

Received:
2 September 2018
Revised:
24 January 2019
Accepted:
7 March 2019

Cite as: Halit Kızılet,
Bilal Yilmaz,
Handan Uysal. Herbal
medicine against genotoxicity
of dimethoate, an insecticide,
in mammalian somatic cells.
Heliyon 5 (2019) e01337.
doi: 10.1016/j.heliyon.2019.
e01337



Herbal medicine against genotoxicity of dimethoate, an insecticide, in mammalian somatic cells

Halit Kızılet^a, Bilal Yilmaz^b, Handan Uysal^{c,*}

^a Department of Cardiology, Erzurum Regional Training and Research Hospital, 25100, Erzurum, Turkey

^b Department of Analytical Chemistry, Faculty of Pharmacy, Atatürk University, 25240, Erzurum, Turkey

^c Department of Biology, Faculty of Science, Atatürk University, 25240, Erzurum, Turkey

* Corresponding author.

E-mail address: hauysal@atauni.edu.tr (H. Uysal).

Abstract

In this study, the genotoxic effects of dimethoate (DIM) were investigated with the *in vitro* micronucleus test in human peripheral lymphocytes. The ethanol extracts of *Rosa canina* and *Salvia lavandulifolia* were used to remove possible genotoxic effects of these substances. For this purpose, different concentrations (0.5–1–2 µg/mL) of dimethoate, DIM + RC_{eta} and DIM + SL_{eta} (1:1 v/v) application groups were prepared and applied to the blood culture. The obtained data were compared with the negative control group that was prepared with dimethyl sulfoxide (DMSO) as solvent and a well-known genotoxic effects of ethyl methanesulfonate (EMS) as positive control group. It was observed in lymphocyte cells that the frequency of MN considerably increased depending on the increasing dose of DIM whereas the nuclear division index (NBI) decreased according to the control group, especially in the last concentration (2 µg/mL). But, as the MN frequency decreased, NBI values approached to control group with 2 µg/mL DIM + RC_{eta} and 2 µg/mL DIM + SL_{eta} according to DIM application group ($P < 0.05$). Additionally, RC_{eta} and SL_{eta} were analyzed by gas chromatography-mass spectrometry (GC-MS).

Keywords: Toxicology, Genetics

1. Introduction

The farmed areas of agriculture in the world is fixed at 1.400.000 ha from 1950 to the present day, while the human population has from 2.600.000 reach to 7.600.000 since 1950 to 2017 (Info, 2017; UN, 2017). The increasing population together with the environmental pollution in the world is also increasing in different ecosystems. Especially in agricultural areas, plants are under the threat of microorganisms, harmful insects, rodents and weeds from the sowing to harvest time and from storage to processing stage. Pesticides are biologically active chemical substances used against harmful organisms to increase the amount of product that can be taken from the unit area. For this reason, pesticides and insecticides —a sub-group of pesticides—have been developed against plant diseases and pests to increase the yield obtained from the unit area in agriculture. These substances have been shown to cause destruction of useful organisms and endangering genetic diversity as they are used more and unconsciously against pests, weeds and diseases every day for the purpose of agricultural struggle (Ozkaraova Gungor, 2008).

Organophosphorus compounds (sub-group of insecticides) may induce oxidative stress leading to the generation of free radicals (Sharmaa et al., 2005). Dimethoate (*O,O*-dimethyl-*S-N*-methylcarbamoylmethyl)-phosphorodithioate [CAS registry no.60-51-5] is an organophosphorus insecticide with a contact and systemic action. It is widely used against a broad spectrum of insects, mites and houseflies. The main risk groups of higher-dose dimethoate exposure are the producers, pesticide workers, farm owners and households.

In many studies, it has been determined that insecticides cause genomic instability, which leads to pathological cases and even aging with various diseases including cancer (Soltani et al., 2009). In order to prevent this situation, studies related with the determination of antimicrobial, antioxidant, antimutagenic, anticancerogenic and even antiaging characteristics of natural compounds originated from plants containing different phytochemicals are being continued (Arora et al., 2002; Simopoulos, 2004; Uysal et al., 2009a, 2015, 2009b; Kızılet et al., 2013; Kasımoğlu and Uysal, 2014). According to Jain et al. (2007), although the science of medicine has grown tremendously in the 20th century, the use of plants still continues in traditional medicine/alternative medicine.

A knowledge of the chemical constituents of rosehip (*Rosa canina*) and sage (*Salvia lavandulifolia*) (which grows naturally in Turkey) are desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. Hence a thorough validation of the herbal drugs has emerged as an

emerging issue of interest emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemical have complementary and overlapping mechanism of action. Mass spectrometry coupled with chromatographic separations such as gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants.

