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The Determination of the Effects of Wild Thyme (*Thymbra spicata L.*) and Cumin (*Cuminum cyminum L.*) Extracts on SCE (Sister Chromatid Exchange) in Human Lymphocyte Chromosomes

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Abstract

The purpose of this work is to determine of *T. spicata* L. and *C. cyminum* L. plant extracts on sister chromatid exchange rate in human peripheral lymphocyte culture as *in vitro*. Human blood lymphocyte cells, *T. spicata* L. and *C. cyminum* L. plant extracts were allowed to interact with doses of 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL for 24 hours. When trial groups where plant extracts were applied were compared with negative control and mitomycin-C (MMC) which was used as positive control, it was determined that the extract doses applied led to an increase in sister chromatid exchange rate. Also, it was found that increasing concentrations of plant extracts caused cell replication index to decrease. Between *C. cyminum L.* doses and RI, a negative correlation (r = -0.95) was observed and between *T. spicata* L. doses and RI a negative correlation was observed (r = -0.94) respectively. As a result of this study; *T. spicata* L. and *C. cyminum* L. plant extracts were found to have genotoxic and clastogenic effects on human peripheral lymphocytes.

Key Words: Mitomycin-C, Replication Index, Genotoxic, Clastogenic.

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1. Introduction

In the genetic information of the living species, hereditary variations that occur suddenly due to factors other than gene recombination are called mutations. In addition to spontaneous mutations in nature, mutagen can also be caused by physical and chemical factors. Mutagenes in DNA molecules cause many damages and some of these damages are tried to be repaired in the cell by special mechanisms. Various damages to DNA can sometimes be incorrectly repaired so that the cell can survive death. Damages in DNA may remain unrepaired in the presence of mutations in the genes controlling the functioning of the repair mechanisms, or by adverse effects such as age, disease, nutrition, and heat. As a result, that cell also causes a mutation, which results in various disorders. Cancer and cell death are among these disorders (Galloway et al., 1998; İpek et al., 2003)

Mutations in the DNA of living things are determined by *in vitro* mammalian cell gene mutation test and back mutation tests using bacteria. *In vitro* mammalian, Sister

Chromatide Exchange, Chromosomal Aberration and Micronucleus tests are among the most widely used methods for cytogenetic investigation of mutations and genotoxic effects in DNA (Natarajan and Obe, 1982).

The goal of this work is to research the effects of plant extracts of cumin and wild thyme on sister chromatid Exchange (SCE) in human lymphocyte chromosomes *in vitro*.

2. Material and Method

The method developed by Speit and Haupter (1985) to provide different staining (Sister Chromatid Differentiation = SCD) of a sister chromatid belonging to a chromosome was modified and used.

In our study, peripheral blood from 10 females (20-25 years old) and 10 males (20-25 years old) was used as material. Wild thyme (*T. spicata*) and Cumin (*C. cyminum*) plant essential oils were used as test substances. Mitomycin-C (MMC) was used as positive control and Acetone (C_3H_6O) was used as test control (Uzun, 2007).

Plant extracts of wild thyme (*T. spicata)* and cumin (*C. cyminum*) were extracted from the leaves and seeds of the plants. NON ASBESTOS brand Clevenger device was used for extraction. For 40 g of plant, 400 mL of distilled water was added to remove essential oil in about 3 hours. This process was repeated until sufficient essential oil was obtained. 100 µL of the obtained essential oil was mixed with 900 µl of acetone. As a result, 1 part of oil and 9 parts of acetone were mixed.

2.1. Test Materials and Solutions

In this study, cumin and wild thyme essential oils were used as test substances.

Acetone

Merck acetone was used in this study. Acetone to be added to 5 mL of medium was calculated as 2.5 µL/mL.

Chromosome Medium

Chromosome Medium B (Cat. No. F5023) from Biochrom was utilized as cell culture in this work.

5"-Bromo-2"-deoxyuridine (BrdU)

BrdU from Sigma (Cat. No. B 5002) was dissolved in 10 ml Chromosome Medium B (50 µg/10 mL medium). 10 µg/mL of SCE was added to the prepared solution (100µL) for SCE study.

Mitomisin-C (MMC) 2 mg (Sigma, M 0503)

MMC (Mitomycin-C) was used as a positive control by dissolving bidistyl in water. Mitomycin-C, weighing 0.6 mg, was dissolved in distilled water to a volume of 1.5 mL.

