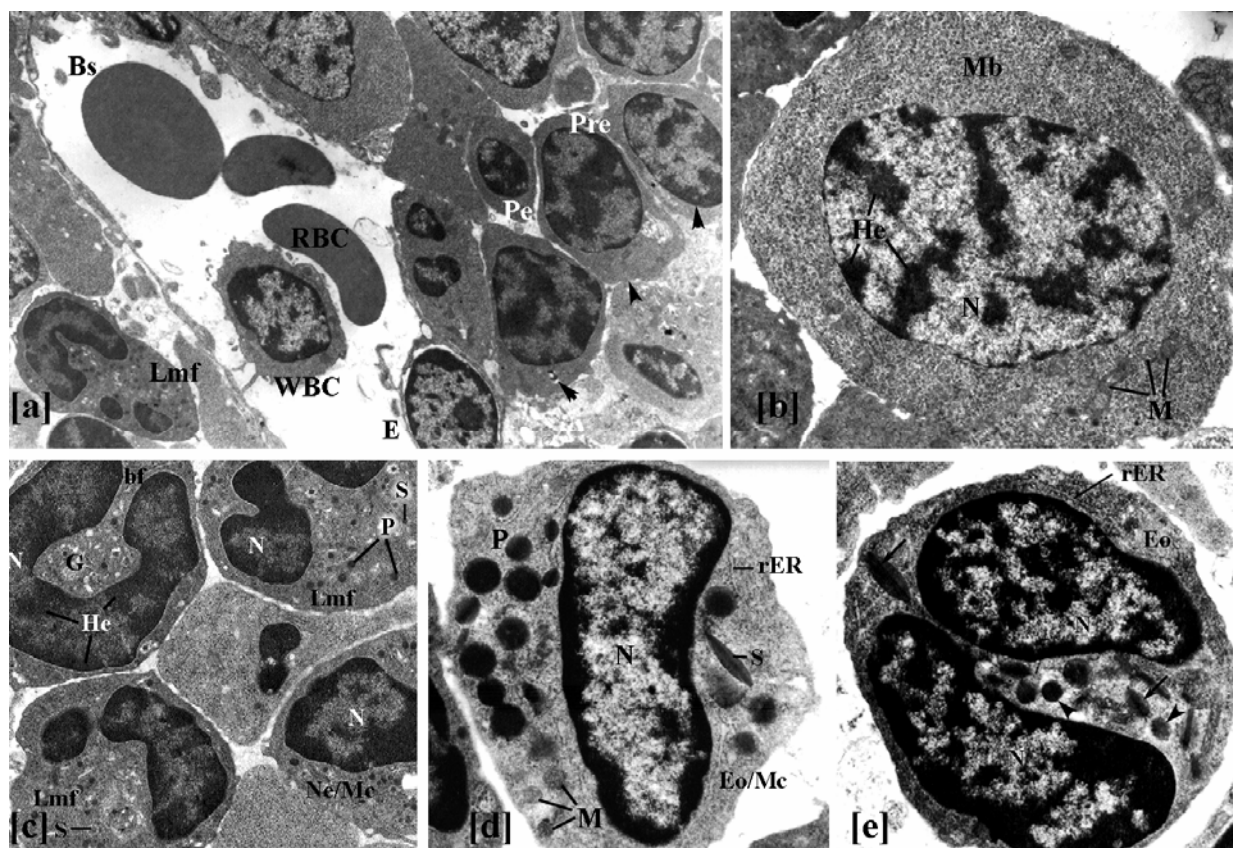


Fig.(2): Electron micrographs of bone marrow sections of control mice. (a) haematopoietic cells arranged in cords (arrowheads), Bs: blood sinusoid, E: endothelial cell, RBC: red blood cell, WBC: white blood cell, Pre: proerythroblast, Pe: polychromatophilic erythroblast with nucleus having condensed chromatin, Lmf: later myeloid form, X4000. (b) Mb: myeloblast with nucleus having dispersed masses of heterochromatin, X7500. (c) Ne/Mc: neutrophil myelocyte, bf: band form, Lmf: later myeloid forms of early stage of nuclear segmentation, note the cytoplasm with some dense primary granules (P) and many less dense spherical specific granules (S), X5000. (d) Eo/Mc: eosinophil myelocyte with large eccentric nucleus having coarse peripheral clumps of heterochromatin, note spherical more electron dense primary granules (P), and elliptic-shape specific granules (S) with central dense crystal, X7500. (e) Eo: mature eosinophil, arrows indicate specific granules, arrowheads indicate dense azurophilic granules, X7500. N: nucleus, He: heterochromatin, M: mitochondria, G: Golgi apparatus, rER: cisternae of rough endoplasmic reticulum.

