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ORIGINAL ARTICLES

Modulatory effect of *Nigella sativa* extract on *Vicia faba* L. toxicity induced by potassium dichromate

Fawzia I. Mohamed

Department of Genetics and Cytology, National Research Centre, Dokki, Giza, Egypt

ABSTRACT

Hexavalent chromium trioxide Cr^{6+} is the most active biologically, because it is capable of passing through cell membranes. It induces oxidative stress and lead to formation of stable Cr-DNA adducts that contribute to its cytotoxic and genotoxic effects. In the present study, the antigenotoxic effects of *Nigella sativa* extract was evaluated using the *Vicia faba* root chromosomal aberrations assay against potassium dicromate. Roots were given five kinds of treatments. In treatment, roots were first treated with distilled water (control). The second treated with crud extract of *Nigella sativa* .The third treated with different concentrations of $K_2Cr_2O_7$ (10,25,50,100 and 200mg/L) for 6 hr. In simultaneous treatment, fourth group the root -tips were treated with different concentrations with $K_2 Cr_2O_7$ and *N.s.* extract simultaneously for 6 hr. In post-treatment roots were treated with different concentrations of $K_2 Cr_2O_7$ followed by *N.s.* extracts. The mitotic index was generally reduced in the treated roots with the different concentrations of $k_2Cr_2O_7$. It reached the lowest value as 3.12% after treatment with 200mg/L $K_2Cr_2O_7$ compared with the control. The limit of mitotic inhibition reached to 71.76%. The increased number of chromosomal aberrations like stickiness, laggards, chromosome bridges, C-metaphase, fragmentation and micronuclei cells were observed with the increasing concentrations of $K_2Cr_2O_7$. The effects of post- and simultaneous treatment of *N.s.* extracts resulted decrease the chromosomal aberrations frequency and mitotic activity. The results indicated the repair role of *Nigella sativa* extract against potassium dichromate injuries during *Vicia faba* L. mitosis.

Key words: Chromium**,** *Nigella sativa***,** *Vicia faba,* Antigenotoxic, Mitotic division.

Introduction

Pollution of water is a major environmental problem facing the modern world. The global heavy metal pollution is increasing in the environment due to increase in number of industries. Many industrial wastewaters contain heavy metals like cadmium, lead, zinc, cobalt and chromium.

Among heavy metals, chromium plays a major role in polluting our water environment. Chromium can co-exist in the environment in two oxidation states *viz.*, trivalent chromium and hexavalent chromium. The hexavalent chromium is released from various industries such as electroplating, leather tanning, textile printing, textile preservation and metal finishing. The compounds of chromium have been known to be strong carcinogens and mutagens that can reach the target organs of human through drinking water. The chromium element in very low amounts is useful for organisms but at higher concentrations, it is toxic and considered as a pollutant.

Chromium (Cr) is often admixed with industrial effluents that are used for irrigation. The uptake of excess concentrations of heavy metals, reduced the growth of plants(Abdel-Azeem and El-Nahas,1996). Thus, Cr loaded effluent used for irrigation disrupts several physiological and cytol**o**gical processes in cells.

Shanker *et al*., (2005) found that chromium reduced root growth, biomass, seed germination, early seedling development and induces chlorosis, photosynthetic impairment and finally leading to plant death (Seoccianti *et al*., 2006; Chidambaram *et al*., 2009 and Akini and Akini, 2010).

Several *in vivo* and *in vitro* studies showed that chromium compounds caused damage DNA in a variety of ways, including DNA single and double-strand breaks (SDSBs) generating chromosomal aberrations, micro-micro-nucleus formation, sister chromatid exchanges, formation of DNA adducts and alteration in DNA replication and transcription (O'Brien *et al*., 2001 and Matsumoto *et al*., 2003).

Plants and plant extracts are inescapable part of many of them exhibit valuable medicinal properties. In the recent years, there has been an increasing interest in antimutagenesis (Calomme, 1996) and antioxidant activity (Yagi *et al*., 2002). These components may be useful in preventing cancer and other mutation related diseases by fortifying physiological defense mechanisms (De Flora, 1996). Antimutagenic agents are natural or synthetic compounds capable of lowering the frequency of mutation by diverse mechanisms .(De Flora and Ramel, 1988).

Corresponding Author: Fawzia I. Mohamed, Department of Genetics and Cytology, National Research Centre, Dokki, Giza, Egypt.
E-mail: adam_fwz@yahoo.com.

