Cytogenetic effects of nine *Helichrysum* taxa in human lymphocytes culture

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Abstract *Helichrysum* Mill. (Asteraceae) species have been used in folk medicine for thousands of years in the world. The in vitro cytogenetic effects in human lymphocytes of nine *Helichrysum* taxa used in Turkey folk medicine were investigated. Blood samples were obtained from healthy donors, non-smoking volunteers, which were incubated and exposed to increasing concentrations of methanol extracts of *Helichrysum* taxa (0.01, 0.05, 0.1, 0.5 and 1 mg/mL). The inhibitory effects of *H. stoechas* (L.) Moench subsp. *barrelieri* (Ten.) Nyman, *H. armenium* DC. subsp. *armenium*, *H. armenium* DC. subsp. *araxinum* (Kirp.) Takht., *H. plicatum* DC. subsp. *plicatum*, *H. compactum* Boiss. and *H. artvinense* P.H.Davis & Kupicha on the mitotic index and replication index indicate that these taxa can have genotoxic and mutagenic effects. They should therefore not be used freely in alternative medicine although their antiproliferative activity may suggest anticarcinogenic properties. Increase effects of *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*,

H. chasmolycicum P.H.Davis, *H. plicatum* subsp. *plicatum*, *H. compactum* and *H. artvinense* on the micronucleus rates showed that these taxa can have genotoxic and carcinogenic effects.

Keywords Cytogenetic · *Helichrysum* · Micronucleus · Mitotic index · Replication index

Introduction

Herbal medicines have become a popular form of therapy. Patients who are self-medicated with herbs for preventive and therapeutic purposes may assume that these products are safe because they are natural. Nevertheless, some of them can cause adverse effects or have the potential to interact with other medications.

Plants of the genus *Helichrysum* Mill. (Asteraceae) are prolific producers of a host of secondary metabolites, including flavonoids, acetophenones, phloroglucinol, pyrones, triterpenoids and sesquiterpenes (Maffei Facino et al. 1990). These are presumably responsible for the remarkable antibacterial (Aslan et al. 2006; Chinou et al. 1997; Cosar and Cubukcu 1990; Kotan et al. 2007; Rios et al. 1991; Roussis et al. 2002; Suzgec et al. 2005), antiviral (Aslan et al. 2006), antifungal (Aslan et al. 2006), antioxidant (Aslan et al. 2007a, b; Carini et al. 2001; Suzgec et al.

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2005; Tepe et al. 2005), anti-inflammatory (El-Dahmy 1994; Recio et al. 1991), anti-allergic (Carini et al. 2001) and antidiabetic (Aslan et al. 2007a, b) properties of extracts from various *Helichrysum* species. *Helichrysum* species have been used in folk medicine for thousands of years in areas as far apart as Europe, Egypt, North America, China, Australia and South Africa (Cosar and Cubukcu 1990; Jakupovic et al. 1989). *Helichrysum* species are traditionally used in the treatment of wounds, infections, and respiratory conditions (Hutchings et al. 1996). Some members of this genus have cholagogue and choleretic activity and stimulate the secretion of gastric juice (Litvinenko et al. 1992). Turkey has a rich flora of *Helichrysum* species that are widely distributed throughout the country. The genus *Helichrysum* is represented by 20 species in Turkey and decoction prepared from various species have been widely used as folk remedy in Turkish folk medicine as diuretics, lithagogues and for treatment of stomachaches (Sezik et al. 2001).

The mitotic index (MI), replication index (RI), and micronucleus (MN) analysis methods are cytogenetic tests that are used both in vivo and in vitro. The MI assay is used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression. RI measures cell division kinetics by counting the percent of cells in first, second, third or more metaphase (Holland et al. 2002). A MN is a small extra nucleus separated from the main one, generated during cellular division by late chromosomes or by chromosome fragments. Because of its association with chromosomal aberrations, MN has been used since 1937 as an indicator of genotoxic exposition based on the radiation studies conducted by Brenneke and Mather (Heddle et al. 1983).

