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GENOTOXIC EFFECTS OF THE HERBICIDE 2,4-D ON MITOSIS AND MEIOSIS OF THE MOUSE AND THE PROTECTIVE EFFECT OF *Nigella sativa* [VAR. BALADY]

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ABSTRACT

The widespread use of pesticides is usually connected with serious problems of pollution and health hazards. The possible mutagenic nature of some agricultural chemicals has been noted. The ability of 2,4-D to induce sister chromatid exchanges (SCE's) and chromosomal aberrations in male mice was investigated. Three dose levels of 2,4-D. (15, 18, 30 mg Kg^{-1} b.wt.) were tested (i.p) for SCE's. The dose 30 mg Kg^{-1} b. wt. induced significant increase in the frequency of SCE's 9.1 ±0.23/cell as compared with 4.3±0.26 for control, but the rate of increase was significantly lower than that with mitomycin C 12±0.5.

Oral treatment with 2,4-D induced a statistically significant increase in the percentage of chromosomal aberrations in mouse spleen cells as well as spermatocytes cells. The aberration frequency increased with the dose and the number of treatments. *Nigella sativa* (var. Balady) decreased the percentage of sister chromated exchanges (SCE) and chromosomal aberrations in spleen cells and germ cells after oral treatment.

The present study showed that 2,4-D has a potential genotoxic effect and advised or adjuvant protective agents such as *Nigella sativa* (var. Balady) must be used with it.

Key words: *cell- protective effect, chromosomal aberrations, Nigella sativa, sister chromatid exchange (SCE)*.

1. INTRODUCTION

The widespread use of pesticides is usually connected with serious problems of pollution and health hazards, the possible mutagenic nature of some agricultural chemicals has been noted (Wild, 1975).

"2,4-D" induced genotoxic effects in rat bone-marrow and was found to be clastogenic (Adhikari and Grover, 1988) and also increased chromosome breakage in the habituated *Nicotiana glauca* tissue cultures (Ronchi *et al.*, 1976).

Several studies revealed that 2,4-D induced a genotoxic effect. El-Najjar and Soliman (1982), reported that 2,4-D increased the percentage of abnormal meiotic cells of wheat significantly and caused changes in mitotic activity, as well as changes during the cell cycle in shallot root-tip cells (Pavlica *et al.*, 1991).

Many substances can reduce or eliminate the activity of mutagens. Some of these antimutagens are natural agents such as *Nigella sativa*, chlorophyllin, Beta-Carotene and garlic (Warner *et al.*, 1991), and others are synthetic such as glutathione and Vit. E. (Tadi *et al.*, 1991).

Nigella sativa is used to protect from negative effects of cisplatin, (Nair *et al.*, 1991). Salomi *et al.*, (1992) demonstrated the anti-tumour effect of *Negella sativa* on Dalton's lymphonia ascites "DLA", Ehrlich ascites carcinoma "E.A.C" and sarcoma-180 *in vitro* and *in vivo*, and it was also demonstrated to act as an anti-oxidants (Houghton *et al.*, 1995).

Khan and Choudhuri (1998) found that in acute metabolic studies using mice as experimental animals, extracts of *Nigella sativa* showed an increase in food intake but almost no change or a slight decrease in defaecation.

In the present investigation, studies were carried out to show the effect of "2,4-D" on mammalian chromosomes using the two cytogenetic parameters, sister chromatid exchange in bone- marrow and chromosomal aberration analysis in somatic cells (spleen) and

germinal cells (spermatocytes) of male mice. Moreover, the protective effect of *Nigella sativa* was studied also.

2. MATERIALS AND METHODS

2.1.Animals

Male Swiss mice (9-12 week-old, weighting 30-35g) were used in all experiments. The animals were obtained from a closed random bred colony at the National Research Centre (NRC), maintained under controlled conditions of temperature and humidity and received feed and water *ad libitum*.

2.2.Chemicals

2,4-Dichlorophenoxy acetic acid (2,4-D) was dissolved in distilled water. The protective *Nigella sativa* (var. Balady) was obtained from the Medicinal and Aromatic plant BreedingGroup. Genetic and cytology Dept.) . one mg/kg b.w from *Nigella sativa* was administered orally to animals.

2.3.Dosage and treatment

Sister chromatid exchange: The experimental procedure was conducted in accordance with the protocol of Allen (1982). The used doses were 15,18 and 30 mg kg^{-1} b. wt. 2,4-D. Brdu tablets, weighing approximately 55±2 mg, were placed subcutaneously.