On the other hand, additions of *N. sativa* to $\frac{1}{2}$ LD₅₀ CCl₄ gave a decrease in stickiness, RCF, centric fission and polyploidy from 6%, 6%, 5% and 6%, respectively in animals treated with $\frac{1}{2}$ LD₅₀ of CCl₄ alone (GV) to 5%, 3%, 3% and 2%, respectively in this

group (GVII). However, treatment with $\frac{1}{2}$ LD₅₀ of CCl₄ plus *Nigella sativa* (GVII) showed significant increase in the percent of chromosomal damages from 0.50 ± 0.84 in control to 3.67 ± 1.21 in treated animals (GVII). While it was found that the percent of

chromosomal damage decreased insignificantly compared to GV.

The, the dose of the *Nigella sativa* used in the present work (50 mg/kg b.wt.) reduced most of the numerical aberrations following the CCl₄ treatment but it was not found to restore the total number of chromosomal aberrations induced by CCl₄ administration to the level of the control group. Aruna and Sivaramakrishnan (1990) reported that cumin seeds significantly suppressed (*in vivo*) the chromosome aberrations caused by benzo(a)pyrene in mouse bone marrow. Similar results were obtained by Aboul-Ela (2002)

who mentioned the protective effect of *N. sativa* extract and its main component thymoquinone (TQ) on the cytogenetic damage caused by Schistosomiasis infection in male albino mice (7-8 week old). Treatment of cultured spleen cells of infected mice with *N. sativa* (16 mg/kg b.w.) or TQ (4 mg/kg b.w.) induced chromosomal aberrations in a decreasing manner with time, whereas in the *in vivo* experiment the percentage of chromosomal aberrations in infected animals reduced to the control values after 7 days of treatment for either *N. sativa* or TQ.

Table (1): Effect of CCl₄ and/or *N. sativa* on mitotic index and chromosomal aberrations in bone marrow cells of Swiss albino mice.

Experimental groups		Mitotic index (%)	Metaphase with chromosomal aberrations / 100 cells	Abnormal metaphases/ 100 cells						Percent of total chromosomal aberrations (% ± S.D.)
				St	B	RCF	CF	Ga	Pp	
Control isotonic saline	GI	12.4±0.15 ^a	3	2	1	0	0	0	0	0.50±0.84 ^a
Olive oil	GII	13.0±0.71 ^a	2	1	1	0	0	0	0	0.33±0.55 ^a
Suspension of <i>N. sativa</i>	GIII	11.6±0.51 ^a	4	2	1	0	0	1	0	0.67±0.88 ^a
¼ LD ₅₀ of CCl ₄	GIV	6.2±0.37 ^b	16	3	2	4	2	3	2	2.67±0.82 ^b
½ LD ₅₀ of CCl ₄	GV	4.6±0.59 ^c	29	6	4	6	5	2	6	4.83±1.60 ^c
¼ LD ₅₀ of CCl ₄ + <i>N. sativa</i>	GVI	8.2±0.37 ^d	18	7	3	2	3	2	1	3.00±2.10 ^b
½ LD ₅₀ of CCl ₄ + <i>N. sativa</i>	GVII	7.6±0.50 ^{bd}	22	5	5	3	3	4	2	3.67±1.21 ^{bc}
Result of one way ANOVA :		P	0.000	0.000						
		F	46.64	12.17						

B : Breaks chromosome (Fragment and Chromatid deletion)

St : stickiness

RCF: Robertsonian Centric Fusion

Ga : Chromatid Gap

CF : Centric Fission

Pp : Polyploidy

One hundred cells were analyzed per animal, for a total of 500 cells per treatment.

Values, within columns, with no common superscripts are statistically different (P<0.05).

