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Investigation of the genotoxic and antigenotoxic properties of essential oils obtained from two *Origanum* species by *Drosophila* wing SMART assay

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Abstract: In this study, essential oils extracted from 2 species of oregano, *Origanum onites* L. and *Origanum minutiflorum* O.Schwarz & P.H.Davis, were assayed for genotoxicity using the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. Results indicated that there is no significant genotoxic effect of the 2 oils tested. In addition, the antigenotoxic activities of these oils against potassium dichromate ($K_2Cr_2O_7$) and cobalt chloride ($CoCl_2$) were tested. Results indicated that essential oil cotreatments were able to abolish the genotoxic effects induced by the 2 metal compounds. It is suggested that the observed effects can be linked to the antioxidant properties of the selected essential oils. Moreover, these in vivo results will contribute to the antigenotoxicity database of the 2 *Origanum* species used in this study.

Key words: *Drosophila*, antigenotoxicity, *Origanum onites*, *Origanum minutiflorum*, cobalt chloride, potassium dichromate

1. Introduction

Origanum is a genus of about 20 species of aromatic herbs in the family Lamiaceae. It is native to warm-temperate western and southwestern Eurasia and the Mediterranean region. The herbal parts of *Origanum* species are used for the production of tea, aromatic water condiments, and essential oils (1). The main components of *Origanum* species, carvacrol, thymol, p-cymene, and γ-terpinene, are closely related with biogenetical processes (2).

Essential oils are complex mixtures of odorous and volatile compounds resulting from secondary plant metabolism. Due to their properties, they are used in many food products and as odorants in fragrances (3,4). Their lipophilic compounds can cross cell membranes and be absorbed through the skin, and for this reason they have been used medicinally (5). Essential oils as a whole or their components display many biological activities including antibacterial, antioxidant, and antifungal, among others (6,7). Nevertheless, few studies have been carried out evaluating their genotoxic, mutagenic, and/or antigenotoxic properties (8).

Antigenotoxicity is a field in expansion due the health concerns associated with human exposure to environmental genotoxins. In particular, the increasing incidence of cancer is of special concern. In this field, the research into using natural plant products to reduce genotoxic and/or carcinogenic effects is continuously growing (9,10). Thus, the antimutagenic properties elicited by some plant species have an array of prospective applications in human health. Previous studies have underlined the chemopreventive activity of several secondary plant metabolites (11), and many investigators have reported the effects of specific compounds, such as polyphenols and triterpenoids (12) or whole plant extracts (13,14), with antimutagenic properties. Anticarcinogenic (15), antigenotoxic (16), and antioxidant (17) activities of the essential oils of different plant species have also been reported. In this context, studies attempting to demonstrate the antigenotoxic potential of plant extracts, or of several of their components, represent a field that deserves much attention due to its potential applications in human health.

The presence of heavy metals in the environment is of particular concern. Chromium and cobalt are well-known

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metals used in many industrial areas, and chromium compounds induce carcinogenic, immunotoxic, and neurotoxic effects (18). Cobalt compounds have been classified as possible carcinogens by the International Agency for Research on Cancer (19), and several studies reported the mutagenicity and carcinogenicity of cobalt compounds (20,21). Exposure to both cobalt and chromium compounds induces formation of reactive oxygen species (ROS), which attack DNA and generate a multiplicity of DNA damage products, including modified bases. Among the ROS, hydroxyl radical (•OH), peroxyl radical (ROO•), and singlet oxygen ($^1O_2$) are the major species that induce membrane damage in cellular systems (22); this damage leads to cytotoxicity and cell death or to DNA damage fixed as mutations. To avoid oxidative damage, antioxidant defenses have evolved to remove most of these oxidant agents. Even if a balance between oxidative damage and protective mechanisms is usually maintained, there are specific situations in which the excessive production of free radicals or deficiencies in antioxidant defenses leads to the appearance of oxidative stress (23).