Mice were injected (i.p.) with 2,4-D.8 hr following Brdu treatment and treated with colchicine 2 hr prior to killing. Metaphase spreads were prepared using the method of Yosida and Amano (1965). Differential staining of sister chromatids was performed according to the fluorescence plus Giemsa method with the use of black lights. Only euploid cells (2n = 40) and well spread chromosomes were selected for scoring. Fourty metaphase spreads per animal were examined microscopically for SCE's (200 metaphases per each treatment group). In all experiments, 2,4-D was dissolved in distilled water.

Chromosomal aberration for spleen and spermatocytes, single oral treatment *via* oral gavage was administered. Oral LD_{50} was determined experimentally. Two different dose levels, 15 and 30 mg

kg^{-1} body wt. were used and mice killed one day and seven consecutive days after treatment. Untreated mice and mice injected with mitomycin C (1 mg kg^{-1} b.wt.) were used as negative and positive controls. Control and treated animals were injected with colchicine 2-3 hr before killing. For spleen cells, fresh spleen was washed with RPMI medium (RPMI, sterile filtered with L-glutamine and $NaHCO_3$ "Sigma chemical made in U.K." and cut into small pieces. The cells were aspirated into a centrifuge tube and 0.075 M KCl was added.

Primary spermatocytes at metaphase I(MI) were prepared from testes according to the air drying technique of Evans *et al.*, (1964). The cells were centrifuged at 1000 rpm for 10 min. and fixed in methanol-acetic acid (3:1) Amer *et al.*, (1993). Slides were stained with 7% Giemsa in phosphate buffer. Around 75 metaphases were analysed per animal in five mice per group scoring the different types of chromosomal aberrations. Significant differences from controls were calculated with student's t-test.

3. RESULTS AND DISCUSSION

The ability of 2,4-dichlorophenoxy acetic acid (2,4-D) to induce sister-chromatid exchange (SCE) in mouse bone-marrow cells was investigated (Table 1). SCE's are currently recognized as being a sensitive indicator of agents which damage mammalian DNA (Latt 1974) and their formation has been compared with that of mutation (Wilkoskey and Rynard 1990). All the tested doses of 2,4-D induced a significant increase in the frequency of SCE's in bone-marrow cells of mice (Table 1). The mean frequency of SCE's increased proportionally as the concentration increased, it reached (9.1±0.23) after injection with the highest tested of dose 30 mg "2,4-D" Kg^{-1} b. wt. compared with (4.3±0.26)/cell for non-treated animals. Such frequency was lower than that induced by the positive control mitomycin C 1 mg Kg^{-1} b. wt. (12±0.5). The results indicated that (2,4-D) is a strong inducer of sister chromatid exchanges in mouse bone-marrow at the high doses.

2,4-D also induced significant increase in the percentage of chromosomal aberrations in spleen cells even after exchange gaps (P

< 0.01) except with the lowest tested dose (single oral treatment) (Table 2). The frequency of chromosomal aberrations increased as the concentration of (2 , 4 -D) goes high. Multiple treatments caused a higher percentage of chromosomal aberrations than single treatment. This may be due to the accumulation effect of 2,4-D. Positive control data showed a high frequency of chromosomal aberrations over those of the treated animals.

Concerning the different types of the aberrations observed, they were mainly of the chromatid type (gap, fragments, deletions). Robertsonian translocation(R.T.)appeared after treatment and increased with the highest tested dose of 2,4-D and after repeated treatments. Tetraploid metaphases were also observed.

The insecticide "dursban" induced chromosome aberrations in bone marrow (Amer *et al.*, 1996) and spleen of the mouse (Amer *et al.*, 1998). Some authors recommended the study of chromosomal aberrations in germinal cells in which the transmissible genetic damage from one generation to another takes place (William and Hsu 1980, Hassan, 1997).

The most common type of abnormality was observed in spermatocytes after treatment with another herbicide(Amer *et al.*, 1998 and Fahmy, 1999).The tested doses of 2,4-D were capable of inducing significant increase in the percentage of chromosomal aberrations in mouse spermatocytes (Table3).Such percentage reached 10.0±1. 9 and 11.2 ± 1.2, respectively after treatment with the doses 15 and 30 mg Kg^{-1} body wt. compared with 3.6 ± 0.48 for the control.Such increase did not exceed the value of mitomycin C (18.6±0.74).Multiple treatments for 7 consecutive days with the same doses caused higher percentage of chromosomal aberrations than single treatment(Table3).Univalent formation increased highly. However,x-y univalents were much more frequent than autosomal univalents.