Ultrastructural patterns observed in bone marrow cells

Examination of samples obtained from the bone marrow of control mice (group I) showed several haematopoietic cells in different stages of maturation were found to be arranged in cords adjacent to or between blood sinusoids (Fig.2a). The ultrastructure study was mainly restricted to erythroid and myeloid elements of the bone marrow (Fig.2a-e). Assessment of the blood and bone marrow has become a routine procedure in the investigation of hematologic disorders in

toxicology and biosafety assessment studies (Perkins, 1999 and Ryan, 2001).

Oral administration of CCl₄ at a dose level of 1.9 ml/kg b.wt. (¼ LD₅₀) every other day for three successive weeks caused marked alterations in the morphology of both erythroid and myeloid elements of bone marrow of the treated mice. The alterations of the erythroid elements involved both the nucleus and the cytoplasm. The nuclei frequently had less heterochromatin content and several blebs in nuclear envelope. The cytoplasm was less electron dense and

occasionally vacuolated (Fig.3a,b). Concerning granulopoietic cells abnormalities, the most impressive observation was the presence of destructed specific granules. These cells had slightly irregular outline and frequently exhibited a decrease in the heterochromatin content (Fig.3a,b). Unclassified cells in mitotic division could be occasionally observed (Fig.3c). The chromatin material in such cells appeared to be clumped in the part of the cell

with uncommon feature and small vacuolated mitochondria in cytoplasm were also observed. Furthermore, blood sinusoids were lined with hypertrophied endothelial cells having heterochromatic nuclei (Fig.3d). In addition, many blood sinusoids were occupied with blood cells displaying abnormal features. The red blood cells appeared with long projections or sickle shape while granulopoietic cells were found in immature stages (Fig.3d).