To detect the suppression of oxidative effects on DNA damage, different biological systems have been used, including *Drosophila melanogaster* (13,15,24). In particular, the wing spot test of *Drosophila*, described by Graf et al. (25) and also known as the somatic mutation and recombination test (SMART), has proven to be a good tool for detecting a broad range of genetic alterations quickly and inexpensively (25,26). In this study we used the wing SMART assay to evaluate the genotoxic and antigenotoxic activity of essential oils from 2 species of oregano, *Origanum onites* and *Origanum minutiflorum*. Their antigenotoxic potential was measured against the effects induced by potassium dichromate ($K_2Cr_2O_7$) and cobalt chloride ($CoCl_2$).

2. Materials and methods

2.1. Extraction and identification of essential oil composition

Plant samples (0.5 kg) were collected at the end of July from their natural habitats. Aerial parts of the plants were air dried, chopped into small pieces using a mill with rotary knives, and subjected to hydrodistillation for 2 h in a Clevenger device to extract the vaporizing essential oils. The obtained oils were stored in dark glass tubes under refrigeration (4 °C) until evaluation.

Essential oils were analyzed by gas chromatography–mass spectrometry (Agilent 6890 GC System 5973 MSD). Helium (1 mL/min) was used as a carrier gas. The initial temperature was 50 °C (2 min), increasing to 200 °C (5 °C/min), remaining for 5 min at 200 °C, and increasing to 250 °C (10 °C/min), with a final stage of 10 min at 250 °C. The essential oils were placed in a glass capillary column (50 m × 0.32 mm × 0.52 µm) filled with HP1 (composition: 100% dimethylpolysiloxane-nonpolar column), and the oven temperature was programmed from 50 to 250 °C at a 5 °C/min dynamic rate, remaining for 15 min at 250 °C. Identification of components was performed by computer searches from commercial reference libraries. The fragmentation patterns of the mass spectra were compared with the WILEY and NIST 05 libraries. The different components isolated from the analyses are listed in Table 1.

2.2. Chemicals

Potassium dichromate ($K_2Cr_2O_7$; CAS No. 7778-50-9), cobalt chloride hexahydrate ($CoCl_2$; CAS No. 7791-13-1), Tween 80 (CAS No. 9005-65-6), and ethyl methanesulfonate (EMS; CAS No. 62-50-0) were purchased from Sigma. Prior to use, the compounds were dissolved in distilled water. The *O. onites* and *O. minutiflorum* essential oils were dissolved in 0.2% Tween 80. Different doses of the essential oils (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) were chosen based on preliminary studies. Selected doses of the mutagens were chosen according to our previously published data (27). Distilled water served as a negative control, and 1 mM EMS was used as a positive control.

2.3. *Drosophila* strains

The 2 *D. melanogaster* strains used were the *multiple wing hairs* strain, with a genetic constitution of *mwh/mwh*, and the *flare-3* strain, with a genetic constitution of *flr*^{3}/*In (3LR) TM3, Bd*^{s}. More detailed information on the genetic symbols and descriptions can be found in the work of Lindsley and Zimm (28).

2.4. Experimental procedure

The wing SMART assay is based on the loss of heterozygosity for the 2 recessive markers affecting wing hair morphology, *mwh* (3-0.3) and *flr* (3-38.8) (25). This test is able to detect a wide spectrum of genetic alterations including point mutations, deletions, mitotic recombination, chromosomal loss, and nondisjunction (26). Transheterozygous larvae were obtained by parental crosses between *flare-3* virgin females and *mwh* males. Eggs were collected from this cross during 8-h periods. After 72 h, third instar larvae were floated off with tap water and transferred to plastic vials containing 1.5 g of dry *Drosophila* instant medium (Carolina Biological Supply Company, Burlington, NC, USA), and were then rehydrated with 9 mL of freshly prepared test solutions (essential oils, mutagens, mutagens plus essential oils, and distilled water or 0.2% Tween 80 as negative controls). The larvae were fed with different concentrations of the test compound, and feeding ended with pupation of the surviving larvae. All experiments were performed at 25 ± 1 °C and approximately 65% relative humidity. The percentage of mutation inhibition was calculated according to the method of Abraham (24).