In this study, the genotoxicity of dimethoate insecticide was determined by micronucleus technique in human peripheral lymphocyte cells. In addition, these genotoxic effects were eliminated by ethanol extracts of *R. canina* (RC_{eta}) and *S. lavandulifolia* (SL_{eta}). Also, in this study, the chemical contents of the *R. canina* and *S. lavandulifolia* plants were determined by GS-MS method.

2. Materials and methods

2.1. Preparation of plant extracts

R. canina and *S. lavandulifolia* used in this study were collected from Palandöken Mountain of Erzurum province, and Aşkale district, Koşapınar village, respectively. All above-ground structures (stem, leaf, flower etc.) of *S. lavandulifolia* and just fruits of *R. canina* had been dried and ground in a cool and clean environment without exposure to direct light. 50 g of ground plant was extracted with 150 mL of ethanol. The ethanol and plant mixture were passed through filter paper after standing at room temperature for 24 hours. In order to increase the amount of solution, this procedure was repeated three times. The mixed or unified filtrates were concentrated with a Soxhlet extractor at 50 °C. 2 µg/mL ethanol extracts (SL_{eta} and RC_{eta}) were dissolved in 2% dimethyl sulfoxide (DMSO) during the application.

2.2. GC-MS system

Chromatographic analysis were carried out on an Agilent 7820A gas chromatography system equipped with 5977 series mass selective detector, 7673 series autosampler and chemstation (Agilent Technologies, Palo Alto, CA). HP-5 MS column with 0.25 µm film thickness (30 m × 0.25 mm I.D., USA) was used for separation. The temperatures of the inlet, transfer line and detector were 250, 250 and 300 °C, respectively.

2.3. GC-MS conditions

Different temperature programs were investigated for GC-MS method. The end of this investigation, the temperature program of the GC/MS was as follows: initial temperature was 50 °C, held for 1 min, increased to 100 °C at a rate of 20 °C/min held for 1 min, increased to 180 °C at a rate of 10 °C/min held for 1 min,

increased to 220 °C at rate of 5 °C/min held for 5 min, and finally to 300 °C at a rate of 10 min and held for 5.5 min. The injector volume was 1 µl in splitless mode and the carrier gas was helium at a flow rate of 1 ml/min.

2.4. Identification of components

The spectrum of the unknown component was compared with the spectrum of the component stored in the National Institute of Standards and Technology Library Version (2005), Software, Turbomass 5.2. The components were identified by comparing linear Kovats retention index and mass spectra with those obtained from the MS library. Interpretation on mass spectrum GC-MS was conducted using the database on National Institute Standard and Technology having more than 62,000 patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The name, molecular weight and structure of the components of the test materials were ascertained.

2.5. Donors with peripheral blood assays

The study involved control and experimental group composed of 3 healthy volunteers with the age of 25–30 years. None of the individuals in the study were habitual smokers or drinkers, and had not been exposed to any known toxic agents, drugs or X rays.

The ethics committee permission for the study was received from Erzurum Regional Training and Research Hospital Local Ethics Committee (Number: 37732058-53/2467/ BEAH KAEK 2015/9-67) and the rules of the committee were followed during the investigations. Written informed consent was obtained from all patients who participated in this study.

2.6. *In vitro* micronucleus (MN) test

A newly modified version of micronucleus test developed by Fenech (2000) and Kirsch-Volders et al. (1997) was used in this study. Preliminary studies were conducted to determine the DIM application doses in which cell division was not blocked and 0.5, 1.0 and 2.0 µg/mL were determined as doses used throughout the study. Enough number of divided cells could not reach at higher doses than these application groups. In our study, distilled water and 2% DMSO, the solvent of DIM, were used as negative controls and 10 mM ethyl metansulfonate (EMS) was used as a positive control apart from DIM application groups. In the second part of the study, another experimental setup with 2.0 µg/mL DIM + RC_{eta} and 2.0 µg/mL DIM + SL_{eta} (1:1 v/v equal amount of insecticide and plant extract) application groups were prepared. Experiments were repeated three times with different donors for each treatment group. All treatment groups were incubated for 72 hours with blood

from appropriate donors. Two nucleated cells (binucleat) were obtained with cytochalasin-B added at 48th hour in each tubes. At the end of the incubation, prepared smear slides were stained with Giemsa and investigated under 10X40 magnification.

1000 cells were randomly counted and the Nuclear Division Index (NDI) was calculated by determining the ratio of one, two, three and four nuclei cells for each repeat. In addition, 1000 two nucleated (binucleat) cells were counted and MNs were recorded. According to the results, DMSO and EMS compared with distilled water, all DIM applications compared with DMSO, and plant extract + DIM applications compared with 2 µg/mL DIM. SPSS 13.0 program was used for statistical analysis related to MN and NBI values obtained as a result of the studies. Univariate analysis of variance (ANOVA) and Turkey test were applied to compare the data obtained from control and treatment groups.