2.2. Experiments

The method developed by Speit and Haupter (1985) to provide different staining (Sister Chromatid Differentiation = SCD) of a sister chromatid belonging to a chromosome was modified and used. 12 drops of heparinized blood samples taken from 10 healthy, close aged and non-smoking 10 women and 10 men were added to the chromosome medium (5mL) under sterile conditions. 10 µg/mL (100 µL of the prepared BrdU solution) from each BrdU solution prepared under sterile conditions were mixed thoroughly under sterile conditions (50 µg BrdU/100 µL medium). The prepared cell culture was incubated in an oven at 37°C for 72 hours.

Solvent Control Group (Acetone)

Acetone to be added to 5 ml of medium was calculated as 2.5 µL/mL. Acetone was put in medium tubes during the last 24 hours of incubation. (72 hours).

Negative Control Group

Both experiment groups were compared with this group. No additions were made to this group. Incubation of 72 hours was performed.

Positive Control Group

0,3 µg/mL of Mitomiycin-C was put in to the medium tubes during the last 24 hours of incubation. (72 hours).

Experiment 1: Essential oil of C. cyminum (72 hours incubation)

Group 1. 0.05 µL/mL Cumin
Group 2. 0.10 µL/mL Cumin
Group 3. 0.15 µL/mL Cumin
Group 4. 0.20 µL/mL Cumin

Experiment 2: Essential oil of T. spicata (72 hours incubation)

Group 1. 0.05 µL/mL Wild Thyme
Group 2. 0.10 µL/mL Wild Thyme
Group 3. 0.15 µL/mL Wild Thyme
Group 4. 0.20 µL/mL Wild Thyme

At the 70th hour of culture (2 hours before the end of the culture period), the solution of colchicine was put in each tube (0.06 µg/mL) and the tubes were slowly shaken to mix well. For 2 hours at 37°C, the cells were pre-treated with colchicine. The culture tubes were centrifuged at 2000 rpm for 10 min at the end of the culture period (at the end of 72 hours). The supernatant was eliminated on the centrifuged culture tubes. After stirring 0.5-0.7 ml of liquid remaining at the lower part of the tube, the hypotonic solution maintained in the oven at 37°C was added to the tubes.

To prevent clustering of the cells, 5 ml of colchicine were added to the tubes and tubes were placed in the incubator in a reserved way after turning upside-down. The tubes with hypotonic solution were kept in the oven at 37°C for 30 minutes. At the end of the period, the supernatant was discarded by centrifugation at 2000 rpm for 10 min. 5 ml of cold fixative were added to each tube with gentle stirring. Cells treated with fixative at 2000 rpm for 10 min were centrifuged and the supernatant was discarded. This procedure was repeated 3 times to completely clear the liquid remaining in the tube. At the end of the centrifugation, the supernatant was discarded so that 0.5-0.7 mL of liquid remained at the lower part of the tube and the slides were prepared with the remaining liquid. Cells collected at the bottom of the tube with a Pasteur pipette were homogenized by mixing and 4-5 drops were drawn from this cell suspension to the pasteur pipette. On the previously cleaned slides, 1 drop of the pasteur pipette was dropped to different areas from 50 cm height (3-4 drops). During the dropping of the cell suspension on the slides, it was ensured that the cells and thus chromosomes were spread on the slide by taking care of not overlapping the drops. Preparations were kept at room temperature for 24 hours to dry.

2.3. Staining of Preparations

The method developed by Speit and Haupter (1985) in order to provide different staining (Sister Chromatid Differentiation = SCD) of sister chromatids belonging to a chromosome was used by modifying it. The dried preparations were put into the irradiation vessel and covered with Sorenson buffer to cover them like a film. Irradiation solution (pH = 6.8) was prepared by taking 5 ml of buffer A and 5 ml of buffer B and this mixture was covered to 100 ml with distilled water. The difference in contrast between the sister chromatids was found to be significantly influenced by the low or high irradiation solution. For this reason, a thin layer of the preparations was covered with irradiation solution. These preparations were irradiated with a single ultraviolet lamp from a height of 15 cm for 30 minutes in the dark which could emit light at a wavelength of 254 nm at 30 W. At the end of irradiation, the preparations were incubated in a 1xSSC solution for 60 minutes at 58-60°C in an oven. The 5% Giemsa dye solution was prepared by mixing 5 mL of Giemsa, 5 ml of buffer A and 5 mL of buffer B 15 minutes before the completion of the incubation period (pH =