2.5. Preparation and microscopic analysis of wings

After metamorphosis, transheterozygous flies were collected and stored in a 70% ethanol solution at 4 °C. Afterwards, the wings were removed and mounted on slides in Faure's solution (30 g gum arabic, 30 mL glycerol, 50 g chloral hydrate, and 50 mL distilled water). They were scored at 400× magnification using a Nikon YS100 light microscope for the presence of clones of cells showing malformed wing hairs. Wings were scored for: a) small (1–2 cells) single spots; b) large (>2 cells) single spots, both with *mwh* and *flr*^{3} phenotypes; and c) twin spots (phenotypes *mwh* and *flr*^{3} in adjacent clones) (25). While single spots originate from recombination, nondisjunction, deletion, and point mutation, twin spots originate only from somatic recombination.

2.6. Statistical analysis

Data were evaluated according to the multiple-decision procedure (29,30). These tests have 2 alternative hypotheses: the mutation frequency in the treated group is no higher than the mutation frequency in the control group, or the frequency in the treated group is no less than *m* times as high as the observed spontaneous mutation frequency in the control. For statistical calculations, the conditional binomial test according to Kastenbaum and Bowman (31) was used at 5% significance levels. Single *flr*^{3} cells were not observed so as to avoid experimental errors because small, single *flr*^{3} spots with modification may occur. According to Graf et al. (25), the *flr*^{3} allele used in the present experiments is not expressed or is not fully expressed in clones smaller than 4 cells per clone. Therefore, we have used large, single spot *flr*^{3} mutations for the calculation of total spots.

3. Results and discussion

Essential oils are complex mixtures with many constituents. In Table 1 there is a description of the different chemicals found in samples obtained from both *Origanum* species. Carvacrol, linalool, thymol, *p*-cymene, and γ-terpinene are among the main constituents. *Origanum* species are regarded as oil rich (>2%) on the basis of their essential oil contents (32). Several *Origanum* species have been characterized, and in general, carvacrol, p-cymene, γ-terpinene, thymol, borneol, and myrcene are among the most frequently described components (33). Our experimental results confirm these findings; carvacrol was the major constituent of our extracts, representing 32%–74% of the total components. The phenolic monoterpene compounds, thymol and carvacrol, are considered responsible for many of the biological activities attributed to this genus (34).

Results obtained in the experiments testing for the genotoxicity of the 2 essential oils are summarized in Table 2. Third instar larvae (72-h old) were given 5 concentrations ranging from 0.1% to 0.5%. Results demonstrated that the 2 tested oils were not genotoxic at the tested concentrations. This agrees with the few studies carried out using several species of *Origanum*. Samples from *O. majorana* were tested in *Vicia faba* root cells and were found to be nongenotoxic as determined by the frequency of chromosome aberrations (35). In addition, essential oils of this species were unable to induce micronuclei or chromosome aberrations in mouse bone marrow cells (36). *O. compactum* extracts evaluated for genotoxicity were likewise unable to induce nuclear genetic effects in the yeast *Saccharomyces cerevisiae* (37).

Results from experiments testing the possible antigenotoxic potential of *Origanum* essential oils from *O. minutiflorum* and *O. onites* are indicated in Tables 3 and 4, respectively. The 2 selected metals induced significant increases in the frequency of mutant clones in the wings of treated flies. This increase is mainly due to the induction of *mwh* clones. With respect to the cotreatment data, the *O. minutiflorum* (Table 3) cotreatment reduced the genotoxic effect induced by the 2 selected metal compounds. The only divergent data are observed in the 0.5% OM + $CoCl_2$ treatment, where an increase was observed. Nevertheless, taking into account the overall data, this result should be considered marginal. Similar results were obtained in the experiments with *O. onites* essential oil extract. Negative results were obtained for all the cotreatments carried out with both $CoCl_2$ and $K_2Cr_2O_7$ (Table 4). All experiments confirmed that the selected oil extracts act as antigenotoxic compounds, significantly reducing the overall genotoxic effects induced by $CoCl_2$ and $K_2Cr_2O_7$ and decreasing, mainly, the frequency of small single and large single *mwh* spots.