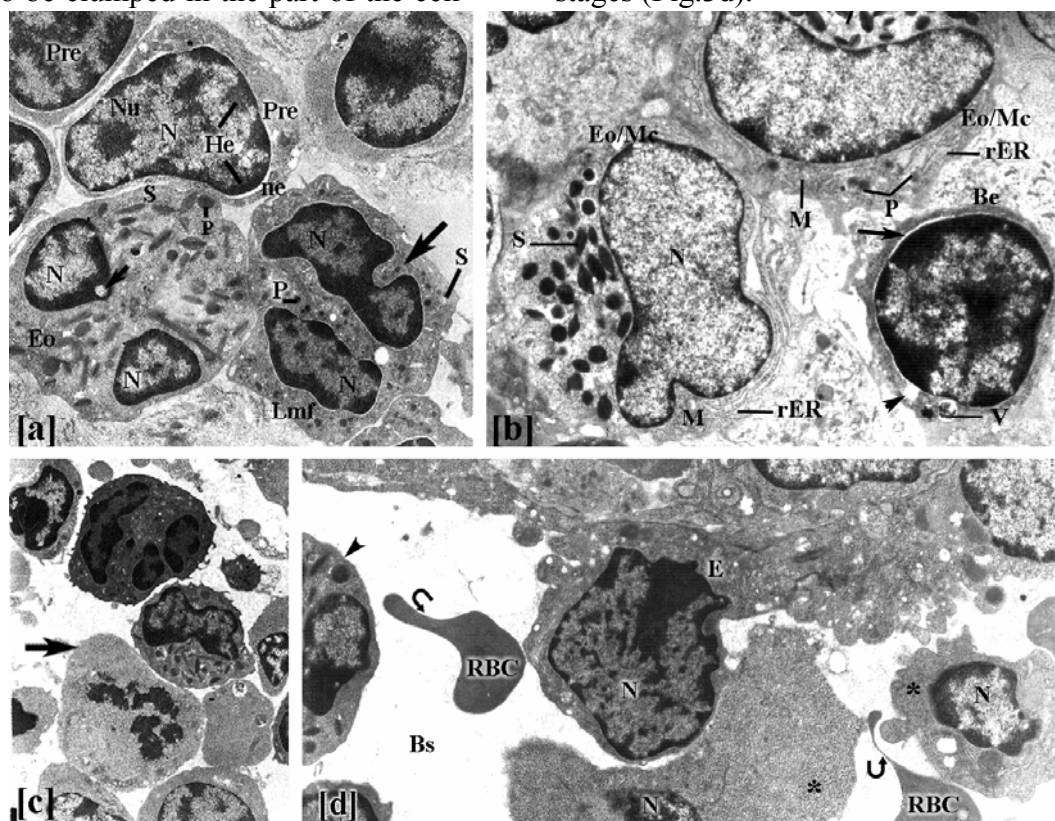


Fig.(3): Electron micrographs of bone marrow sections of mice treated with $\frac{1}{4}$ LD₅₀ of CCl₄. (a) Pre: proerythroblast with dilated nuclear envelope (ne), Lmf: later myeloid form with invaginated nuclear envelope (arrow), Eo: mature eosinophil with intranuclear vacuole (short arrow), X5000. (b) Be: basophilic erythroblast with slightly dilated nuclear envelope (arrow), arrowhead indicates bleb in nuclear envelope, V: cytoplasmic vacuole, Eo/Mc: eosinophilic myelocytes with marked decrease in heterochromatin content, X5000. (c) unclassified cell displaying mitotic division (arrow), note eccentric chromatin material clumped in part of the cell, X3000. (d) Bs: blood sinusoid, E: hypertrophied endothelial cell, erythroblasts (*) with abnormal nuclear feature and less electron dense cytoplasm, part of myeloid element (arrow head), RBC: red blood cell with elongated projections (curved arrows), X5000. N: nucleus, Nu: nucleolus, He: heterochromatin, M: mitochondria, G: Golgi apparatus, rER: cisternae of rough endoplasmic reticulum, S: specific granules, P: primary granules.