6.8). This prepared dye was filtered through filter paper in a vertical trough. When the incubation period was completed, the preparations in 1xSSC solution were taken directly into the giemsa dye solution and left for 20 minutes. At the end of the period, the preparations extracted from the dye were passed through the pure water, put into three different containers and the excess dye on the preparations was allowed to flow and dry. The dried preparations were closed with entellan to make them permanent. With the drying of Intella, the preparations were examined under a microscope.

2.4. Microscopic Examination

Permanent preparations were examined under the Binocular light microscope (10×100 = 1000 magnification) with an immersion objective.

2.5. Replication Index (RI) Detection

RI was calculated to determine the effects of wild thyme (*T. spicata)* and cumin (*C. cyminum*) plant extracts on DNA replication. For these calculations, 100 randomly selected cells were examined in each preparation. By counting the cells in the first, second and third metaphase circuits seen during these investigations, RI was calculated by the following formula.

RI = (1×M1 + 2×M2 + 3×M3) / 100
M1: 1. Number of cells in mitosis
M2: 2. Number of cells in mitosis
M3: 3. Number of cells in mitosis

2.6. Taking Photo wih a Microscope

Photos were taken with an Olympus microscope at 1000X magnification. Photos of SCE samples were taken.

3. Results and Discussion

3.1. Effect of Cumin (C. cyminum) and Wild Thyme (T. spicata) Plant Essential Oils on SCE in Acetone Mixed Human Peripheral Lymphocytes

The mean sister chromatid numbers of *C. cyminum* and *T. spicata* essential oils in human peripheral lymphocytes treated with doses of 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL are shown in table 1. When compared with both controls, in the 100 cells counted, *C. cyminum* and *T. spicata* essential oils increased the number of SCE in human lymphocytes (Tables 1 and 2). R = 0.94 positive correlation was found between cumin dose and SCE, and r = 0.99 positive correlation was found between wild thyme dose and SCE (Graphs 1 and 2).

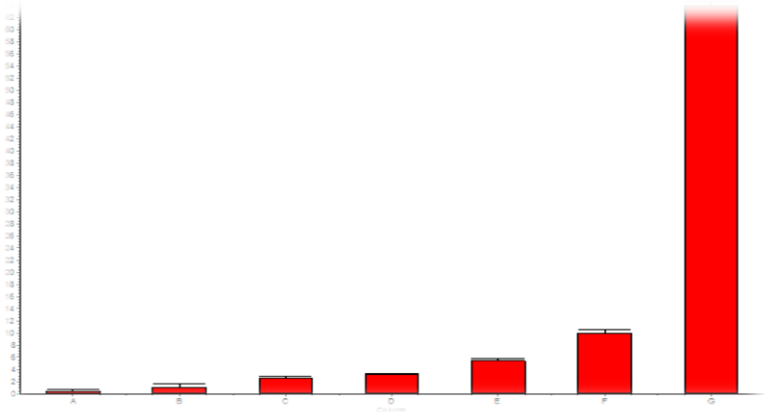
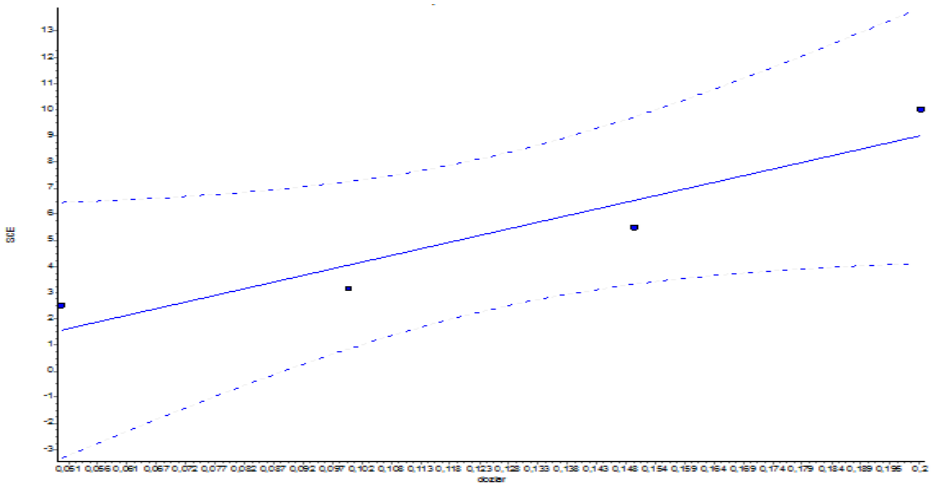