Chromium and cobalt are 2 metals considered to have genotoxic potential. Our results show that the tested concentrations of both chemicals were able to induce significant increases in the frequency of mutant clones (Tables 3 and 4). Chromium compounds induce mutagenic effects in different test systems (38,39), including *Drosophila* (40), and it is assumed that they cause DNA damage by generating ROS (39,41). On the other hand, cobalt is genotoxic in different assays (20,38), including the wing spot test in *Drosophila* (42). It is assumed that cobalt compounds react with H_2O_2 and produce $^1O_2$ and $^{\bullet}OH$, probably via the cobalt–oxygen complex (21). Thus, both selected genotoxic agents seem to act mainly by inducing oxidative damage.

In the wing SMART assay with *Drosophila*, which is the same assay used in our study, 2 different *Origanum* species were evaluated, with no genotoxicity reported for extracts from *O. vulgare* (43) or from *O. compactum* (44). For the species selected in our study, the only available data was from a study with *O. onites*. In that study, negative results

Table 1. Chemical constituents (%) contained in *Origanum onites* and *Origanum minutiflorum* essential oils.

Compound	RT (min)	*O. onites*	*O. minutiflorum*
α-Thujene	12.18	0.5	-
α-Pinene	12.44	0.4	0.2
Camphene	12.88	0.2	0.3
Sabinene	13.57	0.3	-
β-Pinene	13.75	0.1	-
Myrcene	14.03	0.6	0.2
α-Phellandrene	14.55	0.1	-
p-Cymene	15.03	4.40	8.7
Limonene	15.34	0.5	-
γ-Terpinene	16.22	2.3	3.9
trans-Sabinene hydrate	16.40	0.2	-
Terpinolene	17.17	0.2	-
Linalool	17.52	30.7	-
cis-para-Menth-2-en-ol	18.13	0.1	-
Borneol	19.43	1.5	2.4
Terpinen 4-ol	19.77	1,5	-
Thymol	22.80	17.1	0.4
Carvacrol	23.08	32.6	74.6
Eugenol	24.48	0.2	-
Carvacrol acetate	24.86	0.2	-
E-Caryophyllene	26.96	1.5	-
β-Caryophyllene	29.97	-	2.3
Aromadendrene	27.47	0.2	0.2
α-Humulene	27.80	0.1	-
Germacrene D	28.42	0.1	-
Bicyclogermacrene	28.80	0.4	-
β-Bisabolene	28.90	0.7	-
Cadinene	29.14	0.1	-
Spathulenol	30.18	0.3	0.2
Caryophyllene oxide	30.76	1.0	-
Other minor components		2.3	6.5

Retention time (RT) was measured on a polar column.

Table 2. Results from the *Drosophila* wing spot test evaluating the genotoxic potential of *Origanum minutiflorum* and *Origanum onites* essential oils. Results were obtained with *mwh/flr*^{3} wings.

