Effect of Black Cumin (\textit{Nigella sativa}) Extracts in Inhibiting of the Mutagenic Activity of Mitomycin C in Albino Mice

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Abstract. The aim of this study is to investigate the ability of black cumin (\textit{Nigella sativa}) aqueous extracts in reducing the genotoxic effects induced by mitomycin C (MMC) in male mice \textit{Mus musculus} (balb/c strain). \textit{Nigella sativa} (\textit{N. sativa}) has been widely used as a medicinal plant that treats different diseases. To determine the genotoxic effects of mitomycin C, the mitotic index of somatic and germ cells and chromosomal aberrations (chromatid breaks, chromosome breaks, and ring chromosomes) were tested. Twenty-five animals were divided into three main groups: group one was used as a negative control treated with buffer saline, group two was used as a positive control treated orally with mitomycin C (2 mg/kg b. w.) for two days; group three, which was divided into three sub groups, was treated orally with three different doses of \textit{N. sativa} extracts (50, 100, 150 mg/kg b. w.) for one week, and then treated with mitomycin C for two days. The results of this study showed a protective role of \textit{N. sativa} effecting a significant increase (P<0.05) of the frequency of mitotic index of both somatic and germ cells. Additionally, \textit{N. sativa} may improve therapeutic efficiency of mitomycin C by inhibiting the chromosomal aberrations.

1. Introduction

Many medical plant products play an important role in the control of different types of diseases development [1]. About 40\% of chemicals consist of natural compounds [1]. Moreover, medical plants contain antioxidants, vitamin E, vitamin C and carotenoids [2]. For many years, many medicinal plants play an important role in the treatment of diseases in the world, like dates and olive by regulating of genetic and biological pathway [3-5]. \textit{N. sativa}, also known as black cumin or black seed, is one of the herbal medicines belonging to the Ranunculaceae family which grows in the Middle East and the South of Mediterranean [6]. It consists of essential oils, alkaloids, saponin and proteins, amino acids, lipase, minerals and vitamins [7, 8]. Thymoquinone (TQ) is an active component of its essential oils that shows antioxidant potentials and plays a crucial role in cancer prevention by controlling molecular cascade [9]. Many studies demonstrated that the \textit{N. sativa} has many therapeutic properties, including anti-inflammatory, antiviral activates, antimicrobial, hepatoprotective, anti-fungal and anti-tumor effects [7, 8, 11].

In another study, \textit{N. sativa} seeds and their oil have been widely used to treat various diseases, such as asthma, diarrhea, skin disorder, rheumatism and immune system support, throughout the world [19]. Several modern scientific techniques were carried out on \textit{N. sativa} since it has pharmacognostical characteristics and pharmacological activities. Several chemicals mutagens induce chromosomal
aberration and mutations inducers in organisms [10]. *N. sativa* reduces the cisplatin, toxicity, and it is used as a chemotherapy drug [11]. The present study tries to investigate the protection effects of *N. sativa* extracts on the cytogenetic damages caused by mitomycin C (MMC) to treat the deleterious diseases.

2. Materials and Methods

2.1 Animals

The experiments were carried out on mature and healthy white Swiss male mice (*Mus musculus*) 11-12 weeks old, weighting 22-25 g. The animals (5 mice/group) were maintained under standard laboratory conditions, such as temperature, hour light/dark were suitable. They were housed in plastic boxes and bedded with wood shavings. During the experience, water and food were provided.

2.2 Experimental design

In this study, the twenty five albino male mice were divided mainly into three groups: group one was used as negative control (5 mice) and it was treated daily and orally with phosphate buffer saline (PBS) (0.5 ml/kg b. w.); group two was used as positive control (5 mice) treated orally (0.1 ml) with mitomycin C (2 mg/kg b. w. for two days); the third group which was further divided into three sub groups (5 mice/sub group) was treated orally(0.5 ml) and daily with three different doses of *N. sativa* extracts (50, 100, 150 mg/kg b. w.) for one week and then treated with mitomycin C (2 mg/kg b. w. for two days).

2.3 *N. sativa* aqueous extracts preparation

*N. sativa* seeds were purchased from a local herb store in Al-Qadisiyah, Iraq. The seeds were cleaned and dried in shadow and then powdered. The seed powder was boiled for 10 minutes with hot water and filtered.

2.4 Mitotic index and chromosomal aberrations tests

In order to test the effects of *N. sativa* extracts on mitotic index (somatic and germ cells) and chromosomal aberrations (chromatid breaks, chromosome breaks, and ring chromosomes). Chromosomal preparations from bone marrow cells were done by the standard method of Evans et al., (1964) [12]. To calculate the mitotic index (MI) of somatic and germ cells, 1000 cells were examined for each replicator and then the cell division was calculated by calculating the percentage between the number of dividing cells and the total number of cells. (MI) = (number of split cells / total number of cells×1000) × 100 [20].

3. Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Science (SPSS). One-way analysis of variance (ANOVA) was used to compare the groups. The student t-test was used to determine if there are significant differences between groups; p values<0.05 were considered statistically non-significant. All data are presented as means ± Standard Error of Mean (SEM).
4. Results and Discussion

Effects of N. sativa extracts against mitomycin C on mitotic index of somatic and germ cells

Table (1) shows the results of mitotic index of somatic and germ cells which indicates significant difference (P<0.05) between negative control and treated groups. In the mitomycin C (positive control) treatments, the percentage of mitotic index of both somatic (bone marrow) and germ cells significantly decreased (P<0.05) compared with the negative control. In the third concentration (150 mg/kg b. w.) of N. sativa plus mitomycin C treatments, it was observed that the mitotic index inhibited by mitomycin C significantly increased (p<0.05) compared with the first and the second doses (50 mg/kg b. w. and 100 mg/kg b. w.).