Nigella sativa L.(black seed) is commonly known as which belongs to the botanical family of Ranunculaceae. *N. sativa* seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world (Al-Ghamdi, 2001). *N. sativa* is considered a natural food additive and a condiment. Also, it had been used for medicinal purposes as a natural remedy in many ancient cultures, as those of Egypt's, Greece and Rome (Al-Haider, *et al*.,1 993). The *Nigella sativa* is the black seed medicine for every disease except death(Takruri,2003). Recently, many biological activities of *Nigella sativa* L. seeds have been reported, including: antioxidant, anti-inflammatory, anticancer and antimicrobial. *Nigella sativa* L. seeds contain a large amount of fixed oils and the main constituent of the seed extract is thymoquinone (Aboul-Ela,2002 and Kökdil and Yilmaz, 2005). Several pharmacological effects have been attributed to active principles of *Nigella sativa* L. which includes thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine and alpha-hedrin (Aljabre *et al*., 2005). The seeds or its oil is believed to have carminative, diuretic, lactagoge and vermifuge (Houghton *et al*., 1995). *N.sativa* seeds have a wide spectrum of medicinal properties including antimicrobial, antihelminthic, antiinflammatory, analgesic,hypoglycemic, smooth muscles relaxant and immunostimulant activities (Houghton *et al*., 1995 and Hassieb *et al*., 2006).

The present study aimed to evaluate protective effect extract of *Nigella sativa* L. against toxicity of chromium on *Vicia faba* L.

Materials And Methods

Vicia faba L.var. Giza 2. Was used in the present study his varity was supplied by the Legwne Research Section, Agricultural Research Centre, Giza, Egypt.

Potassium dichromate (99.5%) powder was purchased from S.D. FINE. Powder (Fluka).

Treatment:

1-Prepare the extract of *Nigella sativa* L

Nigella sativa seeds were obtained from the local seed supplier; the seeds were crushed manually in a mortar with a pestle. A volume of 100 ml of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged for 15 min. The supernatant (brownish-orange in color) was filtered through Whatman filter No.1using Buchner funnel and stored at 4 °C in sterile tubes until use (Mohamed, 2012).

2-Chromium stock solution was prepared by dissolving 141.4 mg of potassium dichromate ($K_2Cr_2O_7$) in 100 mL distilled water. From the potassium dichromate stock solution, different concentrations of 10, 25, 50, 100 and 200 mg/L of chromium solution were prepared freshly.

Methods of mitotic study:

Faba bean seeds (Giza.2) were grown until the roots reached 1.5-3.00cm. in length, five groups of roots were treated for a duration of six hours.

These groups as shown in Table (1).The roots were cut and fixed in 3 absolute ethyl-alcohol: 1acetic acid (v/v) for 24 hrs., and hydrolysis in 1N HCl for 10 min and stained using Feulgen squash technique. Three replicates were selected for each treatment and control and three roots were examined/ replicate. All experiments were conducted at room temperature (22±1ºC). The mitotic index and the mitotic inhibition were estimated as follows:

Mitotic index = No. of tatal dividing cells / No. of total counted cells X100

The mitotic inhibition= (mitotic index in control- mitotic index in treated)/ mitotic index in control X 100

Chromosome abnormalities were scored in the pro- meta- and ana-telophase stage.

Table 1: Treatment groups of *Nigella sativa* extract and potassium dichromate and abbreviations used in the text.

Plant groups	Treatment for 6 hr	Abbreviated in the text
Group 1	Distill water	Control (C)
Group 2	*Nigella sativa* L. extract	*N.s.*extract
Group 3	potassium dichromate	$K_2Cr_2O_7$
Group 4	potassium dichromate + *Nigella sativa* extract	$K_2Cr_2O_7$+ *N.s.*extract Simultaneously
Group 5	Potassium dichromate for 3h then *Nigella sativa* extract for 3h	$K_2Cr_2O_7$→ *N.s.*extract Post-treatment

Statically analysis:

All cytological data were reported as mean values and Standard Error (SE) of the mean. SPSS computer software was used to estimate the t-test for significance at $p \leq 0.05$ and $p \leq 0.01$ level.

