



Assessment of *In Vitro* Antigenotoxic Effect of *Nigella Sativa* Oil

Nigella Sativa Yağının *In Vitro* Antigenotoksik Etkisinin Değerlendirilmesi

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ABSTRACT

Objectives: Cyclophosphamide (CP) is an alkylating agent widely used as an antineoplastic and immunosuppressive agent. The genotoxicity of CP has been studied in a variety of *in vivo* and *in vitro* systems and is routinely used as a positive control in genotoxicity tests. Traditional medicine *Nigella sativa* L., (*N. sativa*), Ranunculaceae family, especially in the Eastern Mediterranean countries, especially in many countries, and is widely used in many countries as a spice and folk medicine since the time of Dioscorides used as a plant. In this study, it was aimed to show the protective effects of *N. sativa* oil at different concentrations against the genotoxic effects of CP by micronucleus test.

Materials and Methods: For this purpose, healthy cells were treated *in vitro* with *N. sativa* oil at concentrations of 1, 5, 10 µg/mL and CP as positive control for 68 hours. The micronuclei were then counted.

Results: No significant increase in micronucleus frequency was observed when the application of *N. sativa* oil at concentrations of 1, 5, 10 µg/mL compared with the negative control. There was a decrease in the number of micronucleus in all three concentrations (1, 5, 10 µg/mL) compared to the CP group in the groups treated with *N. sativa* oil and CP.

Conclusion: It has been shown that *N. sativa* oil may have protective effects against genotoxicity agents *in vitro*. But more work is needed to understand the mechanism of the genotoxicity effects of *N. sativa* oil.

Key words: Cyclophosphamide, *Nigella sativa* oil, micronucleus, genotoxicity

ÖZ

Amaç: Siklofosfamid, antineoplastik ve immünoşüpresif ajan olarak yaygın olarak kullanılan alkilleyici bir ajandır. Siklofosfamid'in genotoksitesi çeşitli *in vivo* ve *in vitro* sistemde çalışılmış ve rutin olarak genotoksitesite testlerinde pozitif kontrol olarak kullanılmaktadır. Geleneksel tıp da *Nigella sativa* L. (*N. sativa*), Ranunculaceae familyasından olup günümüzde başta Doğu Akdeniz ülkeleri olmak üzere birçok ülkede yaygın olarak yetişen ve hem baharat hem de halk ilacı olarak Dioscorides zamanından beri kullanılan bir bitkidir. Bu çalışmada Siklofosfamid'in genotoksik etkilerine karşı çörek otu yağının değişik konsantrasyonlardaki koruyucu etkilerinin mikronükleus testi ile gösterilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu amaçla, sağlıklı hücreler 1, 5, 10 µg/mL konsantrasyonlarında *N. sativa* yağı ile ve pozitif kontrol olarak Siklofosfamid ile 68 saat boyunca *in vitro* muamele edildi. Daha sonra ise mikronükleuslar sayıldı.

Bulgular: *N. sativa* yağının 1, 5, 10 µg/mL konsantrasyonlarındaki uygulaması negatif kontrolle karşılaştırıldığında mikronükleus frekansında anlamlı bir artış gözlenmedi. *N. sativa* yağı ve Siklofosfamid'in birlikte uygulandığı gruplarda her üç konsantrasyonda da (1, 5, 10 µg/mL) Siklofosfamid grubuna göre mikronükleus sayısında azalma olduğu görüldü.

Sonuç: *N. sativa* yağının genotoksitesite ajanlarına karşı *in vitro* koruyucu etkilerinin olabileceği gösterilmiştir. *N. sativa* yağının genotoksitesiteyi giderici etkilerinin mekanizmasının anlaşılması için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Siklofosfamid, *Nigella sativa* yağı, mikronükleus, genotoksitesite

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INTRODUCTION

Cyclophosphamide (CP) is a oxazophosphorine derivative of nitrogen mustard and is an alkylating agent commonly used as an antineoplastic and immunosuppressive agent.¹⁻³ CP is one of the universally known anti-neoplastic drugs whose therapeutic efficacy against hematological and solid malignancies and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis.⁴ CP is used in high doses for the chemotherapy of various forms of cancer, in low doses in the treatment of autoimmune diseases, and also as an immunosuppressant after organ transplants.⁵ CP's chemically reactive metabolic products induce cytotoxicity by alkylating DNA and proteins.³ CP is known as human carcinogen and has an increased incidence of chromosome aberrations in lymphocytes from patients with malignant and non-malignant diseases.⁶ The genotoxicity of CP has been studied in a variety of *in vivo* and *in vitro* systems, mutagenic, teratogenic and carcinogenic, and is routinely used as a positive control in genotoxicity tests.^{1,6}

