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RESEARCH ARTICLE

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AMELIORATIVE ACTION OF NIGELLA SATIVA AGAINST IRON INDUCED CHROMOSOMAL ABERRATIONS IN RAT BONE MARROW CELLS IN VIVO

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ABSTRACT

Iron deficiency is a worldwide problem and is, in general, more frequent than its excess. Unabsorbed iron may generate free radicals and so, it leads to oxidative stress to absorptive cells. Therefore, it is advisable to give iron tablets, along with vitamin C (to convert it into ferrous form) and vitamin E (to prevent free radicals.) *Nigella sativa* may also act as an ROS scavenger and as a metal chelant. To examine these possibilities, we tested *in vivo* the effect of *Nigella sativa* (50 mg/kg b.wt.) against the genotoxicity of $FeSO_4.7H_2O$. The aim of this study was to generate and evaluate genotoxic data for iron induced free radicals using *in vivo* chromosomal aberration assay in bone marrow cells of rats. Bone marrow cells were obtained from the rats using the technique described by Preston *et al*. (1987). The results of treatments with *Nigella sativa* indicated that it statistically significantly decreases the number of chromosomal aberrations with aberrations induced by $FeSO_4.7H_2O$, but it cannot completely protect cells from damage. *Nigella sativa* showed the most efficient anticlastogenic effect during simultaneous treatment with $FeSO_4.7H_2O$. The result further strengthened the findings about the protective and antigenotoxic properties of *Nigella sativa*.

KEYWORDS

Nigella sativa; Chromosomal aberrations; Ferrous sulfate; Genotoxicity.

INTRODUCTION

Iron is essential for normal cellular functions. However, iron has the potential to cause significant oxidative damage leading to mutagenesis[1]. Iron's toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury. Iron sulfate is a common chemical element that is present in foods and beverages and that is used to treat iron deficiency anemia[2]. The precise mechanism of iron induced oxidative damage to DNA is not known but is believed to involve free iron that catalyzes the formation of hydroxyl radicals by the Fenton reaction[3]. DNA damage may result directly by the oxidation of nucleoside bases or indirectly via the formation of lipid peroxides. *Nigella sativa* L. (black cumin; kalonji) belongs to the Ranunculaceae family comprising many annual herbs. Unfortunately very few people are aware of its medicinal properties discovered by the modern scientific techniques. The effect of NS has been evaluated in animal studies. There are many reports on its biological activities including antihypertensive, anti-diabetic, anti-bacterial[4], anti-tumour and as immunomodulator[5]. *Nigella sativa* oil (crude oil obtained by squeeze) was orally administered to animals at a dose level of 1ml/kg[6]. *Nigella sativa* seeds contain 36-38% fixed oils, proteins, alkaloids, saponin and 0.4- 2.5%-essential oil[7]. Black seed components display a remarkable array of biochemical, immunological and pharmacological actions[8-12].

Therefore, the aim of the present study was to investigate the possible protective effect of *Nigella sativa* seeds in reducing the iron induced free radicals using *in vivo* chromosomal aberration assay in bone marrow cells of rats.

MATERIALS AND METHODS

(i) Chemical Treatment

Ferrous sulfate ($FeSO_4$) (CAS 7782-63-0) was obtained from Merck Specialities Private Limited. Ferrous sulfate solution was freshly prepared by dissolving in distilled water and was given intraperitoneally in doses of 36.1 to 28.9 mg per kg to the test animals with or without the administration of antioxidant. The doses used for iron are equal to 1/8 and 1/10 of rat intraperitoneal LD_{50}. *Nigella sativa* seeds (Black seed) are purchased from the local market and seeds are cleaned, air-dried and are then powdered mechanically to prepare a suspension in isotonic saline solution.

(ii) Chromosomal Aberrations

The animals were sacrificed by cervical dislocation after the exposure of test chemical with or without the administration of *Nigella sativa* 24 and 48 hr later, with colchicine (0.16%) injected intraperitoneally (0.5 ml/100g b.wt.) an hour and a half before sacrifice. Bone marrow cells were obtained from the rats using the technique described by Preston *et al*.[13].

Rats were distributed into 6 groups (6 animals in each) as follows:

Group 1 (Negative Control): normal rats as a control group received 0.5ml distilled water.

Group 2 (Positive Control): normal rats as a positive control group received Cyclophosphamide intraperitoneally.

Group 3 (1/8 of the LD_{50} $FeSO_4.7H_2O$): 36.1 mg per kg $FeSO_4.7H_2O$

Group 4 (1/10 of the LD_{50} $FeSO_4.7H_2O$): 28.9 mg per kg $FeSO_4.7H_2O$

Group 5 ($FeSO_4.7H_2O$ & NS) - Administration of 1/8 of the LD_{50} of $FeSO_4.7H_2O$ intraperitoneally with NS orally at a dose level of 4ml/kg b.wt. (50 mg/kg b.wt.).