Chart 1. Average number of sister chromatid exchange (SCE) in human peripheral lymphocytes treated with a mixture of Acetone Mixture of Cumin Extracts at different doses for 24 hours. *(A: Negative Control, B: Acetone (2.5 µL/mL), C: Cumin (0.05 µL/mL), D: Cumin (0.10 µL/mL), E: Cumin (0.15 µL/mL), F: Cumin (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg / mL).*



Graph 1. A positive regression was found between cumin dose and SCE (r = 0.94).

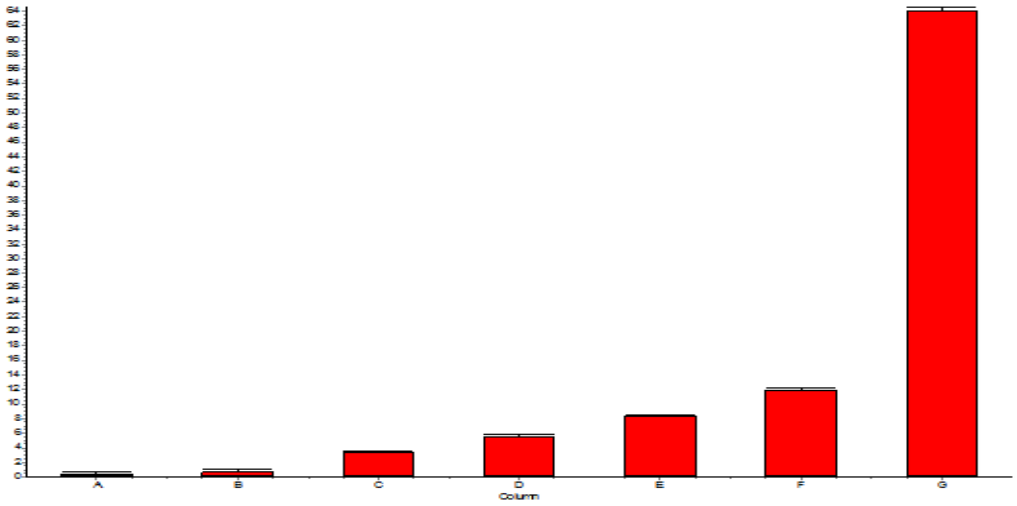
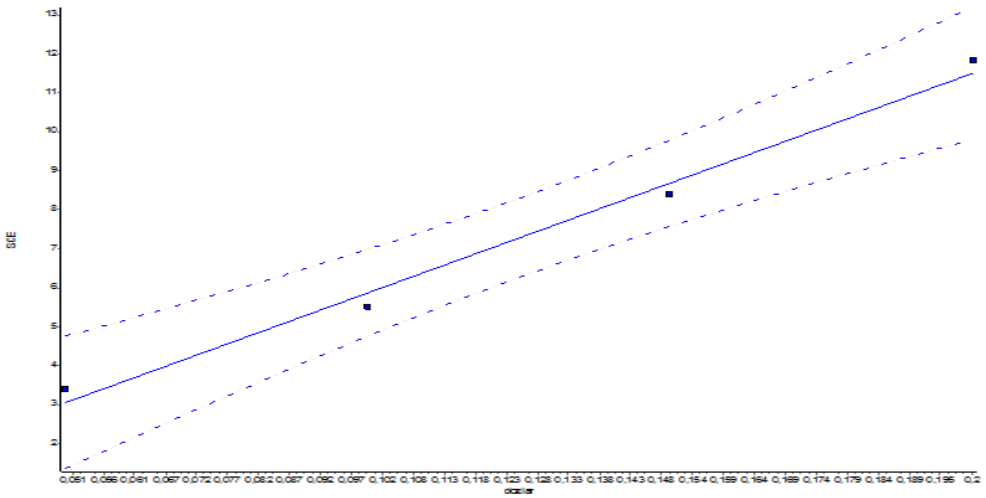


Chart 2. Average number of sister chromatid exchange (SCE) changes in human peripheral lymphocytes treated with Acetone mixture of Wild Thyme Extracts at different doses for 24 hours. *(A: Negative Control, B: Acetone (2.5 µL/mL), C: thyme (0.05 µL/mL), D: thyme (0.10 µL/mL), E: thyme (0.15 µL/mL), F: thyme (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg / mL).*



Graph 2. A positive regression was found between thyme dose and SCE (r = 0.99).