3. Results

As a result of our experiments, the total MN percentages were found as 0.70 ± 0.38 in the distilled water and 0.83 ± 0.65 in the DMSO. In the EMS positive control group, this value was obtained as 5.63 ± 1.60 . The difference between the distilled water and the DMSO control group was insignificant ($P > 0.05$), while the difference between the EMS positive control and the negative control group distilled water was found to be significant ($P < 0.05$). NDI values of control groups were obtained as 1.52 ± 0.15 for distilled water group; 1.54 ± 0.17 for DMSO group and 1.29 ± 0.25 for EMS group. When NDI results of the DMSO and EMS groups were compared to those of the distilled water group, it was determined that EMS has a cytotoxic effect ($P < 0.05$) but DMSO not ($P > 0.05$).

The results obtained from DIM applications are shown in Table 1. The MN percentages due to DIM applications at 0.5 and 1.0 µg/mL were found as 0.90 ± 0.72 and 0.97 ± 0.63 , respectively. Moreover, NDI values were obtained as 1.54 ± 0.20 and 1.54 ± 0.23 respectively. The differences between the MN and NBI values of these two DIM groups and the DMSO control group was non-significant ($P > 0.05$). The MN percentage and NDI value for 2.0 µg/mL DIM application were obtained as 2.10 ± 0.13 and 1.29 ± 0.32 , respectively (Table 1). According to these results, the differences in the MN percentage and NDI values for 2.0 µg/mL DIM application and the DMSO control group were found to be significant ($P < 0.05$).

To be able to determine the antigenotoxic effects of the plant ethanol extracts, the application of DIM + RC_{eta} and DIM + SL_{eta} (1:1 v/v) was performed. However, RC_{eta} and SL_{eta} treatments were not performed in the 0.5 and 1.0 µg/mL DIM treated groups, since the MN frequencies were found to be extremely low in these groups (Table 1). On the other hand, the MN frequency values with DIM + RC_{eta} and

Table 1. Statistical significance of MN and NDI values after the exposure to DIM, DIM + RC_{eta} and DIM + SL_{eta}.

	Application groups	Concentration (µg/mL)	BNC	MN within binucleat			%MN±S.E.	NDI±S.E.
				(1)	(2)	(3)		
Control	Distilled water	-	3000	21	-	-	0.70 ± 0.38	1.52 ± 0.15
	DMSO	%2	3000	25	-	-	0.83 ± 0.65	1.54 ± 0.17
	EMS	10mM	3000	107	16	10	5.63 ± 1.60*	1.29 ± 0.25*
Insecticides application groups	DIM	0.5	3000	27	-	-	0.90 ± 0.72	1.54 ± 0.20
	DIM	1.0	3000	29	-	-	0.97 ± 0.63	1.54 ± 0.23
	DIM	2.0	3000	63	-	-	2.10 ± 0.13*	1.29 ± 0.32*
Insecticides + plant extracts	DIM + RC _{eta}	2.0 + 2.0	3000	13	-	-	0.43 ± 0.16**	1.35 ± 0.41**
	DIM + SL _{eta}	2.0 + 2.0	3000	25	-	-	0.83 ± 0.20**	1.38 ± 0.28**

BNC: Investigated of binucleated cells, MN: micronucleus, NDI: nuclear division index, *: The mean difference is significant at the 0.05 level, **: The mean antioxidant difference is significant at the 0.05 level.

DIM + SL_{eta} applications were significantly decreased to 0.43 ± 0.16 and 0.83 ± 0.20 with respect to the 2.0 µg/mL DIM exposed group (2.10 ± 0.13) ($P < 0.05$). Moreover, The NDI values for DIM + RC_{eta} and DIM + SL_{eta} groups were measured as 1.35 ± 0.41 and 1.38 ± 0.28 respectively. The differences in the NDI values of DIM, DIM + RC_{eta} and DIM + SL_{eta} groups were found to be significant ($P < 0.05$).

The components present in the *R. canina* and *S. lavandulifolia* are listed in Tables 2 and 3. 33 components (92.97%) and 17 components (90.63%) were identified RC_{eta} and SL_{eta}, respectively.

4. Discussion and conclusion

The imbalance between the antioxidant defense of the body and the free radical formation, which causes peroxidation of the lipid layer of cells, is called oxidative stress. Oxidative stress causes changes in the membrane fluidity and DNA damages. It has also carcinogenic effects (Singh and Pandey, 1989; Bagchi et al., 1995). As in the current study, the balance between oxidant and antioxidant status in the cell is disrupted due to the insecticides, free radicals increase, and this leads to oxidative stresses and DNA damages. DNA damage and oxidative stress play an important role in cancer, aging, telomere shortening, various diseases and pathological conditions (Martin-Ruiz et al., 2004; Soltani et al., 2009). Recent studies have reported that the oxidative stress and lipid peroxidation increased due to enhanced free radicals formation in the pesticide poisonings (Oruç and Üner, 2000; Hazarika et al., 2003; Shadnia et al., 2005; Lukaszewicz-Hussain, 2010; Roberts and Reigart, 2013). In the current study, a statistically significant differences in MN percentage and NBI values were obtained between 2.0 µg/mL DIM treated and control groups ($P < 0.05$). According to these results, it is highly

Table 2. Composite composition values for the *R. canina* ethanol extract.