Group 6 ($FeSO_4.7H_2O$ & NS) - Administration of 1/10 of the LD_{50} of $FeSO_4.7H_2O$ with NS intraperitoneally at a dose level of 4ml/kg b.wt. (50 mg/kg b.wt.).

Bone marrow cells were flushed from both femurs in 0.075 M KCl and cells were then resuspended in 5 ml of a hypotonic solution of 0.075 M KCl for 20 min at 37°C. The cells were centrifuged at 1000 rpm for 10 min and fixed with three changes of 5 ml each of ice – cold Carnoy's fixative (methanol - acetic acid, 3:1, v/v). The cells were then dropped onto clean, grease free microscope slides which were air-dried and stained with 5% Giemsa for 15 min. Cytogenetic analysis of the slides was performed with a light microscope using a 100x oil immersion for structural chromosome aberrations. 100 well spread complete metaphases were scored per slide and 4 slides were prepared per rat for each concentration.

(iii) Statistical Analysis

Data are expressed as the mean ±S.E. Student's two tailed "t" test was used for calculating the statistical significance for CA with the help of SPSS 18. The level of significance was set at $P < 0.05$.

RESULTS

Table I and II present the data showing genotoxic effects of $FeSO_4.7H_2O$ and its amelioration by *N. sativa* at 24 and 48 hr. The mean frequency of cells with aberrations was 0.03±0.01, 0.41±0.17, 0.24±0.10, 0.10±0.04, 0.36±0.15 and 0.19±0.08 for control, positive control, 1/10 of LD_{50} of $FeSO_4.7H_2O$, 1/10 of LD_{50} of $FeSO_4.7H_2O$ + 50 mg/kg *N. sativa*, 1/8 µg/ml of LD_{50} of $FeSO_4.7H_2O$ and 1/10 of LD_{50} of $FeSO_4.7H_2O$ + 50 mg/kg *N. sativa* respectively at 24 hr **(Table-1 and Figure-1)**. The mean frequency of cells with aberrations was 0.03±0.01, 0.41±0.17, 0.23±0.10, 0.11±0.05, 0.34±0.15 and 0.18±0.08 for control, positive control, 1/10 of LD_{50} of $FeSO_4.7H_2O$, 1/10 of LD_{50} of $FeSO_4.7H_2O$ + 50 mg/kg *N. sativa*, 1/8 µg/ml of LD_{50} of $FeSO_4.7H_2O$ and 1/10 of LD_{50} of $FeSO_4.7H_2O$ + 50 mg/kg *N. sativa* respectively 48 hr **(Table-2 and Figure-2)**. The difference between control and treated values are statistically significant showing quite significant amelioration after administration of *N. sativa*. The values mentioned above showed a significant increase when compared to the control (P<0.05).

Table: 1

Chromosomal Aberrations in Bone marrow metaphase chromosomes after $FeSO_4.7H_2O$ Treatment and its amelioration with Nigella sativa for 24 hr Duration

Drug Conc.	Chromatid type Aberrations			Chromosome type Aberrations			No. of cells with Aberrations	Aberrations/ cell ±SE
	Gaps	Breaks	Exchanges	Break	Dicentrics	Rings		
$FeSO_4.7H_2O$								
1/10 of LD_{50}	5	6	3	5	1	4	24	0.24±0.10*
1/10 of LD_{50}+NS	3	2	1	2	0	2	10	0.10±0.04*
1/8 of LD_{50}	7	9	5	7	2	6	36	0.36±0.15**
1/8 of LD_{50}+NS	4	6	2	4	0	3	19	0.19±0.08*
Controls								
Positive (CP)	7	10	6	8	3	7	41	0.41±0.17*
Negative	1	1	0	1	0	0	3	0.03±0.01

*CP = Cyclophosphamide SE = Standard error *Significant at P<0.02 **Significant at P<0.01*

Table: 2

Chromosomal Aberrations in Bone marrow metaphase chromosomes after $FeSO_4.7H_2O$ Treatment and its amelioration with Nigella sativa for 48 hr Duration

Drug Conc.	Chromatid type Aberrations			Chromosome type Aberrations			No. of cells with Aberrations	Aberrations/ cell ± SE
	Gaps	Breaks	Exchanges	Break	Dicentrics	Rings		
$FeSO_4.7H_2O$								
1/10 of LD_{50}	6	6	2	4	1	4	23	0.23±0.10*
1/10 of LD_{50}+NS	2	4	1	2	1	1	11	0.11±0.05*
1/8 of LD_{50}	8	10	2	8	2	4	34	0.34±0.15*
1/8 of LD_{50}+NS	4	5	1	6	0	2	18	0.18±0.08*
Controls								
Positive (CP)	7	10	6	8	3	7	41	0.41±0.17*
Negative	1	1	0	1	0	0	3	0.03±0.01

