CARYOLOGIA Vol. 62, no. 4: 303-308, 2009

Genotoxic Potential of *Senecio trapezuntinus* in Cultured Human Lymphocytes

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Abstract — The *in vitro* genotoxic potential of methanol extract of *Senecio trapezuntinus* in human lymphocytes was investigated. Blood samples were obtained from six healthy donors, non-smoking volunteers, who were incubated and exposed to increasing concentrations of *Senecio trapezuntinus* (0.002, 0.01, 0.05 and 0.1 mg/mL). *Senecio trapezuntinus* induced micronucleus, decreased mitotic and proliferation indexes in human lymphocytes ($P<0.01$ for 0.05 and 0.1 mg/mL). While the decreases of mitotic and proliferation indexes indicate that *Senecio trapezuntinus* is an antiproliferative and antimitotic agent, the rise of micronucleus shows that *Senecio trapezuntinus* at high concentrations may become carcinogenic and genotoxic.

Key words: genotoxic, micronucleus, mitotic index, proliferation index, *Senecio trapezuntinus*.

INTRODUCTION

The genus *Senecio* L., belonging to Asteraceae family and generally known under the names "groundsel", is represented by approximately 1500 species in the world. This genus is represented in Turkish flora by 52 taxa (43 species, 3 subspecies and 6 varieties), of which 21 are endemic. *Senecio trapezuntinus* Boiss. is a narrow endemic species known from Northern Anatolia (Trabzon - Turkey) (Matthews 1975). Figure 1 shows the spread in *Senecio trapezuntinus* in Turkey. In 1875, this species was defined by Boiss. for the first time. As it seems from Figure 1, just because the species is known to be narrow endemic of Trabzon, it has been neither seen nor collected by anyone since it was first defined. So *Senecio trapezuntinus* is in the CR (Critically Endangered) danger category according to Iucn (2008). No one has studied on *Senecio trapezuntinus,* because it hasn't been found and seen for many years. These reasons increase the importance of our study.

A micronucleus (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). Investigations on MN frequencies support the widely accepted assumption that MN is a product of early events in human carcinogenic processes (Desai *et al*. 1996).

Alcohol consumption, smoking, exposure to X- and gamma-rays appear to be the most frequent exogenic risk factors for the development of cancer (Seitz, Poschl and Simannowski 1998). Carcinogenic substances pass into target cells and also systematically lead to decreased cell metabolism, producing a relative immune deficiency (Carvalho 1997). Consequently, the MN test, currently known as the MN assay has been used for screening populations under the risk of mutagenic agents, especially for the identification of pre-clinical steps of the carcinogenic process (Ramirez *et al*. 1999). Also, the mitotic index (MI) and proliferation index (PRI) assay are used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression. Compounds that inhibit mitotic progression result in an increase in the MI of population.

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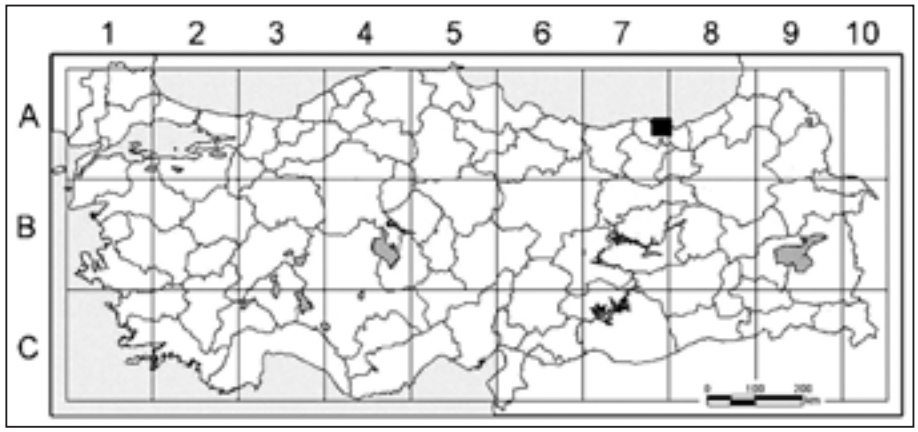

Fig. 1 — The spread area of *Senecio trapezuntinus* (■) in Turkey.