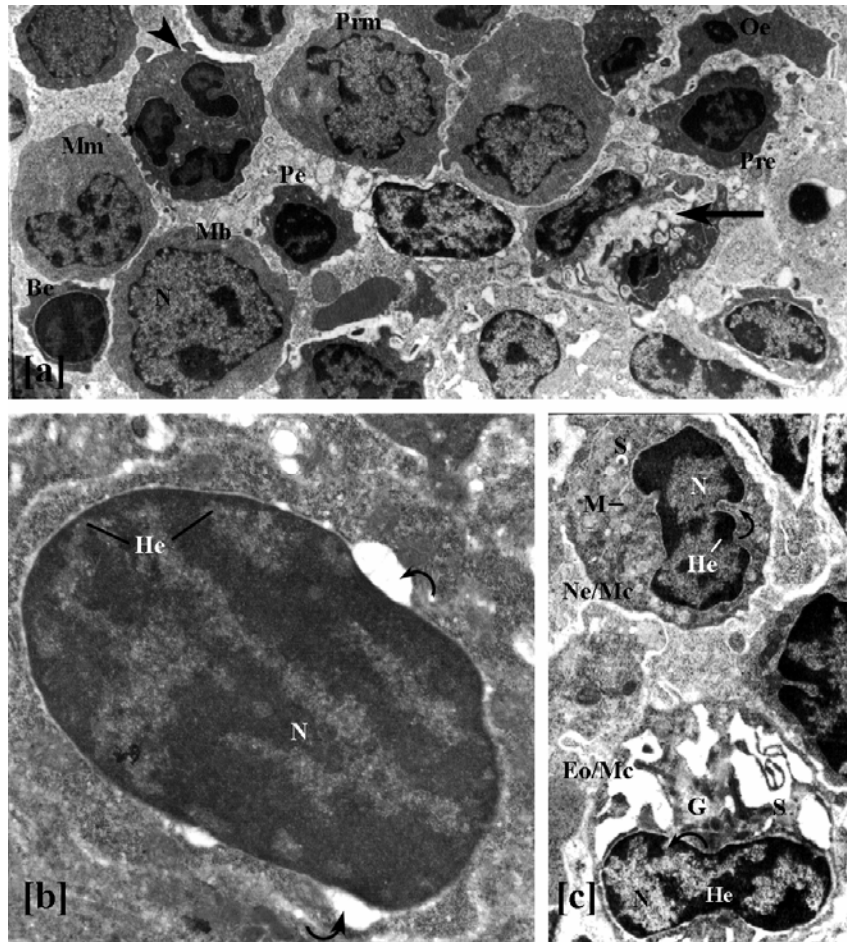


Fig.(4): Electron micrographs of bone marrow sections of mice treated with $\frac{1}{2}$ LD₅₀ of CCl₄. (a) Pre: proerythroblast, Be: basophilic erythroblast having nucleus with conspicuous nuclear envelope, Pe: polychromatophilic erythroblast with nucleus having abnormal heterochromatin distribution, Mb: myeloblast with large irregular euchromatic nucleus, note increase in number of nuclear pores and the less electron dense cytoplasm, Prm: late promyelocyte with marked irregular nucleus, Mm: neutrophilic metamyelocyte, arrow indicates later myeloid form with degenerated cytoplasm, arrowhead indicates later myeloid form with irregular nuclear lobes and cytoplasm with indistinct granules, X3000. (b) Pre: proerythroblast with several blebs in nuclear envelope (curved arrows), X10000. (c) Ne/Mc: neutrophil myelocyte with destructed granules in the cytoplasm, Eo/Mc: eosinophil myelocyte with nucleus having abnormal heterochromatin distribution, note invaginated nuclear envelope (curved arrows), X5000. N: nucleus, He: heterochromatin, M: mitochondria, G: Golgi apparatus, S: specific granules, P: primary granules.

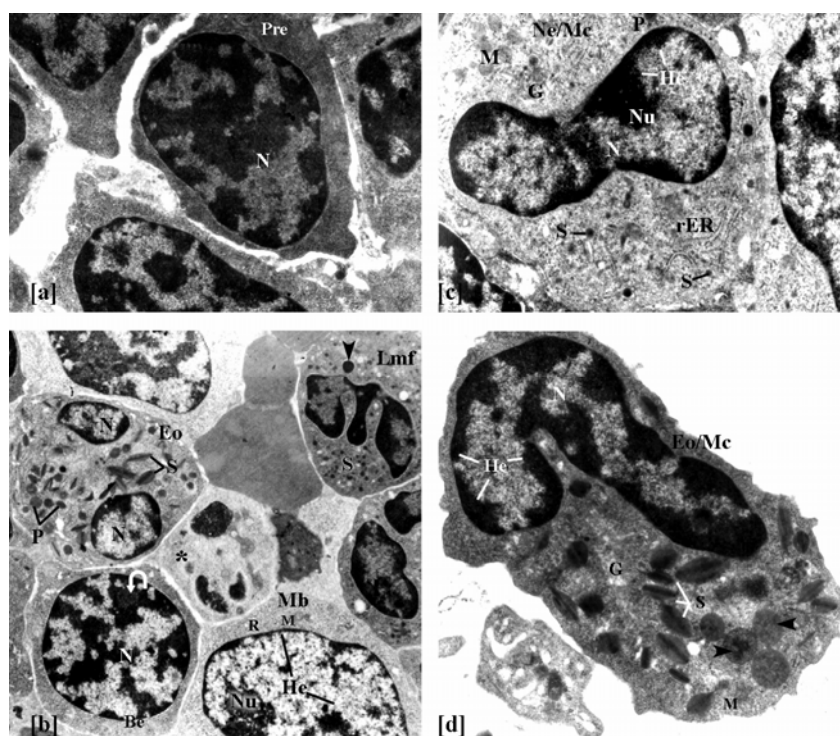


Fig.(5): Electron micrographs of bone marrow sections of mice treated with $\frac{1}{4}$ LD₅₀ of CCl₄ and suspension of *Nigella sativa*. (a) Pre: proerythroblast with normal feature, X7500. (b) Be: basophilic erythroblast with large regular nucleus having evident nucleolus (curved arrow), Mb: myeloblast with nucleus having obvious decrease in the heterochromatin content, segregated nucleolus, its cytoplasm appears with indistinct organelles, note marked decrease in ribosomes (R), and destructed mitochondria, Lmf: later myeloid form having abnormal nuclear feature, note small dense spherical lobe (arrowhead), Eo: eosinophil with bilobed nucleus having nearly normal chromatin content, its cytoplasm appears with less electron density. Unclassified cell (*) having lysed nucleus and pale cytoplasm, X4000. (c) Ne/Mc: neutrophil myelocyte with nucleus having abnormal presented nucleolus, X7500. (d) Eo/Mc: eosinophil myelocyte with normal-looking specific granules, and electron dense membrane-bounded primary granules (arrowheads), X10000. N: nucleus, Nu: nucleolus, He: heterochromatin, M: mitochondria, G: Golgi apparatus, rER: cisternae of rough endoplasmic reticulum, S: specific granules, P: primary granules.