Natural compounds find application in the treatment of refractory diseases, a new trend in modern clinical medicine.⁷ *Nigella sativa* (*N. sativa*) is a short-lived annual plant of the Ranunculaceae family, known as black seed, black cumin and fennel flower.^{8,9} *N. sativa* is an aromatic plant with tremendous therapeutic properties such as hypotensive, gastroprotective, nephrocurative, nephroprotective, antioxidative, antimicrobial, genoprotective, neuroprotective, immunomodulatory, anti-inflammatory, hypoglycemic, hypolipidemic, anticarcinogenic and hepatoprotective.^{9,10} It increases the production of two substances, interferon and interleukin, the first defense shield of the immune system against tumor cells.¹¹ *N. sativa* seeds are very rich in fixed oil, essential fatty acids, alkaloids, phytosterols, glycolipids and phospholipids, saponins and essential oil components. In seed essential oil; thymoquinone, *p*-cymene and thymol are the active components. Thymoquinone has been shown as a cytotoxic agent in several human tumor cell lines resistant to various multidrug drugs.¹ It has also been shown in another study that gastric cancer cells inhibit their growth.¹² The effect of high dose thymoquinone on stomach was found to be equal to Omeprazole.¹³ Molecular mechanisms underlying these anticancer effects; cell cycle arrest, apoptosis, oxidative damage of cellular macromolecules, blockade of tumor angiogenesis and inhibition of tumor migration.¹ Micronucleus analysis methods are widely used in genotoxicity studies at chromosome level. The frequency of micronucleus formation is considered to be an indicator of damage to the genetic material. Micronuclei are those that occur during cell division, originating from centric or ascentric chromosome fragments that are not involved in the core nucleus.¹⁴

In this study, it was aimed to show the healing effects of *N. sativa* oil at different concentrations against the genotoxic effects of CP by micronucleus test.

MATERIALS AND METHODS

Materials

Micronucleus testing is usually performed in peripheral blood lymphocytes to determine genotoxicity in humans. Because in the studies performed, the increase in the micronucleus frequency in peripheral blood lymphocytes from cancer patients was found to be as much as the micronucleus frequency in the target tissue.¹⁵⁻¹⁷ *N. sativa* oil was obtained from a local vegetable urea shop and stored in dark brown bottles until use. It was dissolved in dimethyl sulfoxide and then applied to the cells at a final concentration of 1, 5 and 10 µg/mL. CP, cytochalasin B, RPMI medium, phytohemagglutinin, antibiotic, fetal calf serum, L-Glutamine and Giemsa solution were obtained from Sigma.

Methods

Micronucleus test was performed according to the method described by Fenech and Morley.¹⁸ For the analysis of micronucleus in binucleated lymphocytes, cell culture was established from 0.2 mL of fresh heparinized blood. Cells were treated with *N. sativa* oil at a final concentration of 1 µg/mL, 5 µg/mL, 10 µg/mL. Cytochalasin B was added to each tube at a final concentration of 6 µg/mL at 44 hours of incubation. After 24 hours of incubation at 37°C, the cells were centrifuged and micronucleus test in peripheral lymphocytes was performed.¹⁸ Cells were harvested with hypotonic (0.4% KCl) and fixative (methanol: acetic acid) solution. Cell suspensions were stained with Giemsa after dropping onto clean glass slides. CP was also used as a positive control. CP was given to the tubes at a final concentration of 0.16 µg/mL. Micronucleus scoring was limited to binuclear lymphocytes with cytoplasm according to the criteria determined by Fenech et al.¹⁹ Two thousand binucleated lymphocytes were scored for each donor (8000 binucleated cells per concentration).²⁰

Statistical analysis

Windows for SPSS version 22 statistical software program was used to analyze the data. Experimental and control groups were analyzed with one way Anova. Arithmetic mean (\bar{X}) ± and standard deviation was determined. $P < 0.05$ was considered significant.

RESULTS

Micronucleus data in cells treated with CP and *N. sativa* oil and both are shown in Table 1. Micronucleus frequency for the control group was determined as 3.5. CP treatment increased the micronucleus ratio to 25.2. This value was significantly higher than the control group ($p < 0.05$). No significant increase in micronucleus frequency was observed when the application of *N. sativa* oil at concentrations of 1, 5, 10 µg/mL compared with the negative control. There was a decrease in the number of micronucleus in all three concentrations (1, 5, 10 µg/mL) compared to the CP group in the groups treated with *N. sativa* oil and CP (Table 1).