Peak number	Retention time(min)	Component	Molecular formula	Molecular weight(g/mol)	% ratio in total component
1	3.52	Hexanal	C ₆ H ₁₂ O	100.09	0.69
2	4.31	Pentanoic acid	C ₅ H ₁₀ O ₂	102.07	0.32
3	5.14	2,4-dihydroxy-2,5-dimethyl-3(2h)-furanone	C ₆ H ₈ O ₄	144.04	0.18
4	5.21	Furan, 2-penthyll	C ₉ H ₁₄ O	138.10	0.37
5	5.71	Hexanoic acid	C ₆ H ₁₂ O ₂	116.08	5.21
6	6.69	Heptanoic acid	C ₇ H ₁₄ O ₂	130.10	0.77
7	8.13	Octanoic acid	C ₈ H ₁₆ O ₂	144.11	1.41
8	8.72	2-octanoic acid, trans-	C ₈ H ₁₄ O ₂	142.10	0.40
9	9.09	Butanedioic acid, hydroxy diethylester	C ₈ H ₁₄ O ₅	190.08	0.55
10	9.51	Nonanoic acid	C ₉ H ₁₈ O ₂	158.13	1.44
11	9.64	5-Acetylvaleric acid, methylester	C ₈ H ₁₄ O ₃	158.09	0.68
12	10.25	2-Methyl-oct-2-enedial	C ₉ H ₁₄ O ₂	154.10	1.27
13	12.26	7-Methyl-Z-tetradecen-1-ol acetate	C ₁₇ H ₃₂ O ₂	268.24	0.94
14	12.48	9-Oxononanoic acid	C ₉ H ₁₆ O ₃	172.11	1.18
15	13.04	4-amino-1,5-pentandioic acid	C ₇ H ₁₃ NO ₄	175.08	2.06
16	13.34	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200.18	1.36
17	14.85	Azelaic acid, ethylester	C ₁₁ H ₂₀ O ₄	216.14	1.83
18	15.61	Azelaic acid	C ₉ H ₁₆ O ₄	188.10	5.62
19	16.43	Oleic acid	C ₁₈ H ₃₄ O ₂	282.26	0.83
20	18.98	3-deoxyestradiol	C ₁₈ H ₂₄ O	256.18	0.34
21	19.99	Hexadecanoic acid, ethylester	C ₁₈ H ₃₆ O ₂	284.27	15.44
22	21.31	10-octadecenoic acid, methylester	C ₁₉ H ₃₆ O ₂	296.27	0.98
23	22.59	Trans-13-octadecenoic acid	C ₁₈ H ₃₄ O ₂	282.26	3.01
24	23.21	Oleic acid, ethyl ester	C ₂₀ H ₃₈ O ₂	310.29	26.43
25	23.75	Stearic acid	C ₁₈ H ₃₆ O ₂	284.27	8.27
26	28.03	Cis-13-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310.29	0.95
27	28.59	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312.30	1.39
28	32.87	7-Methyl-Z-tetradecen-1-olacetate	C ₁₇ H ₃₂ O ₂	268.24	0.77
29	32.87	7-Methyl-Z-tetradecen-1-olacetate	C ₁₇ H ₃₂ O ₂	268.24	0.91
30	33.89	13-Decosenamide	C ₂₂ H ₄₃ NO	337.33	1.28
31	34.75	Oleic acid, 3-(octadecyloxy) propyl ester	C ₃₉ H ₇₆ O ₃	592.58	1.95
32	35.68	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436.32	0.24
33	39.59	17-pentatriacontene	C ₃₅ H ₇₀	490.55	3.91

possible that DIM treatment caused oxidative stresses in living systems. For example, it has been reported that DIM led to a chromosomal aberrations in mouse bone marrow, an increased SCE in hamster cells, and it also had mutagenic effects in *Drosophila* (Athwal and Sandhu, 1985). Gomez-Arroyo et al. (1987) reported

Table 3. Composite composition values for the *S. lavandulifolia* ethanol extract.