*CP = Cyclophosphamide SE = Standard error *Significant at P<0.02*

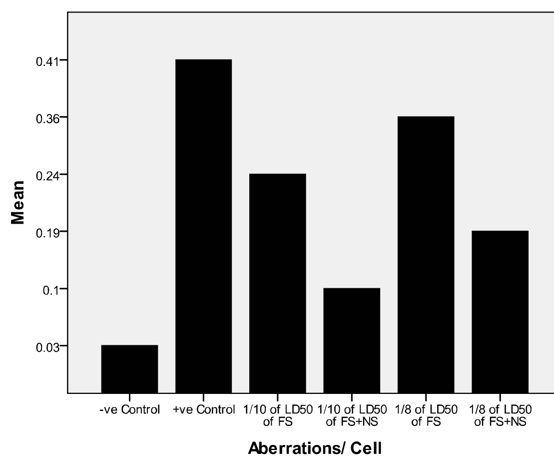

Figure:I

Graphical Representation of Chromosomal Aberrations in Bone marrow metaphase chromosomes after $FeSO_4.7H_2O$ Treatment and its amelioration with Nigella sativa for 24 hr Duration

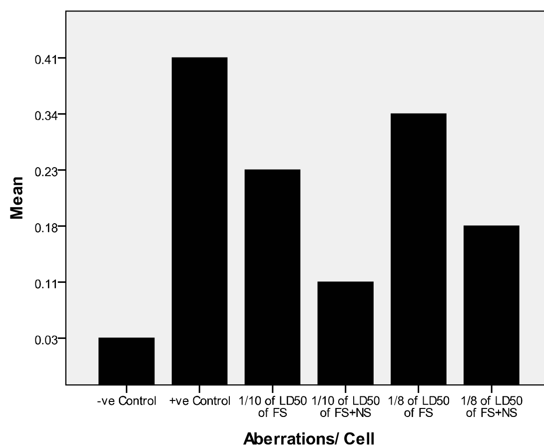

Figure:II

Graphical Representation of Chromosomal Aberrations in Bone marrow metaphase chromosomes after $FeSO_4.7H_2O$ Treatment and its amelioration with Nigella sativa for 48 hr Duration

DISCUSSIONS

Iron sulfate is a common chemical that is present in foods and beverages and that is used to treat iron deficiency anemia[2]. Iron can be toxic because of its role in oxidative stress [14]. Moreover, iron can damage biomolecules mainly through Fenton and Haber-Weiss chemistry, leading to the production of hydroxyl radicals and other reactive oxygen species (ROS)[15]. Iron compounds have been reported to be mutagenic in mammalian culture cells, as detected by Syrian hamster embryo cell transformation/viral enhancement assay[16], base tautomerization in rat hepatocyte cultures[17] and genetic alterations in the mouse lymphoma assay[18].

The seeds of *Nigella sativa* have been used traditionally for centuries in the Middle East, Northern Africa and South Asia for the treatment of various diseases[19, 20]. The plant extracts and essential oil showed a broad range of pharmacological effects such as antidiabetic[21,22], spasmolytic and bronchodialator[23, 24], antioxidant[19, 25], hepatoprotective[26, 27], antihyperlipidemic[28], analgesic and anti-inflammatory[29], antitumor[30, 31] and antiulcer[32, 33] effects in various studies. The extracts also showed *in vitro* and *in vivo* antimicrobial[34], antileishmanial (unpublished data) and anticestodal effects[35]. It is used traditionally in Iran as laxative, carminative and intestinal antiprotozoal drug[36].

In the present study, co-administration of *Nigella sativa* with iron sulfate reduced the number of gaps, breaks, exchanges, dicentrics and rings in the chromatid type aberrations and chromosome type aberrations in bone marrow cells of rats. The protective effect of *Nigella sativa* oil against CCl_4 and D-galactosamine induced hepatic toxicicity in rats was reported by El-Dakhakhny *et al*.[32] and Al- Gamdi[27] whom observed reduction in the activities of serum AST, ALT, alkaline phosphatase, lactate and malate dehydrogenases. In addition, other previous studies demonstrated that *Nigella sativa* may be successful in the protection of liver fibrosis in rabbits[37] and that its oil may play a role against liver damage induced by *Schistosoma mansoni* infection in mice[38]. In conclusion, the present study demonstrated that NS administered in combination with $FeSO_4.7H_2O$ minimized its clastogenic hazards. Consequently, using NS as spice and dietary antioxidant could be beneficial in alleviating $FeSO_4.7H_2O$ toxicity.

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