Effects of N. sativa extracts against mitomycin C on chromosomal aberrations

Table (2) shows the results of chromosomal abnormalities. Chromosomal aberrations, such as chromatid breaks, chromosome breaks and ring chromosomes, were tested and they increased significantly (P<0.05) in mitomycin C group (Positive control) when compared with negative control. However, the treatments with N. sativa extracts showed a significant decrease (p<0.05) of all three doses compared to negative control. The results show that all three doses of N. sativa extracts reduced the harmful effects of mitomycin C. The third concentration (150 mg/kg b. w.) was the highest protective dose compared with the first and the second doses (50 mg/kg b. w. and 100 mg/kg b. w.) respectively.

Table 1. Effects of N. sativa extract and mitomycin C inhibited mitotic index in albino mice (Mean±SEM) (P< 0.05)

| Treatments                      | Mitotic index of somatic cells (bone marrow) (Mean±SEM) | Mitotic index of germ cells (Mean±SEM) |
|--------------------------------|--------------------------------------------------------|---------------------------------------|
| Negative control (PBS.)         | 13±0.23                                                | 7.88±0.31                             |
| Mitomycin C                    | 3.35±0.36                                              | 2.21±0.25                             |
| N. sativa extracts + Mitomycin C | 4.38±0.35                                              | 2.56±0.00                             |
| Mitomycin C                    | 4.67±0.02                                              | 3.47±0.01                             |
| 150 mg/kg                      | 8.56±0.03                                              | 6.50±1.00                             |
Table 2. Effects of the *N. sativa* extracts and mitomycin C on chromosomal aberrations in albino mice (Mean± SEM) (P <0.05)

| Treatments                         | Chromatid breaks (Mean± SEM) | Chr. breaks (Mean± SEM) | Ring chromosomes (Mean± SEM) |
|------------------------------------|------------------------------|-------------------------|-----------------------------|
| Negative control (PBS.)            | 0.13±0.23                   | 0.52±0.35               | 1.01±0.21                   |
| Mitomycin C 2 mg/kg                | 10.23±0.13                  | 9.64±0.66               | 7.52±0.42                   |
| *N. sativa* extracts 50 mg/kg +    | 4.80±1.00                   | 5.00±0.43               | 5.01±1.50                   |
| Mitomycin C 100 mg/kg              | 3.15±0.35                   | 3.23±0.73               | 4.00±0.65                   |
| Mitomycin C 150 mg/kg              | 1.15±0.33                   | 1.63±0.00               | 2.84±0.31                   |

Recently, medicinal plants have become a novel approach to control different diseases. The present study was directed to investigate the protective activity of *N. sativa* extracts against mitomycin C induced mutagenic effects in mice. The data in the present study showed that the pre-treatment of mice with three different doses of *N. sativa* extracts for one week and then with single dose of mitomycin C significantly decreased the chromosomal aberrations of bone marrow cells as well as increased the mitotic index of somatic and germ cells compared to positive control. The ability of the extract of *N. sativa* to reduce the chromosomal aberrations and enhance the mitotic index of cell could play an important role to overcome several mutagenic effect of mitomycin C.

Mitomycin C is used as a chemotherapeutic drug, but it produces chromosomal damage in different types of cells. The cytotoxicity of the mitomycin C is produced by an electrophilic attack on the DNA nucleophilic site [13]. In our study, mitomycin C has enhanced the percentage of cell damages, including chromosome abnormality and reducing the mitotic index (table 1 and table 2), suggesting that the direct effects of the mitomycin C induces severe aspects in the cells. Therefore, this medical plant, *N. sativa*, was used to reduce these defects in present work.

Many researchers found that the *N. sativa* has different roles to treat several diseases. The higher dose of *N. sativa* extract increases the fertility potential and testosterone concentration in male rats [14]. The beneficial role of *N. sativa* was documented as DNA protective against the Mancozeb genotoxic effects by inhibiting the purine-catabolizing enzymes in renal tissues [15]. The *N. sativa* extracts antimicrobial activity against different microorganisms has been studied by different researchers. *N. sativa* seed extracts inhibit the growth of *Escherichia coli*, *Bacillus subtilis* and *Streptococcus fecalis* [16]. Moreover, the antibacterial activity of *N. sativa* extracts are reported to be against multi-drug-resistant organisms, including Gram-positive bacteria like *Staphylococcus aureus* and Gram-negative bacteria like *Pseudomonas aeruginosa* [17]. *N. sativa* has been used to promote health and prevent many diseases. One gram of *N. sativa* can decrease the level of blood glucose of oral treatment after two weeks [18]. Also, *N. sativa* is reported to enhance the immune system.

In addition, the present study shows that the *N. sativa* extracts have an effective role in preventing the mutagenic effects of mitomycin C by reducing the chromosomal aberrations and enhancing mitotic index in mice (table 1; table 2), suggesting that the interaction of *N. sativa* extracts with mutagen mitomycin C...
should be better explored because this effect can affect the treatments efficacy or may be used as a protective approach to inhibit the harmful effects caused by mitomycin C.

5. Conclusion
From the present study, it can be concluded that *N. sativa* extracts may play an important role as a protective agent from genotoxic effects of mitomycin C. The results show that the *N. sativa* extracts are beneficial in enhancing mitotic index and lowering chromosomal aberrations (chromatid breaks, chromosome breaks, and ring chromosomes). Future investigation will have to study *N. sativa* extracts in animals with cancer and study its active compounds to investigate its role in immune system.

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