Compound concentration	Number of wings (N)	Small single spots (1–2 cells) (m = 2)			Large single spots (>2 cells) (m = 5)			Twin spots (m = 5)			Total *mwh* spots (m = 2)			Total spots (m = 2)			Frequency of clone formation per 10^5 cells
		No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	
Control water	80	16	(0.20)		1	(0.01)		0	(0.00)		17	(0.21)		17	(0.21)		0.87
1 mM EMS	80	163	(2.04)	+	89	(1.11)	+	32	(0.40)	+	273	(3.41)	+	284	(3.55)	+	13.99
Tween 80 (0.2%)	80	10	(0.13)	-	4	(0.05)	i	0	(0.00)	i	14	(0.18)	-	14	(0.18)	-	0.72
Origanum minutiflorum essential oil (%)																	
0.1	80	18	(0.23)	i	3	(0.04)	i	0	(0.00)	i	20	(0.25)	i	21	(0.26)	i	1.02
0.2	80	12	(0.15)	-	3	(0.04)	i	0	(0.00)	i	15	(0.19)	-	15	(0.19)	-	0.77
0.3	80	11	(0.14)	-	3	(0.04)	i	1	(0.01)	i	15	(0.19)	-	15	(0.19)	-	0.77
0.4	78	15	(0.19)	-	6	(0.08)	i	0	(0.00)	i	21	(0.27)	i	21	(0.27)	i	1.10
0.5	80	21	(0.26)	i	0	(0.00)	i	1	(0.01)	i	22	(0.28)	i	22	(0.28)	i	1.13
Origanum onites essential oil (%)																	
0.1	80	18	(0.23)	i	1	(0.01)	i	0	(0.00)	i	19	(0.24)	i	19	(0.24)	i	0.97
0.2	80	17	(0.21)	i	1	(0.01)	i	0	(0.00)	i	18	(0.23)	-	18	(0.23)	-	0.92
0.3	80	16	(0.20)	-	1	(0.01)	i	0	(0.00)	i	17	(0.21)	-	17	(0.21)	-	0.87
0.4	80	24	(0.30)	i	5	(0.06)	i	1	(0.01)	i	30	(0.38)	i	30	(0.38)	i	1.54
0.5	80	21	(0.26)	i	4	(0.05)	i	2	(0.03)	i	27	(0.34)	i	27	(0.34)	i	1.38

No: number, Fr: frequency, D: statistical diagnosis according to Frei and Würgler (29,30), +: positive, -: negative, i: inconclusive, *m:* multiplication factor; probability levels $\alpha = \beta = 0.05$.

Table 3. Inhibitory effect of *Origanum minutiflorum* (OM) essential oil on the genotoxicity induced by $CoCl_2$ and $K_2Cr_2O_7$. Results were obtained with *mwh/flr*^{3} wings.

Compound concentration (%)	Number of wings (N)	Small single spots (1–2 cells) (m = 2)			Large single spots (>2 cells) (m = 5)			Twin spots (m = 5)			Total *mwh* spots (m = 2)			Total spots (m = 2)			Frequency of clone formation per 10^5 cells	Inhibition (↓) and induction (↑)
		No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D		
Control water	80	16	(0.20)		1	(0.01)		0	(0.00)		17	(0.21)		17	(0.21)		0.87	
8 mM $CoCl_2$	80	42	(0.53)	+	5	(0.06)	i	2	(0.03)	i	49	(0.61)	+	49	(0.61)	+	2.51	
0.5 mM $K_2Cr_2O_7$	80	29	(0.36)	+	17	(0.21)	+	1	(0.01)	i	44	(0.55)	+	47	(0.59)	+	2.25	
0.1% OM + $CoCl_2$	80	36	(0.45)	-	6	(0.07)	-	1	(0.01)	i	43	(0.54)	-	43	(0.54)	-	2.20	↓ 11.48
0.2% OM + $CoCl_2$	80	38	(0.48)	-	8	(0.10)	i	1	(0.01)	i	46	(0.58)	-	47	(0.59)	-	2.36	0
0.3% OM + $CoCl_2$	80	38	(0.48)	-	2	(0.03)	-	0	(0.00)	-	40	(0.50)	-	40	(0.50)	-	2.05	↓ 18.03
0.4% OM + $CoCl_2$	80	35	(0.44)	-	8	(0.10)	i	1	(0.01)	i	44	(0.55)	-	44	(0.55)	-	2.25	↓ 9.84
0.5% OM + $CoCl_2$	68	62	(0.91)	+	6	(0.09)	-	1	(0.01)	i	69	(1.01)	+	69	(1.01)	+	4.16	↑ 65.57
0.1% OM + $K_2Cr_2O_7$	80	11	(0.14)	-	7	(0.09)	-	2	(0.03)	i	20	(0.30)	-	20	(0.30)	-	1.03	↓ 49.15
0.2% OM + $K_2Cr_2O_7$	80	19	(0.24)	-	9	(0.11)	-	4	(0.05)	i	30	(0.38)	-	32	(0.40)	-	1.54	↓ 32. 20
0.3% OM + $K_2Cr_2O_7$	80	19	(0.24)	-	10	(0.13)	-	0	(0.00)	i	26	(0.33)	-	29	(0.36)	-	1.33	↓ 39.00
0.4% OM + $K_2Cr_2O_7$	80	30	(0.38)	-	7	(0.09)	-	0	(0.00)	i	36	(0.45)	-	37	(0.46)	-	1.84	↓ 22.03
0.5% OM + $K_2Cr_2O_7$	80	14	(0.18)	-	4	(0.05)	-	1	(0.01)	i	16	(0.20)	-	19	(0.24)	-	0.82	↓ 59. 32