Peak number	Retention time(min)	Component	Molecular formula	Molecular weight(g/mol)	% ratio in total component
1	3.37	2-octanol	C ₈ H ₁₈ O	130.14	0.22
2	3.63	Methoxyformamide	C ₂ H ₅ NO ₂	75.03	0.37
3	5.16	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144.04	0.65
4	6.01	Erythritol	C ₄ H ₁₀ O ₄	122.06	3.28
5	7.65	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	144.04	2.39
6	9.00	6-Acetyl-β-d-mannose	C ₈ H ₁₄ O ₇	222.07	3.46
7	10.76	10-heptadecen-8-ynoic acid, methyl ester	C ₁₈ H ₃₀ O ₂	278.22	0.82
8	11.70	Benzofuran-2-carboxaldehyde	C ₉ H ₆ O ₂	146.04	7.47
9	13.28	B-hydroxydodecanoic acid	C ₁₂ H ₂₄ O ₃	246.17	1.23
10	19.77	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.24	8.54
11	19.92	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.27	3.89
12	22.15	1-heptatriacotanol	C ₃₇ H ₇₆ O	536.59	1.75
13	23.14	Linolenic acid	C ₁₈ H ₃₀ O ₂	278.22	26.38
14	30.92	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C ₁₉ H ₃₈ O ₄	330.23	3.24
15	33.33	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C ₂₁ H ₄₂ O ₄	358.31	6.13
16	33.87	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO	337.33	19.84
17	34.11	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436.32	0.98

that methyl parathion and dimethoate insecticides increased sister chromatid exchanges in human peripheral lymphocytes as compared to the control group, and statistically positive results were observed. Moreover, [Maiti and Kar \(1997\)](#), and [Maiti et al., \(1996\)](#), demonstrated that dimethoate intoxication produced oxidative stress through the generation of free radicals and induced hepatic lipid peroxidation in chicken and mice. According to [Ündeğer and Başaran \(2005\)](#), dimethoate and methyl parathion at 100 and 200 µg/mL significantly increased DNA damage in human lymphocytes. In addition, *in vitro* exposure to dimethoate induced a concentration-dependent increased sister chromatid exchange (SCE) frequency in toadfish lymphocytes ([Ellingham et al., 1986](#)). In another study, after exposure to dimethoate, the level of superoxide dismutase declined while lipid peroxidation, glutathione, induction of micronucleus and DNA damage were increased in *C. punctatus* as the concentration and exposure time increased ([Daoud et al., 2014](#)). It is also found to increase the incidence of numerical but not structural chromosomal aberration (CA) in male Wistar rats ([Ündeğer et al., 2000](#)). Moreover, it is reported that dimethoate was mutagenic in *Escherichia coli*, *Saccharomyces*

cerevisia and *Salmonella typhimurium* TA 100, but non-mutagenic in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 102 (Mohn, 1973; Bianchi et al., 1994). According to Dogan et al. (2011), it can be concluded that oxidative stress condition evoked by dimethoate could not be responded effectively and genotoxic nature of pesticide was proven by determined clastogenic effect possibly via being an alkylation agent or stimulating the production of reactive species.

In addition, it has been shown that profenofos, an organophosphate insecticide, increased the micronucleus frequency in human peripheral lymphocyte cells and decreased the nuclear division index (0.25, 0.5, 0.75 and 1.0 µg/mL) at different concentrations (Kasımoğlu and Uysal, 2014). In another study of antioxidant enzyme levels with chlorpyrifos-ethyl from organophosphate insecticides, it has been found that the paraoxonase activity and total antioxidant levels decreased while the levels of oxidative stress index and total oxidant increased (Deveci et al., 2015). Gültekin et al. (2000) showed that chlorpyrifos, an organophosphate insecticide, increased the level of malondialdehyde (MDA) lipid peroxidation product in erythrocytes. Similarly, Gültekin et al. (2001) reported that chlorpyrifos-ethyl increased lipid peroxidation in rats and the antioxidant defense decreased together with increased oxidative stress. Many studies on plant-derived natural compounds demonstrated that plant compounds exhibit a protective activity against genotoxicity caused by oxidative stress (Plazar et al., 2008). Medicinal plants are a potential source of antioxidants and ROS scavenging molecules (Arora et al., 2005) and contain high amount of diverse vitamins and minerals. Many studies over the past three decades have focused on the assessment of anticarcinogenic and antimutagenic activities of plants. One of these is the rosehip fruit, which contains plenty of various vitamins including vitamin C, B1, B2, E, K and P (permeability vitamins) and carotene. It is also rich in mineral substances and flavonoids (Gao et al., 2000; Hvattum, 2002; Demir et al., 2014). The water extracts of *Salvia* species, the other plant used in the current study, are known to be rich in rosmarinic acid and luteolin-7-glucoside (Ramos et al., 2010). In our study, the ethanol extracts of *R. canina* and *S. lavandulifolia* were applied as much as 2 µg/mL DIM and the results were found to be important for their antigenotoxicity ($P < 0,05$). These obtained findings indicated that the oxidative damages caused by DIM treatment are eliminated by the free radical scavenging activity of SL_{eta} and RC_{eta}. Similar studies in the literature reported that there was a strong association between the phenolic compounds and the antioxidant activity in *R. canina*, *S. lavandulifolia* and many plant species.