Table 1. Different doses of cumin and wild thyme essential oils treated for 24 hours with a mixture of acetone average human peripheral lymphocytes number of sister chromatid exchange (SCE)

Groups	Concentration (µl/ml)	Treatment Time (hour)	Total Cells	Number of SCE	±SEM (%)
Negative Control	-	-	100	2.57	±0.02
Acetone	2.5 µL/mL	24	100	1	±0.57
	0.05 µL/mL	24	100	2.5*	±0.28
Cumin	0.10 µL/mL	24	100	3.16*	±0.16
Essential Oil	0.15 µL/mL	24	100	5.5*	±0.28
	0.20 µL/mL	24	100	10*	±0.57
	0.05 µL/mL	24	100	3.4*	±0.20
Wild Thyme	0.10 µL/mL	24	100	5.5*	±0.28
Oil	0.15 µL/mL	24	100	8.4*	±0.20
	0.20 µL/mL	24	100	11.83*	±0.44
Positive Control (MMC)	0.3 µg/mL	24	100	64	±0.57

*Compared with Dunnett T test. No difference between negative control and solvent control (p> 0.05). * Important compared to control and solvent control (p<0.01).*

Table 2. Replication ındex of control and wild thyme essential oil groups in human lymphocyte cells

Groups	Concentration (µl/ml)	Treatment Time (hour)	Total Cells	M1	M2	M3	Number of RI	±SEM (%)
Negative Control	-	-	100	10	25	65	2.57	±0.02
Acetone	2.5 µL/mL	24	100	35	20	45	2.25*	±0.08
Wild	0.05 µL/mL	24	100	12	38	50	2.44	±0.05
Thyme	0.10 µL/mL	24	100	48	12	40	1.90*	±0.05
Essential	0.15 µL/mL	24	100	59	11	30	1.75*	±0.02
Oil	0.20 µL/mL	24	100	60	17	23	1.58*	±0.02
Positive Control (MMC)	0.3 µg/mL	24	100	89	5	6	1.20	±0.02

*Compared with Dunnett T test. No difference between negative control and solvent control (p> 0.05). * Important compared to control and solvent control (p<0.01).*

3.2. Effect of Wild Thyme (T. spicata) Essential Oil on SCE in Human Peripheral Lymphocytes Treated with Acetone Mixture

Replication index (RI) and M1, M2 and M3 ratios of human peripheral lymphocytes treated with doses of 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL of *T. spicata L.* essential oil are given in Table 2. When compared with both controls in 100 cells count, *T. spicata* extract appears to reduce RI in human lymphocytes (Chart 3).

The doses of 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL of *T. spicata* extract were found significant when compared with control and solvent control ($p < 0.01$). There was a negative correlation between wild thyme dose and RI ($r = -0.94$) (Graph 3).

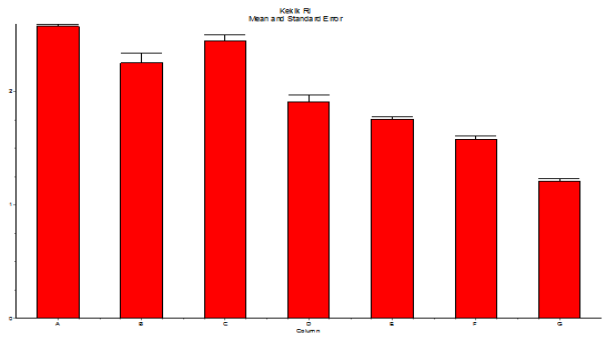


Chart 3. Replication Index Rates of Human Lymphocyte Cells of Control and Wild Thyme Extract Groups *(A: Negative Control, B: Acetone (2.5 µl/mL), C:Oregano (0.05 µL/mL), D:Thyme (0.10 µL/mL), E:Thyme (0.15 µL/mL), F:Thyme (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg/mL).*