No: number, Fr: frequency, D: statistical diagnosis according to Frei and Würgler (29,30), +: positive, -: negative, i: inconclusive, *m:* multiplication factor; probability levels $\alpha = \beta = 0.05$.

Table 4. Inhibitory effect of *Origanum onites* (OO) essential oil on the genotoxicity induced by $CoCl_2$ and $K_2Cr_2O_7$. Results were obtained with *mwh/flr*^{3} wings.

Compound concentration (%)	Number of wings (N)	Small single spots (1–2 cells) (m = 2)			Large single spots (>2 cells) (m = 5)			Twin spots (m = 5)			Total *mwh* spots (m = 2)			Total spots (m = 2)			Frequency of clone formation per 10^5 cells	Inhibition (%)
		No	Fr	D	No	Fr	D	No	Fr	D		Fr	D	No	Fr	D		
Control water	80	16	(0.20)		1	(0.01)		0	(0.00)		17	(0.21)		17	(0.21)		0.87	
8 mM $CoCl_2$	80	42	(0.53)	+	5	(0.06)	i	2	(0.03)	i	49	(0.61)	+	49	(0.61)	+	2.51	
0.5 mM $K_2Cr_2O_7$	80	29	(0.36)	+	17	(0.21)	+	1	(0.01)	i	44	(0.55)	+	47	(0.59)	+	2.25	
0.1% OO + $CoCl_2$	80	29	(0.36)	-	4	(0.05)	-	2	(0.03)	i	33	(0.41)	-	35	(0.44)	-	1.69	↓ 27.87
0.2% OO + $CoCl_2$	80	22	(0.28)	-	4	(0.05)	-	1	(0.01)	i	26	(0.33)	-	27	(0.34)	-	1.33	↓ 44.26
0.3% OO + $CoCl_2$	80	36	(0.45)	-	3	(0.04)	-	1	(0.01)	i	40	(0.50)	-	40	(0.50)	-	2.05	↓ 18.03
0.4% OO + $CoCl_2$	80	33	(0.41)	-	5	(0.06)	-	3	(0.04)	i	41	(0.51)	-	41	(0.51)	-	2.10	↓ 16.39
0.5% OO + $CoCl_2$	80	31	(0.39)	-	6	(0.08)	-	0	(0.00)	i	37	(0.46)	-	37	(0.46)	-	1.89	↓ 24.59
0.1% OO + $K_2Cr_2O_7$	80	22	(0.28)	-	3	(0.04)	-	2	(0.03)	i	27	(0.34)	-	27	(0.34)	-	1.38	↓ 42.37
0.2% OO + $K_2Cr_2O_7$	80	17	(0.21)	-	3	(0.04)	-	1	(0.01)	i	21	(0.26)	-	21	(0.26)	-	1.08	↓ 55.93
0.3% OO + $K_2Cr_2O_7$	80	22	(0.28)	-	0	(0.00)	-	4	(0.05)	i	26	(0.33)	-	26	(0.33)	-	1.33	↓ 44.07
0.4% OO + $K_2Cr_2O_7$	80	22	(0.28)	-	4	(0.05)	-	6	(0.08)	+	32	(0.40)	-	32	(0.40)	-	1.64	↓ 32.20
0.5% OO + $K_2Cr_2O_7$	78	18	(0.23)	-	3	(0.04)	-	2	(0.03)	i	23	(0.29)	-	23	(0.29)	-	1.21	↓ 50.85