The present study examined the in vitro cytogenetic effects in human lymphocytes of the nine *Helichrysum* taxa: *H. stoechas* (L.) Moench subsp. *barrelieri* (Ten.) Nyman, *H. armenium* DC. subsp. *armenium*, *H. armenium* DC. subsp. *araxinum* (Kirp.) Takht., *H. chasmolycicum* P.H.Davis, *H. heywoodianum* P.H.Davis, *H. plicatum* DC. subsp. *plicatum*, *H. compactum* Boiss., *H. graveolens* (M.Bieb.) Sweet, *H. artvinense* P.H.Davis & Kupicha are used in folk medicine. *H. chasmolycicum*, *H. heywoodianum*, *H. compactum* and *H. artvinense* are endemic species of the Turkish flora.

Materials and methods

Collection of plant materials

Collection information and vernacular names of the nine plant species which are individually numbered are listed below:

1. *H. stoechas* subsp. *barrelieri* (Gold flower, Gud-deme flower)—Aydın: between Ortaklar and Söke, 5th km, 51 m, (37°48.484N, 27°28.773E), 14.IV.2006, Budak 1998, Hamzaoglu & Aksoy.
2. *H. armenium* subsp. *armenium* (Yellow flower, Straw flower)—Sivas: between Suşehri and Zara, between 11 and 12th km, 1,200 m, (40°06.329N, 38°04.491E), 13.VI.2006, Hamzaoglu 3904, Aksoy & Budak.
3. *H. armenium* subsp. *araxinum* (Yellow flower, Straw flower)—Erzincan: between Erzincan and Kelkit, between 18 and 22nd km, 1,850 m, (39°51.951N, 39°21.137E), 18.VII.2006, Hamzaoglu 4320, Aksoy & Budak.
4. *H. chasmolycicum* (Plateau flower, Plateau rose, Snake rose)—Isparta: Eğirdir, Electric Central of Kovada, 800–850 m, (37°37.834N, 30°51.736E), 22.VI.2006, Hamzaoglu 4037, Aksoy & Budak.
5. *H. heywoodianum* (Gold flower, Bee flower, Fadeless flower)—Aydın: between Söke and Didim, exit to right from Boynak village, 820 m, (37°40.831N, 27°13.711E), 5.VI.2006, Hamzaoglu 4070, Aksoy & Budak.
6. *H. plicatum* subsp. *plicatum* (Bee flower, Plateau flower, Bozoğlan)—Yozgat: between Akdağmadeni and Kızılcaova, Urus İsmail fountain, 1,950 m, (39°31.845N, 36°00.664E), 11.VII.2006, Hamzaoglu 4217, Aksoy & Budak.
7. *H. compactum* (Yellow flower, Yellow savran, Mantuvar flower)—Antalya: Elmalı, north of Vahabi Ümmi Tomb, 1480 m, (36°45.255N, 29°54.612E), 12.VI.2007, Budak 2151, Hamzaoglu & Aksoy.
8. *H. graveolens* (Moth herb)—Bursa: Uludağ, north of Aydın Yıldız Hotel, 1,840–1,900 m, (40°06.225N, 29°07.775E), 29.VII.2006, Hamzaoglu 4387, Aksoy & Budak.
9. *H. artvinense* (Fadeless flower, Immortal flower)—Artvin: Ardanuç, Ardanuç Castle, 645 m, (41°07.450N, 42°03.137E) 17.VII.2007, Hamzaoglu 4834, Aksoy & Budak.

Ergin Hamzaoglu, Ahmet Aksoy and Ümit Budak authenticated the plant material. Voucher specimens have been deposited at the Herbarium of the Department of Biology, Bozok University, Yozgat-Turkey.

Preparation of methanol extracts

Dried plant at room temperature was ground to powder with a grinder. Then the powdered plant material (10 g) was extracted in a Soxhlet extractor with 100 mL methanol (MeOH) at 60 °C for 6 h. The extract was filtered and concentrated to dryness under reduced pressure at 40 °C with a rotary evaporator. Finally, the extracts were kept at +4 °C until tested.

Subjects

After getting approval from the Local Ethics Committee, heparinized blood samples obtained from volunteers. Six healthy subjects (three female and three male) were examined for each plant sample. Peripheral blood was obtained from people who did neither smoking nor drinking alcohol and had no viral infection. Heparinized blood samples were collected for peripheral lymphocyte cultures.