Electron micrographs of different erythroid cells of mice treated orally with CCl₄ at a dose level of 3.8 ml/kg b.wt. ($\frac{1}{2}$ LD₅₀) confirmed the presence of marked nuclear aberrations involving shape, chromatin distribution, and nuclear envelope (Fig.4a,b). The cytoplasm was vacuolated and contained damaged organelles. Concerning granulopoietic cells, severe to mild decrease in heterochromatin was evident in different stages of maturation. Invaginated nuclear

envelope was also a common feature. Most myeloid elements exhibited vacuolated cytoplasm with less electron density and with less distinct organelles (Fig.4a,c). Immature or destructed specific granules were frequently observed. In general, eosinophils appeared more affected by treatment. Many eosinophil myelocytes with shrunken cytoplasm having completely degenerated organelles were observed (Fig.4c). In such cytoplasm, well developed Golgi apparatus was noticed.

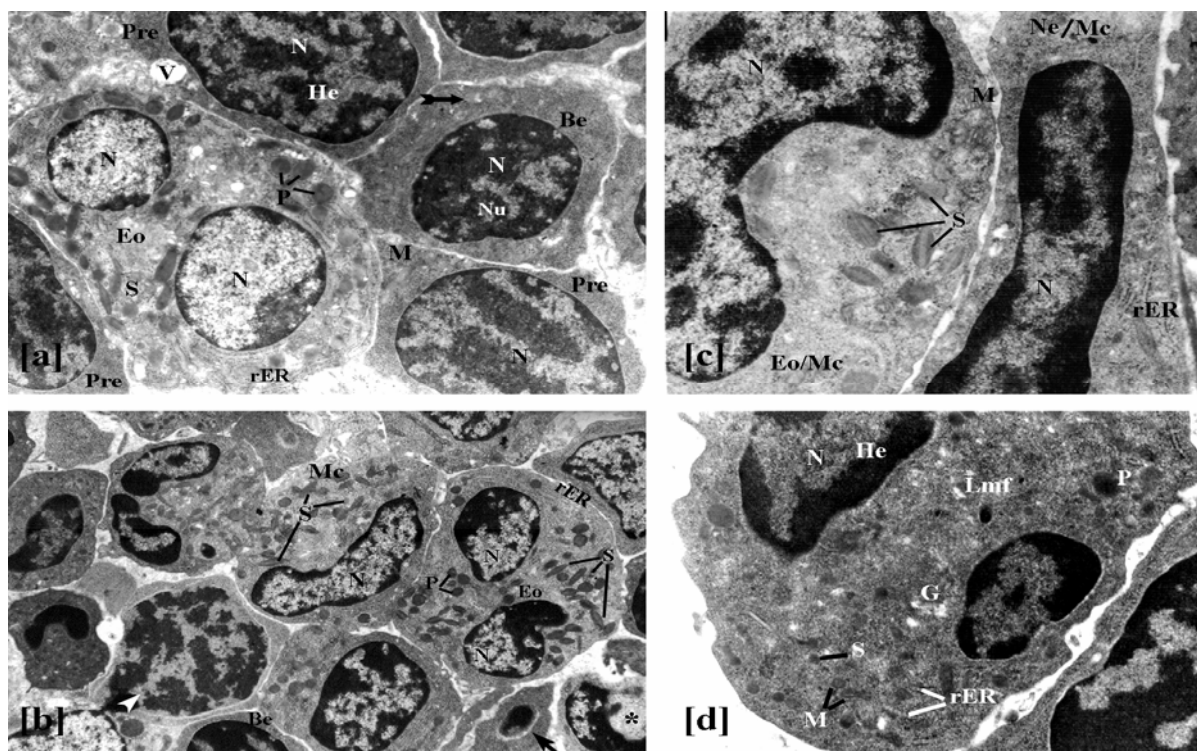


Fig.(6): Electron micrographs of bone marrow sections of mice treated with $\frac{1}{2}$ LD₅₀ of CCl₄ and suspension of *Nigella sativa*. (a) Pre: proerythroblasts with normal nuclei, Be: basophilic erythroblast having abnormal presence of nucleolus, note disintegrated mitochondria (thick arrow), Eo: mature eosinophil with vacuolated cytoplasm containing specific granules without central crystals, X5000. (b) Be: basophilic erythroblast with nucleus having slight decrease in heterochromatin content, arrow indicates orthrochromatophilic erythroblast with nucleus having dilated nuclear envelope, Mc: eosinophil myelocyte with cytoplasm containing many specific granules with abnormal feature, Eo: mature eosinophil with nucleus having heterochromatin condensed in part of the nuclear lobe. Note necrotic cell (*) with pyknotic nucleus and destructed cytoplasm. Arrowhead indicates cell in mitotic division, X4000. (c) Ne/Mc: neutrophil myelocyte, Eo/Mc: eosinophil myelocyte, X10000. (d) Lmf: later myeloid form, X10000. N: nucleus, Nu: nucleolus, He: heterochromatin, M: mitochondria, G: Golgi apparatus, rER: cisternae of rough endoplasmic reticulum, S: specific granules, P: primary granules.

These findings presented show the first description of the ultrastructural changes of the bone marrow after CCl₄ administration. Elmore (2006) indicated that the majority of bone marrow changes that are observed in toxicological studies are the physiological responses of the bone marrow to hematological changes or lesions elsewhere in the