In a study done by Kılıçgün (2008), it was determined that *R. canina* inhibited liver damage in rats even at low concentrations. The ascorbic acid, which was found as large amount of *R. canina*, was demonstrated to reduce the genotoxic effects of

EMS, MMS (methyl methanesulfonate) and ENU (N-nitroso N-ethylurea) in *D. melanogaster* (Kaya, 2003). Moreover, the other compound, beta carotene in *R. canina* was shown to decrease the genotoxic effects of doxorubicin in this fruit fly (Dias et al., 2009). It was found that the ethanol and water extracts of *R. canina* reduced the genetic damages in the human lymphocyte cells induced by cypermethrin and fenvalerate insecticides exposures and in the *D. melanogaster* induced by EMS exposure (Kızılet et al., 2013; Kasimoğlu and Uysal, 2014). Ramos et al. (2010) demonstrated that the extracts of *S. officinalis*, *S. fruticosa*, and *S. lavandulifolia* and their major components; rosmarinic acid and luteolin-7-glucoside protected the DNA of Caco-2 and HeLa cells exposed to oxidative agents against oxidative DNA damage and stimulated DNA repair. Eidi and Eidi (2009) investigated the antidiabetic effects of *S. officinalis* leaves on normal and diabetic mice. It was shown that the ethanol extract of these leaves led to a reduction in the amount of serum glucose, triglyceride and total cholesterol in diabetic mice, while it had no effects in normal mice. In another study performed by Uysal et al. (2015), it was observed that the methanol extract of *Echium amoenum* reduced the genotoxic effects of EMS.

In RC_{eta} sample (Table 2), 92.97% of the total extract was identified, predominating oleic acid (29.21%), hexadecanoic acid (15.44%), stearic acid (8.27%), azelaic acid (7.45%) hexanoic acid (5.21%), octadecenoic acid (3.99%) and pentatriacontene (3.90%). In SL_{eta} sample (Table 3), 90.63% of the total extract content was identified and the main components were linolenic acid (26.38%), docosenamide (19.84%), hexadecanoic acid (15.67%), benzofuran-2-carboxaldehyde (7.47%), octadecanoic acid (6.13%), 6-acetyl- β -D-mannose (3.46%) and erythritol (3.28%). Major fatty acids has biological activities such as being anti-inflammatory, anti-androgenic, cancer preventing, dermatogenic, hypocholesterolemic, anaemiagenic, insectifuge as well as being a 5-alpha reductase inhibitor (Duke, 1998). n-hexadecanoic acid has been reported to show selective toxicity to human leukemic cells as well as anti tumor activity and is suggested to be a lead compound in anti cancer drugs (Harada et al., 2002).

These results MN demonstrate that DIM can cause genotoxic effects even at very low doses. Since genotoxicity induced in somatic cells is highly likely to occur gametically, plants with medical prescription as alternative genomic preservatives should be taken into consideration in the daily nutritional regime. As a result, we can say that these plant components, belonging to *R. canina* and *S. lavandulifolia*, protect the cells against oxidative DNA damage and stimulate DNA repair. Our obtained results were consistent with the similar studies on plant extracts. According to the results obtained from our study, it can be inferred that the scavenging effects of *R. canina* ve *S. lavandulifolia* on the DIM was arisen from the antioxidative properties of their compounds.

Declarations

Author contribution statement

Halit Kızılet, Bilal Yilmaz, Handan Uysal: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Arora, A., Sairam, R.K., Srivastava, G.C., 2002. Oxidative stress and antioxidative systems in plants. *Curr. Sci.* 82, 1227–1238.
- Arora, R., Gupta, D., Chawla, R., Sagar, R., Sharma, A., Kumar, R., Prasad, J., Singh, S., Samanta, N., Sharma, R.K., 2005. Radioprotection by plant products: present status and future prospects. *Phytother. Res.* 19 (1), 1–22.
- Athwal, R.S., Sandhu, S.S., 1985. Use of human x mouse hybrid cell line to detect aneuploidy induced by environmental chemicals. *Mutat. Res.* 149, 73–81.
- Bagchi, D., Bagchi, M., Hassoun, E.A., Stohs, S.J., 1995. *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology* 104 (1-3), 129–140.
- Bianchi, L., Zannoli, A., Pizzala, R., Stivala, R.A., Chiesara, E., 1994. Genotoxicity assay of five pesticides and their mixtures in *Saccharomyces cerevisia* D7. *Mutat. Res.* 321, 203–211.
- Daoud, A., Gend Kumar, P., Kumar, S., Mukhtar, A., 2014. Evaluation of genotoxic and oxidative stress response to dimethoate in freshwater fish *Channa punctatus* (Bloch). *Chem. Speciat. Bioavailab.* 26 (2), 111–118.
- Demir, N., Yildiz, O., Alpaslan, M., Hayaloglu, A.A., 2014. Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in

Turkey. LWT - Food Sci. Technol. (Lebensmittel Wissenschaft Technol.) 57 (1), 126–133.