Chemicals

Peripheral blood (PB) karyotyping medium (Biological Industries), colcemid (Sigma) and giemsa stain (Merck) were used in peripheral blood culture. PB Karyotyping Medium is based on RPMI-1640 basal medium supplemented with L-glutamine, fetal bovine serum, antibiotics (gentamycin) and phytohemagglutinin (PHA-M).

Lymphocyte cultures and cell harvesting

Using an aseptic technique, heparinized blood samples (0.4 mL), obtained from healthy donors, were placed in sterile culture tubes containing 5 ml of PB karyotyping medium. Then, *Helichrysum* extract preparations were added to obtain the five final concentrations (0.01, 0.05, 0.1, 0.5, and 1 mg/mL). After mixing the contents of each culture tube by gently shaken, the culture tubes were incubated in a slanted position at 37 °C for 72 h. After 70 h of incubation, 0.1 mL colcemid solution (10 µg/mL)

was added to each culture tube and mixed by shaking gently. 72 h of incubation, cells were harvested by centrifugation, by giving hypotonic treatment (0.075 M KCl) and they were fixed in fresh fixative solution (methanol:acetic acid, 3:1). This fixation step was repeated for three times. Slides were air-dried and stained with giemsa (Ozkul et al. 2005).

Examination of mitotic index and replication index

MI was calculated as the proportion of metaphase for 2,000 cells for each donor and concentration.

A total of 500 cells were scored for the determination of the RI. RI was calculated by using the following formula: $RI = (1 \times M1 + 2 \times M2 + 3 \times M3)/500$, where M1, M2 and M3 are the cells containing 1, 2, 3 or more nuclei (Rojas et al. 1993).

Staining and examination of micronucleus

The MN staining was performed according to Ozkul et al. (2005). The slides were scored by a single observer. About 500 cells were examined at 600 check magnification from each slide and when MN cells were located they were examined under 1,000 magnification. The criteria suggested by Scarpato and Migliore (1996) for recognizing micronuclei were followed. Dead or degenerating cells were excluded from evaluation. Nuclear blebbing (MN-like structure connected with the main nucleus with a bridge) was not considered. Only micronuclei equal to or smaller than one-fifth of the main nucleus were assumed. Multimicronucleated cells were also scored but not included in the evaluation of MN frequency (Scarpato and Migliore 1996).

Statistical analysis

The computer software program SPSS 10.0 was used to analyze the data. The statistical significance of the effects of *Helichrysum* taxa on MI, RI and MN was tested by the repeated measures of the analysis of variance (ANOVA) and differences between groups were determined by the least significant differences (LSD) test and were considered significant for with $p < 0.01$.

Results

Helichrysum species (especially *H. stoechas*, *H. graveolens*, *H. plicatum*) have been used to alternative medicine for many years in Turkey (Baytop 1997). Their increasing use in recent years is clear evidence of public interest in having alternatives to conventional medicine. Five different concentrations (0.01, 0.05, 0.1, 0.5, and 1 mg/mL) and in vitro three different parameters (MI, RI, MN) were used for determine the cytogenetic effects of nine *Helichrysum* taxa in human peripheral lymphocytes.