Results And Discussion

The cytotoxicity level of a test compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring (Femander,*et al*.,2007)**.**

The ability of $k_2Cr_2O_7$ to affect the mitotic activity is shown in Table (2). The mitotic index was generally reduced in the treated roots with the different concentrations of $k_2Cr_2O_7$. It reached the lowest value of 3.12% after treatment with 200mg/L $K_2Cr_2O_7$ compared with the control. The limit of mitotic inhibition reached to 71.76%.

Cr caused cytotoxic and inhibition of cell division (Chandra *et al*., 2005). Also, Cr^{6+} may have caused blocking of the entry of cells into mitosis by delaying either the onset of G_2 (Wacchter and Baserga,1982) and/ or S-phase (Mitchison and Creanar,1971).

After treatment with *Nigella sativa* (*N.s*)extract only, the mitotic index showed no significant change and limit of mitotic inhibition was 5.52%. Also, the concentration 10mg/L of $K_2Cr_2O_7$ didn't affect the mitotic index and limit of mitotic inhibition was 8.87%. Treatment with *N.S* extract and $K_2Cr_2O_7$ simultaneously showed a marked protective effect aginest the mito-inhibition effect of the $K_2Cr_2O_7$ at the lower concentrations (10,25mg/L). However, when *N.s.* extract was added at the end of the treatment (post- treatment), the improvement in division rate was much lower (Mohamed, 2002, El-Ashry and Mohamed, 2012).On the other hand, the effect of the highest concentrations on cell division remained almost lower after *N. s.* extract treatment. This mean that the limit of mitotic inhibition improved after treatment with *N.s.* extract in groups 4 and 5. Several investigators found that heavy metals inhibited the cell division in different plants such as *Allium cepa* (Liu *et al*., 1995; Babu and Uma Maheswari, 2006) in *Vicia faba* (Duan and Wan,1995).

In the present study, the different concentrations of hexavalent chromium could lead to various forms of chromosomal aberrations. The aberration rate depends upon the chromium concentrations. The frequency of total abnormalities (0.50, 1.86, 4.22, 9.56, 12.89 and 15.97%) was observed in various concentrations (control, 10, 25, 50, 100 and 200 mg/L) of chromium, respectively. The number of abnormal cells having disturbed, bridge, laggard, stickiness ,fragment and micro-nuclei cells are gradually increased from control to 200 mg/L onwards. The similar findings were observed by Chidambaram,*et al*., (2009). The high doses of chromium supply have a toxic effect on cell division attributes. The increasing concentrations of the heavy metal induced a marked reduction in the mitotic activity of root meristems *Vicia faba* where as the total percentage of chromosomal aberrations were found to be increased.

Table 2: Mitotic index, percentage of limited of mitotic inhibition in *Vicia faba* root-tip meristems after root-treatment for 6 hr with potassium dichromate, then *Nigella sativa* extract or both.

Experimental groups	Concentration (mg/l)	%of MI±S.E.	% limited of Mitotic inhibition
Group 1 Control		11.05± 0.69	
Group 2: *N.s.* extract		10.44± 0.48	5.52
Group 3: $k_2Cr_2O_7$			
	10	10.07±0.63	8.87
	25	9.11± 0.47*	17.56
	50	5.81± 0.29**	47.42
	100	4.03±0.23**	63.52
	200	3.12± 0.19**	71.76
Group 4: $k_2Cr_2O_{7+}$ *N.s* extract			
	10	10.78±0.12	2.44
	25	9.98 ±0.21	9.68
	50	8.99±0.19**	18.64
	100	8.13±0.25**	26.43
	200	7.04±0.09**	36.29
Group 5: $k_2Cr_2O_7 \rightarrow$ *N.s* extract	10	10.89±0.38	1.45
	25	10.76±0.33	2.62
	50	9.76±0.31*	11.67
	100	9.03±0.24*	18.28
	200	8.56±0.15*	22.53

*: Significant at (P< 0.05) level. **: Significant at (P<0.01) level.