Ford(1969) reported that autosomal univalents may result from asynapsis(absence of Zygotene pairing between homologous segments) or from desynapsis (sparation due to the failure of chiasma formation).Also,breaks,fragments and gaps were observed. Translocation in the form of a chain of IV was observed after treatment with different doses. It was observed that the percentage

Table (1): Frequency of sister chromatid exchanges (SCE`s) in mouse bone-marrow cells induced after i.p. treatment with different concentrations of 2,4-D.

	Treatment and doses	Mice No.	Metaphases SCE's No.	SCE's No.	SCE's/cell Mean ± S.E.
I-	Control (non treated)	5	200	860	4.3 ± 0.26
	Mitomycin C 1 mg Kg^{-1} body wt. (+ve control)	5	200	2398	12 ± 0.5
II-	2,4-D				
	a) 15 mg Kg^{-1} body wt.	5	200	1060.	5.3 ± 0.17
	b) 18 mgKg^{-1} body wt.	5	200	1242	6.21 ± 0.35*
	c) 30 mgKg^{-1} body wt.	5	200	1794	9.1 ± 0.23**
III-	Protection *Nigella sativa* (var. Balady) 1 mgKg^{-1} body wt. added with 30 mg 2,4-D, Kg^{-1} body wt.	5	200	998	5.1 ± 0.2

* Significant at 0.05. ** Significant at 0.01/level

Table (2): Number and mean percentage of metaphases with different types of chromosomal abnormalities in mouse spleen cells induced after oral treatment with 15 and 30 mg "2,4-D" kg^{-1} body wt.

Treatment and dose	Harvest time after the last treatment	Total number of examined metaphases (n)	No. of metaphases						Metaphases with chromosomal abnorm.	
			No. of abonormal metaphases (n)	Gaps	Frag. and/or break	Robertson trans.	Dele.	Tetraploid chrom. No.	Including gaps mean %± S.E.	Excluding gaps mean ± S.E.
I- Control (non-treated)	-	500	16	4 (0.8)	12 (2.4)	-	-	-	3.2±0.4	2.4±0.4
II- Positive control mitomycinC 1mg Kg^{-1} body. wt.	one day	500	112	20 (4)	39 (7.8)	11 (2.2)	28 (5.6)	14 (2.8)	22.4±0.8	18.4±1.1
III- 2,4-D										
a) 15 mgKg^{-1} body wt.	one day	500	46	16 (3.2)	13 (2.6)	3 (0.6)	9 (1.8)	5 (1)	9.2±1.3*	6.0±0.8
	7 days	500	66	20 (4)	17 (3.4)	8 (1.6)	15 (3)	6 (1.2)	13.2±1.35**	9.2±1.1*
b) 30 mgKg^{-1} body.wt.	one day	500	82	22 (4.4)	43 (8.6)	4 (0.8)	7 (1.4)	6 (1.2)	16.4±0.97**	12±1.26**
	7 days	500	118	39 (7.8)	58 (11.6)	7 (1.4)	8 (1.6)	6 (1.2)	23.6±0.97**	15.8±0.49**
IV. Protection *Nigella sativa* mgKg^{-1} b.wt. (var. Balady) with 30 mg 2,4-D Kg^{-1} b.wt.	7 days	500	30	5 (1)	12 (2.4)	-	9 (1.8)	4 (0.8)	6±0.74	5±0.73

* Significant at 0.05 level. ** Significant at 0.01 level.

Table (3): Number and mean percentage of metaphases with different types of chromosomal abnormalities in mouse spermatocytes of male mice induced after oral treatment with 15 and 30 mg "2,4-D" Kg^{-1} body wt.

Treatment and dose	Harvest time after the last treatment	Total number of examined metaphases (n)	No. of abnormal metaphases	Univ. % metaphases with:				Metaphases with chromosomal aberrations mean %±S.E.
				x-y univ.	Autos. univ.	Frag.	ChainIV	
I- Control (non-treated)	-	500	18	7 (1.4)	8 (1.6)	3 (0.6)	-	3.6±0.48
II- Positive control mitomycinC 1mg Kg^{-1} body. wt.	one day	500	93	36 (7.2)	37 (7.4)	13 (2.6)	7 (1.4)	18.6±0.74
III- 2,4-D body								
a) 15 mgKg^{-1}body.wt.	one day	500	50	17 (3.9)	21 (4.2)	9 (1.8)	3 (0.6)	10.0±1.09*
	7 days	500	78	28 (5.6)	33 (6.6)	12 (2.4)	5 (1)	15.6±1.16**
b) 30 mgkg^{-1}body.wt.	one day	500	56	20 (4)	26 (5.2)	7 (1.4)	3 (0.6)	11.2±1.2*
	7 days	500	102	40 (8)	49 (9.8)	6 (1.2)	7 (1.4)	20.4±0.8**
IV. Protection *Nigella sativa*,4-D,1mgKg^{1}b.wt. (var. Balady) with 30 mg 2.4D Kg^{-1}b.wt.	7 days	500	31	12 (2.4)	10 (2)	9 (1.8)		6.2±1.44

* Significant at 0.05 level ** Significant at 0.01 level.

of abnormalities in sister chromatial exchange in bone-marrow, spleen cells and germ cells in mice treated with 2,4-D decreased after post-treatment with *Nigella sativa* (var. Balady) (Tables 1,2,3).