The effects of *Helichrysum* species on MI and RI are summarized in Tables 1 and 2. These results showed that increasing doses of *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. heywoodianum* (only RI), *H. plicatum* subsp. *plicatum*, *H. compactum* and *H. artvinense* decreased MI and RI ($p < 0.01$). However, increasing doses of *H. chasmolycicum* ($p < 0.01$) and *H. graveolens* ($p = 0.01$) increased MI and RI. According to control, a significant different in MI was observed in the 0.01 mg/mL concentration range for *H. armenium* subsp. *armenium* (3.41 ± 1.26) and *H. armenium* subsp. *araxinum* (2.93 ± 0.63), in the 0.05 mg/mL concentration range for *H. artvinense* (2.70 ± 0.93), in the 0.1 mg/mL concentration range for *H. chasmolycicum* (5.06 ± 0.74) and *H. compactum* (1.40 ± 0.34), in the 0.5 mg/mL concentration range for *H. stoechas* subsp. *barrelieri* (1.16 ± 0.48) and *H. plicatum* subsp. *plicatum* (1.07 ± 0.45). These significant differences are in the same concentrations of *H. armenium* subsp. *armenium* (0.01 mg/mL – 1.352 ± 0.105), *H. armenium* subsp. *araxinum* (0.01 mg/mL – 1.412 ± 0.141), *H. stoechas* subsp. *barrelieri* (0.5 mg/mL – 1.188 ± 0.058) and *H. plicatum* subsp. *plicatum* (0.5 mg/mL – 1.222 ± 0.048) for RI, too. But these significant differences are in the different concentration range (0.5 mg/mL) for *H. chasmolycicum* (1.506 ± 0.131), *H. heywoodianum* (1.454 ± 0.143), *H. compactum* (1.131 ± 0.089) and *H. artvinense* (1.211 ± 0.140).

The results of MN test are given in Table 3. MN results showed that high concentrations of *H. stoechas* subsp. *barrelieri* (0.5 mg/mL – 8.30 ± 2.06), *H. chasmolycicum* (0.5 mg/mL – 6.16 ± 0.75), *H. plicatum* subsp. *plicatum* (0.5 mg/mL – 3.50 ± 0.53), *H. compactum* (0.5 mg/mL – 3.83 ± 1.19) and low concentrations of *H. artvinense* (0.01 mg/mL

– 2.93 ± 0.66), *H. armenium* subsp. *armenium* (0.1 mg/mL – 8.33 ± 1.50), *H. armenium* subsp. *araxinum* (0.1 mg/mL – 6.83 ± 1.86) increased MN rates ($p < 0.01$). However, there is not a statistically significant in MN rates for *H. heywoodianum* and *H. graveolens* ($p = 0.01$).

Discussion

MI and RI are used as indicators of adequate cell proliferation biomarkers. MI measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or delay in the cell proliferation kinetics (Rojas et al. 1993). Except for *H. graveolens* and *H. heywoodianum* (only MI), there are great differences among MI and RI rates of other *Helichrysum* taxa ($p < 0.01$). These differences were dose-dependent ($p < 0.01$). The present results also show that RI values except for *H. chasmolycicum* and *H. graveolens* were significantly lower in extract concentrations than in controls. This may be attributed to several factors, one of which could be the fact that many cells, during the time remained in the circulation (at G_0), suffered DNA damage and did not survive the first cell cycle in culture to express MN, since they would have entered a process of necrosis or apoptosis before this event. Another factor to be considered could be that mitotic delay, by permitting the repair of genotoxic lesions, might also modify the frequency of cells that undergo mitosis at a given time in culture, thus changing the frequency of cells with two nuclei or more at harvest.

In living creatures, which are exposed to a mutagen factor, the probability of formation of mitotic and meiotic defects is increased and the rate of MN could increase due to this increase (Ramalho et al. 1988). Alcohol consumption, smoking and viral infections increased to MN rates in peripheral blood lymphocytes (Seitz et al. 1998). In this study peripheral blood samples were obtained from people who did neither smoke nor drink alcohol and had no viral infection. Except for *H. heywoodianum* and *H. graveolens*, other *Helichrysum* taxa effected MN rates ($p < 0.01$). We also detected a negative correlation between MN and progression of cell division; namely the higher the MN frequency were detected in exposed individuals, the lower the values of nuclear division progression

Table 1 Mitotic index (%) in human lymphocyte cultures exposed to methanol extracts of *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. chasmolycicum*, *H. heywoodianum*, *H. plicatum* subsp. *plicatum*, *H. compactum*, *H. graveolens*, *H. artvinense*