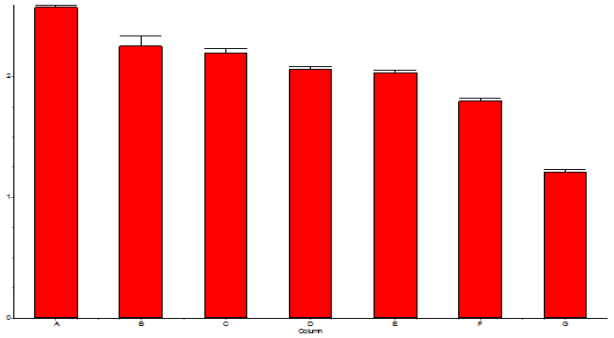
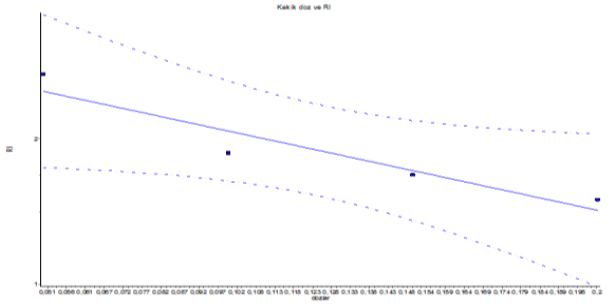
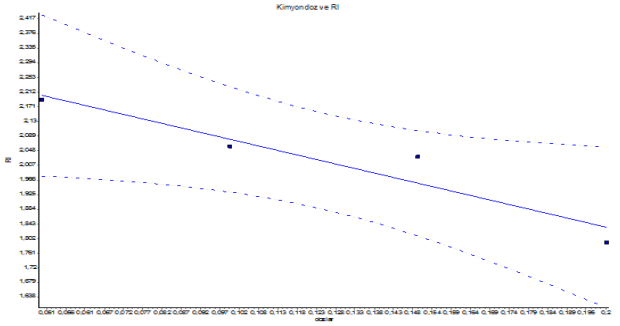


Chart 4. Replication Index Ratios of Control and Cumin Extract Groups in Human Lymphocyte Cells *(A: Negative Control, B: Acetone (2.5 µL/mL), C: Cumin (0.05 µL/mL), D: Cumin (0.10 µL/mL), E: Cumin (0.15 µL/mL), F: Cumin (0.20 µL/mL), G: Positive Control (MMC) (0.3 µg/mL).*



Graph 3. A negative regression was found between wild thyme dose and RI (r = -0.94).



Graph 4. A negative regression was found between the cumin dose and RI (r = -0.95).

3.3. Effect of Cumin (C. cyminum) Essential Oil on SCE in Human Peripheral Lymphocytes Treated with Acetone Mixture

C. cyminum extract 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL doses of 24 hours treated with human peripheral lymphocytes determined in the replication index (RI) and M1, M2 and M3 rates are seen in Table 3. When compared with both controls in 100 cells counted, *C. cyminum* extract appears to reduce RI in human lymphocytes (Chart 4). Doses of *C. cyminum* L. extract 0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL were found significant when compared to control and solvent control ($p < 0.01$). A negative correlation was found between cumin dose and RI (r = -0.95) (Graph 4).

In the literature, in two separate studies conducted with Thymol, the essential oil component of thyme was used as an antiseptic in some diseases such as bronchitis, cough, catarrh and eczema. At the same time, the genotoxic effect of Thymol was determined by the SCE test and Tyhmol significantly increased the number of SCE at high doses, but this result was not found to be statistically significant (Büyükleyla, 2007; Becarano and Emden, 1946). In the study conducted by Yavuz (2005), whether benzol peroxide used as bleaching agent in flour has genotoxic effects on human peripheral lymphocytes or not via *in vitro* SCE test were investigated; benzene peroxide generally increased SCE, but this increase was observed to be statistically significant over 48 hours of administration with only the highest concentration of benzene peroxide (100 µg / ml).