The increased number of chromosomal aberrations like stickiness, laggards, chromosome bridges, C-metaphase, fragmentation and micro-nuclei cells were observed with the increasing concentrations of $K_2Cr_2O_7$ (Table 3). Fig. 1 shows the effect of chromium on mitosis of *Vicia faba* root cells. The chromosomal aberrations might be induced directly by chemical compounds. It may also be induced by disturbing the process of DNA and protein synthesis or during the RNA translocation. It may also be induced by the activities of chemical compounds to prevent the re-establishment of the chromosome under normal repairing of some damage Chidambaram *et al*., (2009). The occurrence of many chromosomal aberrations in the experiment clearly indicate that the hexavalent chromium has a genotoxical effect on root cells of *Vicia faba* . The chromosomal aberrations in meristematic cells of roots show stickiness in chromosomes and disturbances that C-metaphase may affect the spindle apparatus (Grant, 1978). Chromosomal stickiness could also be observed at high frequency in this study. The induction of this type of aberration may be due to the disturbance in nucleic acid metabolism in the cell. Similar findings were observed in earlier reports (Vasquez *et al.*, 1986, Salam *et al.*, 1993, Mansour and Kamel, 2005).

Table 3: Percentage of abnormalities and types of mitotic abnormalities in *Vicia faba* root-tip meristems after root –treatments for 6 hr with different concentrations potassium dichromate, then *Nigella sativa* extract or both.

Experimental groups	Concentration (mg/L)	%of Abn.± S.E.	% 0f types of abnormalities relative to the number of abnormal mitosis Dist. Stick. Bridg. Lagg. Frag. Micro-n
Group 1 Control		0.51±0.20	
Group 2: *N.s.* extract		0.37± 0.48	
Group 3: $k_2Cr_2O_7$			
	10	1.86±0.39*	45.45 20.45 13.64 10.64 3.00 6.82
	25	4.22± 0.48**	29.07 34.88 5.81 13.95 4.65 11.62
	50	9.56± 0.89**	26.44 40.23 9.19 12.64 5.75 5.75
	100	12.89±0.23**	35.00 25.00 15.00 15.00 5.00 5.00
	200	15.97± 0.19**	28.57 28.57 14.29 21.29 21.43 7.14
Group 4: $k_2Cr_2O_{7+}$ *N.s* extract			
	10	1.07±0.27	26.32 36,84 26.32 5.26 ----- 2.26
	25	2.21 ±0.15*	30.00 30.00 10.00 20.00 6.00 4.00
	50	6.56±0.79**	33.33 33.33 16.67 11.11 6.11 5.00
	100	8.88±0.48**	36.67 26.67 10.00 10.00 16.67 10.00
	200	9.45 ±0.09**	35.90 25.00 20.00 10.00 5.00 5.0
Group 5: $k_2Cr_2O_7\rightarrow$ *N.s* extract	10	0.99±0.38	23.81 33.33 19.52 19.05 4.29 ----
	25	1.16±0.33	31.25 31.25 9.38 18.75 3.13 6.24
	50	2.76±0.31**	30.41 21.75 21.75 17.39 ---- 8.69
	100	4.03±0.24**	37.50 25.00 18.75 12.50 ----- 6.25
	200	5.56±0.15**	38.88 33.33 11.11 11.11 ----- 5.56

*: Significant at (P< 0.05) level. **: Significant at (P<0.01) level.

It may be attributed to enhance disturbance of spindle function with increase in chromium concentration (Jain *et al.*, 2000). The hexavalent chromium is highly bioactive compound can easily enter the cell through cell membrane and it generates some active oxides. These active oxides can combine with the intracellular DNA and lead to the unreliable intercrossing connection and duplication in DNA and ultimately result in chromosomal aberrations (Li *et al.*, 1995; Qian, 2004). Thus, it has cytotoxic effects which can even lead to DNA damage (Edwards and Karen, 1994).

The number of abnormal cells such as bridge, laggard, stickiness, fragment and micro-nuclei cells are gradually increased from control to 200 mg/L onwards. The similar findings were observed in *Allium cepa* L. (Jayaprakash *et al.* 1994) and sugarcane (Jain *et al.* 2000) due to chromium treatment. *Nigella sativa* extract successfully reduced the effect of $K_2Cr_2O_7$ on the percentage of chromosomal abnormalities in roots treated with $K_2Cr_2O_7$ and *N.s* extract either simultaneously or post –treatment if compared to the treatment with $K_2Cr_2O_7$ alone. *N.s.* extract minimized the induced mito-inhibition and genotoxic effects. Aboul-Ela (2002) found that the seed extract of *Nigella sativa* L. has protective effects against infection with schistosomiasis on mouse cells. Also, Sharma *et al*,(2011) found that the effects of pre-,post- and simultaneous treatment of methanol extract of *Syzygium aromaticum* (MSA) and *Cinnamomum tamala* (MCT) resulted in a dose dependent decrease in chromosomal aberrations frequency in *Allium cepa* after treated with chromium trioxide.