Mean MI $\pm$ SD	Doses (mg/mL)					
Taxa	Control	0.01	0.05	0.1	0.5	1
<i>H. stoechas</i> subsp. <i>barrelieri</i>	4.72 $\pm$ 1.91	4.15 $\pm$ 1.99	4.14 $\pm$ 1.96	3.75 $\pm$ 1.79	1.16 $\pm$ 0.48*	0.96 $\pm$ 0.42*
<i>H. armenium</i> subsp. <i>armenium</i>	5.27 $\pm$ 1.49	3.41 $\pm$ 1.26*	2.86 $\pm$ 1.22*	2.20 $\pm$ 0.73*	0.97 $\pm$ 0.31*	0.94 $\pm$ 0.22*
<i>H. armenium</i> subsp. <i>araxinum</i>	4.74 $\pm$ 1.44	2.93 $\pm$ 0.63*	2.23 $\pm$ 1.17*	1.72 $\pm$ 0.99*	0.95 $\pm$ 0.42*	0.89 $\pm$ 0.14*
<i>H. chasmolycicum</i>	3.86 $\pm$ 1.12	3.40 $\pm$ 0.84	4.43 $\pm$ 0.36	5.06 $\pm$ 0.74*	5.31 $\pm$ 0.56*	5.50 $\pm$ 0.52*
<i>H. heywoodianum</i>	4.42 $\pm$ 2.70	5.56 $\pm$ 2.44	5.45 $\pm$ 2.63	4.32 $\pm$ 2.31	2.88 $\pm$ 1.62	2.37 $\pm$ 1.00
<i>H. plicatum</i> subsp. <i>plicatum</i>	3.46 $\pm$ 1.14	3.44 $\pm$ 1.23	3.44 $\pm$ 1.19	3.20 $\pm$ 0.99	1.07 $\pm$ 0.45*	0.95 $\pm$ 0.41*
<i>H. compactum</i>	3.50 $\pm$ 1.26	2.68 $\pm$ 1.22	2.20 $\pm$ 0.99	1.40 $\pm$ 0.34*	0.75 $\pm$ 0.30*	0.66 $\pm$ 0.22*
<i>H. graveolens</i>	3.32 $\pm$ 0.88	3.49 $\pm$ 0.74	4.53 $\pm$ 1.33	4.46 $\pm$ 1.15	4.59 $\pm$ 1.25	4.60 $\pm$ 1.21
<i>H. artvinense</i>	4.43 $\pm$ 1.26	3.60 $\pm$ 1.13	2.70 $\pm$ 0.93*	1.93 $\pm$ 1.25*	0.97 $\pm$ 0.42*	0.74 $\pm$ 0.30*

MI mitotic index; SD standard deviation

* Significantly different from control ($p < 0.01$)

Table 2 Replication index in human lymphocyte cultures exposed to methanol extracts of *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. chasmolycicum*, *H. heywoodianum*, *H. plicatum* subsp. *plicatum*, *H. compactum*, *H. graveolens*, *H. artvinense*

Mean RI $\pm$ SD	Doses (mg/mL)					
Taxa	Control	0.01	0.05	0.1	0.5	1
<i>H. stoechas</i> subsp. <i>barrelieri</i>	1.723 $\pm$ 0.202	1.626 $\pm$ 0.196	1.627 $\pm$ 0.232	1.550 $\pm$ 0.141	1.188 $\pm$ 0.058*	1.160 $\pm$ 0.045*
<i>H. armenium</i> subsp. <i>armenium</i>	1.560 $\pm$ 0.139	1.352 $\pm$ 0.105*	1.286 $\pm$ 0.075*	1.251 $\pm$ 0.078*	1.103 $\pm$ 0.019*	1.101 $\pm$ 0.065*
<i>H. armenium</i> subsp. <i>araxinum</i>	1.670 $\pm$ 0.135	1.412 $\pm$ 0.141*	1.277 $\pm$ 0.111*	1.270 $\pm$ 0.040*	1.114 $\pm$ 0.019	1.098 $\pm$ 0.020*
<i>H. chasmolycicum</i>	1.350 $\pm$ 0.112	1.256 $\pm$ 0.124	1.430 $\pm$ 0.148	1.448 $\pm$ 0.147	1.506 $\pm$ 0.131*	1.518 $\pm$ 0.101*
<i>H. heywoodianum</i>	1.577 $\pm$ 0.122	1.673 $\pm$ 0.195	1.678 $\pm$ 0.070	1.722 $\pm$ 0.143	1.454 $\pm$ 0.143*	1.368 $\pm$ 0.127*
<i>H. plicatum</i> subsp. <i>plicatum</i>	1.658 $\pm$ 0.188	1.616 $\pm$ 0.122	1.586 $\pm$ 0.156	1.548 $\pm$ 0.136	1.222 $\pm$ 0.048*	1.186 $\pm$ 0.055*
<i>H. compactum</i>	1.373 $\pm$ 0.161	1.335 $\pm$ 0.136	1.310 $\pm$ 0.158	1.244 $\pm$ 0.108	1.131 $\pm$ 0.089*	1.089 $\pm$ 0.052*
<i>H. graveolens</i>	1.340 $\pm$ 0.156	1.425 $\pm$ 0.257	1.496 $\pm$ 0.246	1.502 $\pm$ 0.178	1.522 $\pm$ 0.198	1.529 $\pm$ 0.191
<i>H. artvinense</i>	1.496 $\pm$ 0.190	1.362 $\pm$ 0.178	1.296 $\pm$ 0.130	1.284 $\pm$ 0.170	1.211 $\pm$ 0.140*	1.162 $\pm$ 0.110*