The search for natural therapeutic agents to treat different diseases including cancer,has become a matter of interest to many scientists.*Nigella sativa*(var.Balady)is a natural substance that was reported to be an inhibitor of chemical carcinogen (Salomi *et al.*, 1991).

Hrelia *et al.*, (1987) reported the protective effect of *Nigella sativa* against any mutagenicity in liver, kidney, and lung. Salam *et al.*, (1992) treated mice bearing ascites carcinoma with a single dose of ethanol extract of *Nigella sativa.* They found an increase in the mean survival time of animals by 45%.

El-Banna (1993) concluded that *Nigella sativa* (var. Balady) increased significantly natural killer cell percentage and the level of plasma complement C_3 in patient with liver cirrhosis. So, *Nigella sativa* could be used in the management of patient suffering from liver cirrhosis and prevention of major complications as hepatocellular carcinoma.

Khan and Choudhuri (1998) studied acute and chronic metabolism of *Nigella sativa* l imm, in auto metabolic studies using mice as experimental animals, extracts of *N. sativa* seeds, there was an increase in weight in the cauda epididymides and seminal vesicles. Gross histopathological changes were noticed in the kidney and testes. Possible histopathological changes were noticed in the liver and small intestine.

Badary *et al.*, (1998) studied acute and subchronic toxicity of thymoquinone (TQ) in mice, the main constituent of black seeds of *Nigella sativa.* In the subchronic study, mice received TQ in drinking water (0.01, 0.02 or 0.03%, with approximate daily intakes of 30, 60 and 90 mg/day, respectively) for 90 days with no resulting mortality or signs of toxicity. There were no changes of toxicological significance in body and organ weights, food and water intake, or urine and faeces output. Tissue GSH, plasma concentrations of total proteins, were urea creatinine and triglycerides and enzyme activities of ALT, LDH and CPK were not affected. Histological examination revealed no gross or microscopic tissue damage. TQ did

induce a significant decrease in fasting plasma glucose.

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التأثير الوراثى السمى لمبيد الأعشاب *"توفوردى"* على خلايا نخاع العظام، خلايا الطحال والخلايا التناسلية للفأر والتأثير الوقائى لحبة البركة (البلدى)

كوثر منصور الشربينى

قسم الوراثة والسيتولوجى- المركز القومى للبحوث - الدقى - القاهرة

ملخص

يهدف البحث إلى دراسة قدرة مبيد الأعشاب "التوفوردى" على إحداث الشذوذ الكروموسومى فى خلايا نخاع العظام (تبادل الكروماتيدات الشقيقة)، خلايا الطحال والخلايا التناسلية للفأر.

تم الحقن بالجرعات (١٥، ١٨، ٣٠ ملليجرام "توفوردى"/كجم من وزن الجسم. زادت النسبة المئوية للشذوذ الكروموسومى بزيادة التركيز حيث بلغت النسبة ٩,١ ± ٠,٢٣ عند أعلى تركيز للمبيد (وكانت ذات قيمة إحصائية معنوية عالية) بالمقارنة بالنسبة ٤,٣ ± ٠,٢٦ لمعاملة المقارنة. ولقد تم الحقن بعد ٢٤ ساعة خلال الغشاء البريتونى بالمضادة الضابطة الموجبة الميتوميسين (ج) وكانت النسبة ١٢ ± ٠,٥.

وقد تم تغذية الفئران عن طريق الفم وكان له تأثير ذات قيمة إحصائية معنوية عالية على خلايا الطحال والخلايا التناسلية للفئران.

لقد احدثت حبة البركة (البلدى) تأثيراً ملحوظاً على تقليل نسبة الشذوذ الكروموسومى الذى أحدثه "التوفوردى" فى كل من خلايا نخاع العظام (تبادل الكروماتيدات الشقيقة)، خلايا الطحال والخلايا التناسلية للفئران بعد التغذية عن طريق الفم.

لذلك يجب أن نتخذ الاحتياطات الكافية عند استعمال "التوفوردى" فى الأغراض الزراعية.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٢) العدد الثالث (يوليو ٢٠٠١):٤٩٧-٥١٠.