Finally, from all the above mentioned results it was evident that the tested potassium dichromate affected *Vicia faba* on cytological levels. *Nigella sativa* was found to minimize the effect of potassium dichromate on mitotic activities.

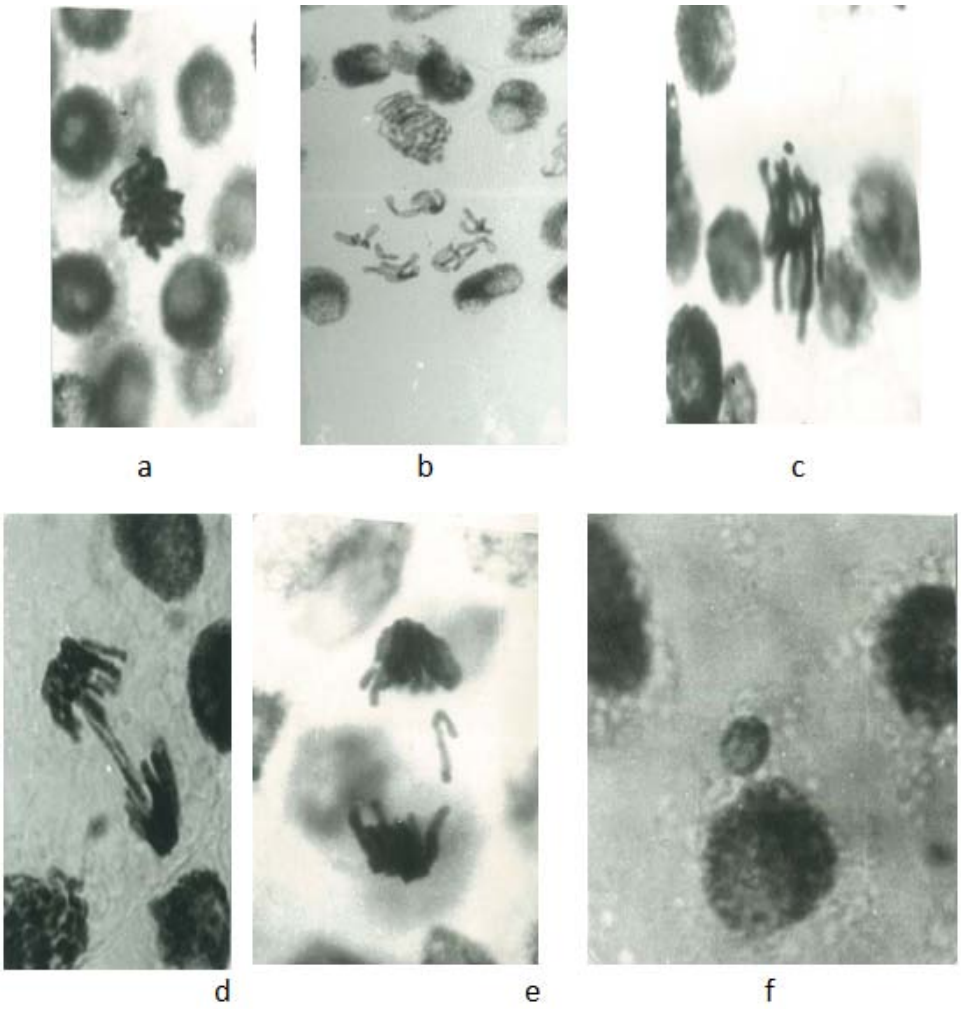

Fig. 1(a-f): Chromosomal abnormalities after root-treated of *Vicia faba* with $K_2Cr_2O_7$. (a): Sticky metaphase after treatment with 50 mg/L. (b): Disturbed metaphase after treated with 25mg/L., (c) metaphase with micro-nuclei after treated with 100mg/L, (d): Anaphase with bridge after treated with 200mg/L, (e):Anaphase with lagging after treated with 100mg/L, (f): interphase with micro-nuclei after treated with 200mg/L.

Conclusion:

The results revealed that potassium dichromate have genotoxic effects on mitotic division. The results also indicated the repair role of *Nigella sativa* extract which succeeded in minimizing the incidence of chromosomal aberrations and mitotic index induced by $K_2Cr_2O_7$ in somatic cells of *Vicia faba* .

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