Table 3. Replication index of control and cumin extract groups in human lymphocyte cells

Groups	Concentration (µl/ml)	Treatment Time (hour)	Total Cells	M1	M2	M3	Number of RI	±SEM (%)
Negative Control	-	-	100	10	25	65	2.57	±0.02
Acetone	2.5 µL/mL	24	100	35	20	45	2.25*	±0.08
	0.05 µL/mL	24	100	36	15	49	2.19*	±0.03
Cumin	0.10 µL/mL	24	100	43	10	47	2.06*	±0.01
Essential Oil	0.15 µL/mL	24	100	42	14	44	2.03*	±0.01
	0.20 µL/mL	24	100	40	36	24	1.79*	±0.02
Positive Control (MMC)	0.3 µg/mL	24	100	89	5	6	1.20	±0.02

Compared with Dunnett T test. No difference between negative control and solvent control ($p > 0.05$). * Important compared to control and solvent control ($p < 0.01$).

In another study using SCE method; as a result of evaluating the role of genomic instability in patients with different types of leukemia; he considered the low incidence of SCE to be an expected condition in patients with leukemia and suggested that it would not be informative in the study of genomic instability (Sevinç, 2006).

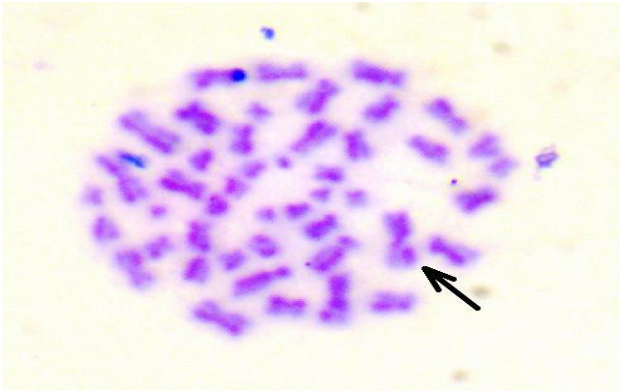


Photo 1. Metaphase plate with sister chromatid exchange.

Genotoxicity tests; In this study, we aimed to determine the role of genomic instability in chromosome aberration, micronucleus and sister chromatid exchange methods and myelodysplastic syndrome (MDS) and to investigate the relationship between these methods. Significant increase in the incidence of MN and SCE in patients with MDS and MN and SCE for genomic instability seem to be an informative biological marker (Nazlıgül, 2009).

By looking at histopathological effects of different doses of TSL (*T. spicata*) grown in Isparta, also known as thyme; and atorvastatin (ATS) on liver, heart and kidney tissues of rats; it was seen that TSL and ATS did not impair routine biochemical tests in general. It was found that TSL may be effective in hypercholesterolemia and 300 mg/kg TSL and ATS show antihypercholesterolemic effect at approximately the same rate. TSL and ATS have very low renal damage, but the amount of damage increases with increasing dose (Demiralay, 2010).

In the study on cinnamon, cumin and sumac which are widely used in our country; water, ethanol-water, methanol and chloroform extracts of each species of spice samples were obtained using appropriate methods to the literature. Extracts' antioxidant activity, phenolic compound amounts and reducing ability were determined; it was concluded that antioxidant activity levels were the highest in cinnamon and cumin extracts, the lowest in chloroform extracts and medium in all other extracts. The total amount of phenolic compounds of the extracts; sumac was the highest in methanol and ethanol-

water extracts, the lowest in chloroform extracts and at varying levels in all other extracts. According to the measurements, the reductive power of extracts was found to be the highest in sumac methanol extract, the lowest in chloroform extracts and at varying levels in all other extracts (Aydın, 2011).

4. Conclusion

In accordance with our *in vitro* study, we demonstrated the genotoxic effects of Wild Thyme (*T. spicata*) and Cumin (*C. cyminum)* plants by SCE test.

As a result, four different doses (0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL) of Cumin plant extract were applied in human lymphocyte culture for 24 hours.

a) There is a negative regression between the replication index and the different doses that reduce the replication index (r = -0.95).
b) Each dose is significant compared to the control and solvent control ($p < 0.01$).
c) When compared to negative control, it increased SCE number.

Wild Thyme (*T. spicata)* Extract was applied by applying four different doses (0.05 µL/mL, 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL) of it in human lymphocyte culture for 24 hours.

a) There was a negative regression between different doses and replication index (r = -0.94) and it was found to decrease the replication index.
b) Doses of 0.10 µL/mL, 0.15 µL/mL and 0.20 µL/mL are significant compared to the control and solvent control ($p < 0.01$).
c) Positive regression was found between doses and SCE number (r = 0.99).