Deveci, H.A., Karapehlivan, M., Kaya, İ., Kükürt, A., Alpay, M., 2015. Akut klorprifos-etil zehirlenmesine karşı kafeik asit fenetil ester'in koruyucu etkisi. Ankara Üniv. Vet. Fak. Derg. 62, 255–260.

Dias, C.D., Araújo, B.C., Dutra, E.S., Nepomuceno, J.C., 2009. Protective effects of β -carotene against the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. Genet. Mol. Res. 8 (4), 1367–1375.

Dogan, D., Can, C., Kocyigit, A., Dikilitas, M., Taskin, A., Bilinc, H., 2011. Dimethoate-induced oxidative stress and DNA damage in *Oncorhynchus mykiss*. Chemosphere 84 (1), 39–46.

Duke, J.A., 1998. Phytochemical and Ethnobotanical Databases. <https://phytochem.usda.gov/phytochem/search/list>.

Eidi, A., Eidi, M., 2009. Antidiabetic effects of sage (*Salvia officinalis* L.) leaves in normal and streptozotocin-induced diabetic rats. Diabetes Metab. Syndr — Clin. Res. Rev. 3 (1), 40–44.

Ellingham, T.J., Christensen, E.A., Maddock, M.B., 1986. *In vitro* induction of sister chromatid exchanges and chromosomal aberrations in peripheral lymphocytes of the oyster toadfish and American eel. Environ. Mutagen. 8 (4), 555–569.

Fenech, M., 2000. The *in vitro* micronucleus technique. Mutat. Res. 455 (1-2), 81–95.

Gao, X., Björk, L., Trajkovski, V., Uggla, M., 2000. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J. Sci. Food Agric. 80 (14), 2021–2027.

Gomez-Arroyo, S., Noriega-Aldana, N., Juarez-Rodriguez, D., Villalobos-Pietrini, R., 1987. Sister chromatid exchanges induced by the organophosphorus insecticides methyl parathion, dimethoate, phoxim and methyl azinfos in cultured human lymphocytes. Contam. Ambient. 8 (1), 63–70.

Gültekin, F., Delibaş, N., Yaşar, S., Kılınç, İ., 2001. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch. Toxicol. 75, 88–96.

Gültekin, F., Öztürk, M., Akdogan, M., 2000. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*). Arch. Toxicol. 74 (9), 533–538.

Harada, H., Yamashita, U., Kurihara, H., Fukushi, E., Kawabata, J., Kamei, Y., 2002. Antitumor activity of palmitic and found as selective cytotoxic substance in a marine red algae. *Anticancer Res.* 22, 2587–2590.

Hazarika, A., Sarkar, S.N., Hajare, S., Kataria, M., Malik, J.K., 2003. Influence of malathion pretreatment an the toxicity of anilofos in male rats: a biochemical interaction study. *Toxicology* 185, 1–8.

Hvattum, E., 2002. Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. *Rapid Commun. Mass Spectrom.* 16 (7), 655–662.

Info, 2017. <https://www.infoplease.com/world/population-statistics/total-population-world-decade-1950-2050>. (Accessed 12 August 2018).

Jain, S., Shrivastava, S., Nayak, S., Sumbhate, S., 2007. Recent trends in *Curcuma longa*. *Pharmacogn. Rev.* 1, 119–128.

Kasımoğlu, C., Uysal, H., 2014. Mutagenic biomonitoring of pirethroid insecticides in human lymphocyte cultures: use of micronuclei as biomarkers and recovery by *Rosa canina* extracts of mutagenic effects. *Pharmaceut. Biol.* 53 (5), 625–629.

Kaya, B., 2003. Anti-genotoxic effect of ascorbic acid on mutagenic dose of three alkylating agents. *T. J. Biol.* 27, 241–246.

Kılıçgün, H., 2008. Antioxidant Potential of Rosehip's Infussions. PhD Thesis, Marmara Üniversitesi. Sağlık Bilimleri Enstitüsü, İstanbul (in Turkish).

Kirsch-Volders, M., Elhajouji, A., Cundari, E., Van Hummelen, P., 1997. The *in vitro* micronucleus test: a multi-endpoint assay to detect simultaneously mitotic-delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. *Mutat. Res.* 392 (1), 19–30.

Kızılet, H., Kasımoğlu, C., Uysal, H., 2013. Can the *Rosa canina* plant be used against alkylating agents as a radical scavenger? *Pol. J. Environ. Stud.* 22, 1263–1267.