RI replication index; SD standard deviation

RI = $(1 \times M1 + 2 \times M2 + 3 \times M3)/500$; M1, mononucleat cells; M2, binucleat cells; M3, tri or polynucleat cells

* Significantly different from control ($p < 0.01$)

were expressed as RI. This may mean that cells with greater chromosomal damage may die before cell division or they may be less capable of entering this phase (Santos-Mello et al. 1974). Multiple MN as the result of the loss of large part of the genome impairs or even prevents the cell division (Nath and Ong 1990).

Micronucleated cells with one MN have a better chance to survive because only a small part of the genome is lost (Hando et al. 1997).

In literature, there are a lot of studies interested in properties of *Helichrysum* species and using folk medicine of theirs. Some members of this genus have

Table 3 Micronucleus (%) in human lymphocyte cultures exposed to methanol extracts of *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. chasmolycicum*, *H. heywoodianum*, *H. plicatum* subsp. *plicatum*, *H. compactum*, *H. graveolens*, *H. artvinense*

Taxa	Doses (mg/mL)					
	Control	0.01	0.05	0.1	0.5	1
<i>H. stoechas</i> subsp. <i>barrelieri</i>	4.66 ± 1.75	5.00 ± 1.67	5.36 ± 1.63	5.60 ± 1.66	8.30 ± 2.06*	8.50 ± 2.38*
<i>H. armenium</i> subsp. <i>armenium</i>	4.30 ± 1.95	6.46 ± 1.44	7.40 ± 1.65	8.33 ± 1.50*	11.36 ± 2.53*	11.36 ± 3.07*
<i>H. armenium</i> subsp. <i>araxinum</i>	4.46 ± 1.25	5.43 ± 0.83	5.96 ± 1.54	6.83 ± 1.86*	8.96 ± 1.53*	9.30 ± 0.80*
<i>H. chasmolycicum</i>	3.60 ± 1.55	4.23 ± 1.64	4.63 ± 0.73	5.26 ± 0.57	6.16 ± 0.75*	6.40 ± 0.43*
<i>H. heywoodianum</i>	3.70 ± 1.94	4.16 ± 1.77	4.46 ± 2.21	4.43 ± 1.88	4.63 ± 2.05	4.80 ± 2.18
<i>H. plicatum</i> subsp. <i>plicatum</i>	1.40 ± 0.88	1.63 ± 0.61	1.73 ± 0.58	1.93 ± 0.64	3.50 ± 0.53*	3.96 ± 0.68*
<i>H. compactum</i>	1.60 ± 0.85	2.06 ± 0.81	2.50 ± 0.91	3.13 ± 1.14	3.83 ± 1.19*	3.93 ± 1.33*
<i>H. graveolens</i>	2.23 ± 0.87	1.96 ± 1.04	2.20 ± 0.65	1.70 ± 0.62	1.70 ± 0.71	1.46 ± 0.61
<i>H. artvinense</i>	1.16 ± 0.44	2.93 ± 0.66*	3.36 ± 0.94*	3.96 ± 0.46*	5.06 ± 1.00*	5.03 ± 0.89*