body. Administration of CCl₄ (0.05 ml/mouse) three times a week (every other day) for five weeks through gastric intubation caused severe anaemia, leucopaenia, lympho-cytopenia, neutophilia, eosinophilia and haemoglobinemia (Mandal *et al.*, 1998).

Electron microscopic studies of bone marrow of mice treated with both doses of CCl₄

($\frac{1}{4}$ or $\frac{1}{2}$ LD₅₀) and *N. sativa* showed ameliorating changes (Figs.5,6). However, erythroid and myeloid cells with variable degrees of alterations could be occasionally detected (Fig.5b,c and Fig.6a,b). Treatment with *Nigella sativa* resulted in significant decrease of haematological disorders induced by aflatoxin (Abdel-Wahhab and Aly, 2005) and cadmium (Demir *et al.*, 2006). No remarkable pathological changes were recorded in bone marrow of animals treated with suspension of *Nigella sativa* or olive oil alone (III and II).

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التأثير الوقائي للحبة السوداء (نيجيلا ساتيفا) ضد الشذوذ الكروموسومي والتغيرات التركيبية الدقيقة في خلايا نخاع العظام

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يهدف هذا البحث إلى دراسة تأثير المعلق المائي للحبة السوداء (٥٠ ملليجرام/كجم) على السمية الوراثة والتغيرات التركيبية الدقيقة في خلايا نخاع عظام الفئران السويسرية البيضاء والمستحثة بجرعتين من رابع كلوريد الكربون تعادلان ٤/١ (١,٩ مللي/كجم) و ٢/١ (٣,٨ مللي/كجم) من الجرعة القمية المميتة لنصف الفئران (oral LD₅₀).

أوضحت النتائج أن رابع كلوريد الكربون قد تسبب في حدوث انخفاض ذي دلالة إحصائية (تعتمد على الجرعة المعطاة) في النشاط الميتوزي لخلايا نخاع العظام وكذلك زيادة في عدد خلايا نخاع العظام التي تحتوي على أنواع مختلفة من الشذوذ الكروموسومي. كما تسبب أيضا حدوث تغيرات تركيبية دقيقة في المراحل المختلفة لخلايا نخاع العظام كانت معتمدة على الجرعة المعطاة و شملت كلا من النواة والسيتوبلازم. كما أوضحت النتائج أن الحيوانات المسممة برابع كلوريد الكربون و المعالجة بالحبة السوداء قد أظهرت تحسنا واضحا ، حيث أرتفع النشاط الميتوزي لخلايا نخاع العظام و أنخفض عدد خلايا نخاع العظام التي تحتوي على شذوذ كروموسومي. أيضا حدث تحسن واضح في التغيرات الباثولوجية التي ظهرت في التركيب الدقيق لخلايا نخاع العظام في الفئران المعالجة بالحبة السوداء. و لهذا فان الحبة السوداء يمكن اعتبارها علاجا واعداء و أمنا للاضطرابات المرضية في خلايا الدم و نخاع العظام.