Lukaszewicz-Hussain, A., 2010. Role of oxidative stress in organophosphate insecticide toxicity—Short review. *Pestic. Biochem. Phys.* 98 (2), 145–150.

Maiti, P.K., Kar, A., 1997. Dimethoate inhibits extrathyroidal 5'-monodeiodination of thyroxine to 3,3',5 -triiodothyronine in mice: the possible involvement of lipid peroxidative process. *Toxicol. Lett.* 91, 1–6.

- Maiti, P.K., Gupta, P., Chaurasia, S.S., Kar, A., 1996. Dimethoate induced lipid peroxidation and inhibition of type-I iodothyronine 5'-monodeiodine activity in young cockerel. *Bull. Environ. Toxicol.* 157, 335–340.
- Martin-Ruiz, C., Saretzki, G., Petrie, J., Ladhoff, J., Jeyapalan, J., Wei, W., Sedivy, J., Von Zglinicki, T., 2004. Stochastic variation in telomere shortening rate causes heterogeneity of human fibroblast replicative life span. *J. Biol. Chem.* 279 (17), 17826–17833.
- Mohn, G., 1973. 5-Methyl tryptophan resistance mutations in *Escherichia coli* K-12. Mutagenic activity of monofunctional alkylating agents including organophosphorus insecticides. *Mutat. Res.* 20, 7–15.
- Oruç, E.Ö., Üner, N., 2000. Combined effects of 2,4-d and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of *Orochromis niloticus*. *Comp. Biochem. Physiol.* 127, 291–296.
- Ozkaraova Gungor, E.B., 2008. Environmental Technologies, Soil Pollution and Remediation Problems in Turkey, 8, pp. 111–134.
- Plazar, J., Filipic, M., Groothuis, G.M., 2008. Antigenotoxic effect of xanthohumol in rat liver slices. *Toxicol In vitro* 22, 318–327.
- Ramos, A.A., Azqueta, A., Pereira-Wilson, C., Collins, A.R., 2010. Polyphenolic compounds from *Salvia* species protect cellular DNA from oxidation and stimulate DNA repair in cultured human cells. *J. Agr. Food. Chem.* 58 (12), 7465–7471.
- Roberts, J.R., Reigart, J.R., 2013. Recognition and Management of Pesticide Poisonings, sixth ed. U.S. Environmental Protection Agency, Washington DC.
- Shadnia, S., Azizi, E., Hosseini, R., Khoei, S., Fouladdel, S., Pajoumand, A., Nasser, J., Abdollahi, M., 2005. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. *Hum. Exp. Toxicol.* 24 (9), 439–445.
- Sharmaa, Y., Bashira, S., Irshadb, M., Datta Guptac, S., Dogra, T.D., 2005. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. *Toxicology* 206, 49–57.
- Simopoulos, A.P., 2004. Omega-3 fatty acids and antioxidants in edible wild plants. *Biol. Res.* 37, 263–277.
- Singh, S.K., Pandey, R.S., 1989. Toxicity of endosulfan on kidney of male rats in relation to drug metabolizing enzymes and microsomal lipid peroxidation. *Indian J. Exp. Biol.* 27, 725–728.

Soltani, F., Mosaffa, F., Iranshahi, M., Karimi, G., Malekaneh, M., Haghighi, F., Behravan, J., 2009. Evaluation of antigenotoxicity effects of umbelliprenin on human peripheral lymphocytes exposed to oxidative stress. *Cell Biol. Toxicol.* 25, 291–296.

UN, 2017. <https://www.un.org/development/desa/publications/world-population-prospects-the-2017-revision.html>. (Accessed 12 August 2018).

Ündeğer, U., Institoris, L., Siroki, O., Nehez, M., Desi, I., 2000. Simultaneous geno- and immunotoxicological investigations for early detection of organophosphate toxicity in rats. *Ecotoxicol. Environ. Saf.* 45, 43–48.

Ündeğer, Ü., Başaran, N., 2005. Effects of pesticides on human peripheral lymphocytes *in vitro*: induction of DNA damage. *Arch. Toxicol.* 79 (3), 169–176.

Uysal, H., Altun, D., Aşkın, H., Aslan, A., 2009a. Effects of water extract of *Usnea longissima* Ach. On Some Development Stages of *Drosophila melanogaster*. *Fresen. Environ. Bull.* 18 (4), 450–455.

Uysal, H., Altun, D., Aslan, A., 2009b. Effect on the length of life of *Lobaria pulmonaria* (L.) Hoffm lichen in *Drosophila melanogaster*. *TÜBAVBilim.* 2 (3), 271–276.

Uysal, H., Kızılet, H., Ayar, A., Taheri, A., 2015. The use of endemic Iranian plant, *Echium amoenum* against the ethyl methanesulfonate and the recovery of mutagenic effects. *Toxicol. Ind. Health* 31 (1), 44–51.