According to the data of our study, when the experimental groups treated with Cumin and Wild Thyme Extract were compared with the negative control, it was seen that the ratio of SCE increased due to the increase in the extract doses applied. In addition, it was observed that the replication index (RI) decreased with increasing extract concentration. As a result of this study; *T. spicata* and *C. cyminum* plant extracts were found to have genotoxic and clastogenic effects on human peripheral lymphocytes. This *in vitro* study may not fully explain the effects of Cumin and Wild Thyme extracts, so *in vivo* studies are also recommended.

Acknowledgments

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Conflict of Interest

None

References

1. Aydın, Ö., 2011. 'Tarçın, Kimyon Ve Sumak Adlı Baharat Türlerinden Elde Edilen Su, Etanol-Su, Metanol ve Kloroform Ekstraktlarının In Vitro Antioksidan Özelliklerinin Belirlenmesi' Atatürk University, Institute of Health Sciences, Pharmacy-Biochemistry Department, Erzurum.
2. Becarano, S. J., Emden, C., 1946. "Pratik Tıbbi Formüller". İsmail Akgün Printing Hause. İstanbul.
3. Büyükleyla, M., 2007. 'Thymol'ün İnsan Lenfositlerinde Kardeş Kromatid Değişimi, Kromozom Anormalliği ve Mikronükleus Oluşumu Üzerine Etkileri'. Çukurova University, Graduate School of Natural and Applied Sciences, Department of Biology, Adana.
4. Demiralay, H., 2010. "Thymbra Spicata Labiatae Oil'in (Karabaş Kekik Yağı) Ve Atorvastatin'in Antihiperkolesterolemik Etkinliğinin Araştırılması' Süleyman Demirel Üniversitesi, Sağlık Bilimleri Enstitüsü Biyokimya Anabilim Dalı Isparta.
5. Galloway, S.M., Miller, J.E., Armstrong, M.J., Bean, C.L., Skopek, T.R., Nıchols, W.W., 1998. "DNA Synthesis İnhibition As An İndirect Mechanism Of Chromosome Aberrations: Comparison Of DNA-Reactive And Nondna-Reactive Clastogens". J. Mutat. Res. 400,169-186.
6. İpek, E., Tüylü, B.A., Zetinoğlu, H., , 2003. "Effects Of Carvacrol On Sister Chromatid Exchanges İn

Human Lymphocyte Cultures". J. Cytotechnology. 43,1-3:145-148.
7. Natarajan, A. T., Obe, G., 1982. "Mutagenicity Testing With Cultured Mammalian Cells: Cytogenetic Assays". In: Heddle JA(ed) Mutagenicity. New Horizons in Genetic Toxicology. Academic Pres, New York, 1-213.
8. Nazlıgül, E., 2009. 'Miyelodisplastik Sendromlu Olgularda Genomik İnstabilitenin Farklı Sitogenetik Yöntemlerle (Kromozom Aberasyonu, Kardeş Kromatid Değişimi, Mikronukleus) Araştırılması', Istanbul University, Institute of Health Sciences, Department of Genetics / Genetics Master Thesis, İstanbul.
9. Sevinç, B., 2006. 'Hematolojik Malign Hastalıklarda Genomik İnstabilitenin Farklı Sitogenetik Yöntemlerle (Kromozom Aberasyonu, Kardeş Kromatid Değişimi, Mikronukleus) Araştırılması'. Istanbul University Institute of Health Sciences, Department of Medical Genetics, Master Thesis, İstanbul.
10. Speit, G., Haupter, S., 1985. On the mechanism of differantial giemsa staining of bromodeoxyuridine substitued chromosomes. II. Differences Between the demonstration of sister chromatid differantiation and replication patterns. J. Hum. Genet. 70, 126-129.
11. Uzun, S., 2007. 'Sodyum Hipoklorite Maruz Kalmış Kişilerin Lenfositlerindeki Mikronükleus Sıklığının Araştırılması', Master Thesis, Erciyes University, Institute of Health Sciences, Department of Medical Biology, Kayseri.
12. Yavuz, A., 2005. 'Benzol Peroksit'in İnsan Periferal Lenfositlerinde *in vitro* Genotoksik Etkileri'. Çukurova University, Graduate School of Natural and Applied Sciences, Department of Biology, Adana.