Table 1. Frequency of MN in cultured human lymphocytes treated with *N. sativa* oil

Test substance	Concentrations	MN (X±SD)
Control	-	3.5±0.57
Cyclophosphamide	0.16 µg/mL	25.2±3.8 ^a
<i>N.sativa</i> oil	1 µg/mL	3.5±1.29 ^b
	5 µg/mL	5.25±0.95 ^b
	10 µg/mL	2.75±0.95 ^b
Cyclophosphamide + <i>N.sativa</i> oil	0.16 µg/mL+1 µg/mL	19.75±2.5 ^{ac}
	0.16 µg/mL+5 µg/mL	17.75±4.64
	0.16 µg/mL+10 µg/mL	20.5±3.55 ^{ac}

SD: Standart deviation, p<0.05, 2000 cells were scored for each tube, ^a: Significant difference from control, ^b: Significant difference from cyclophosphamide, ^c: Significant difference from *Nigella sativa* oil, MN: Micronucleus

DISCUSSION

Phytotherapy is an area that uses plants as health promoting agents to treat diseases. In the conventional use of phytotherapies, the original composition of the plant or a certain percentage of certain components of the plant is generally used.²¹ Medicinal plants are considered to be the main source of potentially therapeutically effective new chemicals. According to the data of the World Health Organization, 70-80% of the population in developing countries relies on plants for primary health care.²² *N. sativa* oil, including the main components of thymoquinone and P-cymene, is considered to have anti-inflammatory, hepatoprotective and reno-protective effects.²³ One of the most commonly used cytogenetic assays for genotoxic evaluation of different agents is Cytokinesis Blocked Micronuclei test in cultured human leukocytes.²² In this study we investigated the effect of *N. sativa* oil on genotoxicity in human leukocytes. *N. sativa* oil was used in different concentrations (1, 5, 10 µg/mL). Unlike our study, Abdel-Moneim et al.²⁴ investigated the protective effects of *N. sativa* seeds against genotoxicity and chromosomal aberrations induced by carbon tetrachloride in mouse spermatocytes. In our study, the therapeutic effects of *N. sativa* oil were investigated in spite of the application of CP in lymphocyte cells from healthy individuals instead of mouse spermatocytes. Abdel-Moneim et al.²⁴ have shown that *N. sativa* is effective in the prevention of CCl₄-induced genetic damage in germ cells and can be used as an adjunct nutritional supplement in the early stages of exposure to mutagens. Similarly, in our study, it was observed that the amount of micronucleus decreased in the group in which CP was used together with *N. sativa* oil compared to the positive control group. In the study of Galhena et al.²⁵, a mixture of 100-600 µg/mL consisting of *N. sativa* seeds, *Hemidesmus indicus* (*H. indicus*) roots and *Smilax glabra* (*S. glabra*) rhizomes was applied to human lymphocyte culture together with bleomycin and chromosome aberrations such as dicentric chromosome, acentric fragment, chromatid fractures were examined. According to the results of this study; *N. sativa* seeds, *H. indicus* roots and *S. glabra* rhizomes showed that the mixture has the potential to protect against cytogenetic damage caused by bleomycin in human peripheral lymphocytes. In our study, *N. sativa* oil was used instead of *N. sativa* seeds and

only micronucleus frequency was examined. However, when the results were examined, similar to the results of Galhena et al.²⁵, Hashem et al.¹⁰ concluded that *N. sativa* oil is potentially protective against carbendazim-induced hematoxicity, hepatotoxicity and genotoxicity. In this study, it was determined that *N. sativa* oil moderately improved in terms of micronucleus percentage and DNA fragmentation when applied together with carbendazim and mancozeb. In our study, positive control CP was used to determine the healing effect of *N. sativa* oil and *in vitro* cell culture experiments were performed. Al-Okbi et al.²³ investigated the effect of using *N. sativa* oil alone and in combination with fish oil in CCl₄ treated rats. According to the results of this study, it was observed that combined oral administration of *N. sativa* oil and fish oil-*N. sativa* oil combined with anti-inflammatory and antioxidant activity reduced liver and kidney damage. Nguyen et al.²⁶ in Morocco investigated the *in vitro* cytotoxicity, genotoxicity and antigenotoxicity of aqueous plant extracts from three different regions (Erfoud, Fkih ben Salah, Settati) by the neutral red uptake test in human C3A cells, the bacterial Vitotox, Ames assays, comet assay and micronucleus test. *N. sativa* seed extracts showed varying degrees of antigenotoxicity depending on where the test specimens came from. Extracts from Fkih ben Salah and Settati were reported to exhibit antigenotoxic effects by significantly reducing the micronucleus number at concentrations of 9 mg/mL. Similarly, in our study, the effects of *N. sativa* oil on micronucleus formation in healthy human lymphocyte cell culture at concentrations of 1, 5 and 10 µg/mL were examined and the genotoxic effects of CP used as positive control were reduced by *N. sativa* oil in all three concentrations. Although *N. sativa* oil concentrations and cell type used in our study were different from those of Nguyen et al.²⁶, similar results were found. However, the location of the samples, the test and other test conditions may affect the research result.

CONCLUSION

In this study, it was shown that *N. sativa* oil may have curative effects against mutation inducing agents. To understand the mechanism of the genotoxicity effects of *N. sativa* oil, molecular tests, testing of different concentrations and *in vivo* experiments are needed.

Conflict of Interest: No conflict of interest was declared by the authors.

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