MN micronucleus; SD standard deviation

* Significantly different from control ($p < 0.01$)

cholagogue and choleretic activity and stimulate the secretion of gastric juice (Litvinenko et al. 1992). Studies regarding *Helichrysum* species used in this study are summarized. Literature showed that antibacterial (Aslan et al. 2006; Chinou et al. 1997; Rios et al., 1991; Roussis et al. 2002), antiviral (Aslan et al. 2006), antifungal (Aslan et al. 2006), anti-inflammatory (El-Dahmy 1994; Recio et al. 1991), antioxidant (Carini et al. 2001) and anti-allergic (Carini et al. 2001) effects of *H. stoechas*; antibacterial (Aslan et al. 2006; Cosar and Cubukcu 1990), antiviral (Aslan et al. 2006) and antifungal (Aslan et al. 2006) effects of *H. armenium*; antibacterial (Cosar and Cubukcu 1990; Erdoğan et al. 2001; Kotan et al. 2007; Smirnov et al. 1982), antioxidant (Aslan et al. 2007b; Tepe et al. 2005) and antidiabetic (Aslan et al. 2007b) effects of *H. plicatum*; antibacterial (Sagdic et al. 2002; Suzgec et al. 2005) and antioxidant (Suzgec et al. 2005) effects of *H. compactum*; antibacterial (Aslan et al. 2006; Cosar and Cubukcu 1990), antiviral (Aslan et al. 2006), antifungal (Aslan et al. 2006), antioxidant (Aslan et al. 2007a) and antidiabetic (Aslan et al. 2007a) effects of *H. graveolens*. However, there are few reports in literature about the genotoxic, cytotoxic and mutagenic effects of *Helichrysum* species. Reid et al. (2006) evaluated mutagenic and antimutagenic effects of South African plants (*H. herbaceum* Sweet., *H. nudifolium* Less., *H. ruderae* Hilliard. & B.L.Burt, *H. rugulosum* Less., *H. simillimum* DC.,

H. umbraculigerum Less.) and showed mutagenic effects of *H. simillimum*, *H. herbaceum* and *H. rugulosum*. It was determined that there was not genotoxic effect of *H. italicum* (Roth) D.Don (Bakkali et al. 2005; Idaomar et al. 2002; Nostro et al. 2003; Rosa et al. 2007). Elgorashi and van Staden (2007) reported mutagenic effects of methanol extracts of *H. simillimum*. In the present study we found that some *Helichrysum* extracts used in alternative medicine induced MN decreased MI and RI in human lymphocytes. We think that *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. chasmolycicum*, *H. plicatum* subsp. *plicatum*, *H. compactum* and *H. artvinense* should not be used in high quantities in the general population because of their genotoxic and mutagenic properties (especially *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. artvinense*). *H. heywoodianum* and *H. graveolens* had no genotoxic effect. We may suggest theirs in alternative medicine (especially *H. graveolens*). *H. graveolens* is one of the most used species in alternative medicine in Turkey. It is a plant widely used for tea. It is used for the treatment of kidney stone and uro-genital disorders (Baytop 1997; Ezer 2006). The decreases of MI and RI in the peripheral blood lymphocytes indicate that *H. stoechas* subsp. *barrelieri*, *H. armenium* subsp. *armenium*, *H. armenium* subsp. *araxinum*, *H. plicatum* subsp. *plicatum*, *H. compactum* and *H. artvinense* may suggest as anticarcinogenic agents.

In contrast, increasing MN rates showed that they could have genotoxic and carcinogenic effects at high concentrations. *H. chasmolycicum* increased rates of MI and RI. This species can be useful in investigations regarding the increase of cell division. Furthermore, there are a lot of compounds in extracts of *Helichrysum* (Chinou et al. 1997; Jakupovic et al. 1989). The use of single constituents would allow more precise interpretation of data and the added effect of the numerous compounds will be lost. It is therefore likely that the mixture of *Helichrysum* compounds may result in synergistic effects. Further studies will be needed to determine the effects of different compounds isolated from *Helichrysum* species and evaluate the synergistic effects on MI, RI and MN.

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