No: number, Fr: frequency, D: statistical diagnosis according to Frei and Würgler (29,30), +: positive, -: negative, i: inconclusive, *m:* multiplication factor; probability levels $\alpha = \beta = 0.05$.

were reported from the Ames test with *Salmonella*, both with and without external metabolic activation (16). All of these results could extend the lack of genotoxic risk to the different species of the *Origanum* genus. Nevertheless, it must be pointed out that when major components such as thymol and carvacrol were tested as individual components, weak but genotoxic effects were obtained in *Salmonella* (16), rat bone marrow cells (45), and *Drosophila* (43). The genotoxicity of these individual components does not correspond with their participation in the essential oils or in mixtures of these 2 phenolic monoterpenes. For this reason, it has been assumed that there are no antagonistic phenomena (43).

Essential oils from different *Origanum* species have been considered as antioxidants and antigenotoxins (7,46). *O. onites* extracts strongly inhibited the mutagenicity induced by 4-nitro-o-phenylenediamine and 2-aminofluorene in *Salmonella* (16), and similar results were obtained in *S. cerevisiae*, where extracts from *O. compactum* were able to abolish the genotoxic effects induced by UVC radiation, 8-methoxypsoralen plus UVA radiation, and methyl methanesulfonate (37). In complex organisms, *O. majorana* extract has antimutagenic potential against sodium azide-induced chromosome aberrations in *V. faba* root meristem cells (35). These properties have also been recorded in mammals, where extracts of *O. majorana* significantly reduced the rate of micronuclei, the number of aberrant cells, and different kinds of chromosome aberrations in the bone marrow cells of mice treated with lead acetate (36). Our results showing that *O. minutiflorum* and *O. onites* extracts inhibit the genotoxicity of chromium and cobalt in *Drosophila* confirm the assumed antigenotoxicity of *Origanum* extracts and the usefulness of *Drosophila* in this kind of study. The lack of a dose–response relationship seems to indicate that the lower dose of both extracts tested (0.1%) is high enough to produce the observed effects. Similar results were observed when the antioxidant effects of ascorbic acid were tested using the same *Drosophila* assay against the effects of $K_2Cr_2O_7$, where the range of doses assayed (25–250 mM) produced a significant reduction in the effects induced by $K_2Cr_2O_7$ (36).

As many of the *Origanum* essential oils were reported to have antioxidant activity (46,47), the antigenotoxicity observed in this study can be attributed to the same mechanisms, including the possibility that essential oils act by blocking the oxidation processes (48). Nevertheless, desmutagenic effects resulting from the scavenging of radicals can also be considered as a potential antigenotoxic mechanism for *Origanum* essential oils. Inhibition of cytochrome P450 activity has been proposed as the major desmutagenic mechanism by which *Origanum* extracts exert their modulating effects (44), as demonstrated by Ganzera et al. (49).

Our study demonstrates that coincubation of larvae with mutagens and *Origanum* essential oils leads to significant reductions in the frequency of induced mutations. Although antioxidant properties of these essential oils are considered the main mechanism, specific experiments should be carried out to confirm the significance of this mechanism in the antigenotoxicity observed in *O. minutiflorum* and *O. onites*.

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