We report a study of healthy donors, evaluating rates of MN, MI and PRI in their peripheral blood lymphocytes with different methanol extract concentrations of the *Senecio trapezuntinus*, compared to a control group.

MATERIALS AND METHODS

Collection of Plant Materials - Collection information of the *Senecio trapezuntinus* is listed below: *Senecio trapezuntinus* – Trabzon: Boztepe, Esentepe district, Masatlık place at 120 m altitude, 21.IV.2006, Budak, Hamzaoğlu & Aksoy.

Ergin Hamzaoğlu, Ahmet Aksoy and Ümit Budak authenticated the plant material. Voucher specimens (Herbarium number: 2011) have been deposited at the Herbarium of the Department of Biology, Bozok University, Yozgat, Turkey.

Preparation of Methanol Extract - Dried plants at room temperature were ground to powder with a grinder. Then the powdered plant materials (10 g) were extracted in a Soxhlet extractor with 100 mL methanol (MeOH) at 60ºC. The extract was filtered and concentrated to dryness under reduced pressure at 40ºC with a rotary evaporator. Finally, methanol extract was kept at +4ºC until tested.

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, foetal bovine serum, antibiotics (gentamycin) and phytohemagglutinin.

Human Lymphocyte Cultures and Cell Harvesting - After getting approval from Local Ethic Committee, heparinized blood samples (0.4 mL), obtained from six healthy donors, three of whom are male and three of whom are female, were placed in sterile culture tubes containing 5 mL of PB karyotyping medium. Then, the extract was added to obtain four final concentrations (0.002, 0.01, 0.05 and 0.1 mg/mL). The concentrations were a result of trial and error. There was no previous literature we could resort to. 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. After 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, SILICI and EROĞLU 2005).

Staining and Examination of Micronucleus - The MN staining was performed according to OZKUL, SILICI and EROĞLU (2005). The slides were randomized and 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 1000 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) were not considered. Only micronuclei equal to

or smaller than one-fifth of the main nucleus were considered. Multimicronucleated cells were also scored but not included in the evaluation of MN frequency.

Examination of Mitotic Index - MI was calculated as the proportion of metaphase for 2000 cells for each donor and concentration.

Examination of Proliferation Rate Index - A total of 500 cells were scored for the determination of the PRI. PRI was calculated by using the following formula: PRI = (1xM1 + 2xM2 + 3xM3)/500, where M1, M2 and M3 are the cells containing 1, 2, 3 or more nuclei, respectively (Siddique, Beg and Afzal 2007).

Statistical Analysis - The computer software program SPSS 10.0 was used to analyze the data. The statistical significance of the effects of *Senecio trapezuntinus* on MN, MI and PRI 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 with $P<0.01$ was considered significant.

RESULTS AND DISCUSSION

In spite of fairly large genus, there are few reports in literature about the genotoxic and mutagenic effects of *Senecio*. SANTOS-MELLO *et al.* (2002) investigated such genotoxic and antimitotic effects of pyrrolizidine alkaloids extracted from *Senecio brasiliensis* (Sprengel) Less. in polychromatic erythrocytes of mouse bone marrow. Alkaloids showed no significant increase in micronucleus frequency in binucleated cells, probably due to the lack of a metabolic activation mechanism. However, an antimitotic effect was observed. LOIZZO *et al.* (2005) evaluated *in vitro* antiproliferative effects on human tumour cell lines of extracts from *Senecio leucanthemifolius* Poiret. LOIZZO *et al.* (2007) reported that *Senecio ambiguus* subsp. *ambiguus* (Biv.) DC. extracts were able to inhibit the *in vitro* proliferation of renal cell adenocarcinoma and hormone dependent prostate carcinoma. POOL (1982) showed the genotoxic activity of an alkaloidal extract of *Senecio nemorensis* subsp. *fuchsii* in *Salmonella typhimurium* and *Escherichia coli* systems. For this reason, it is of importance to determine the genotoxic effects of *Senecio* species. *In vitro* three different cytogenetic parameters (MN, MI and PRI) were used.

The results of MN test are given in Table 1. When MN formation was analyzed after treatment with different concentrations of methanol extract of *Senecio trapezuntinus*, significant changes in the percentage of MN were detected for 0.05 and 0.1 mg/mL ($P<0.01$). Other extract concentrations (0.002 and 0.01 mg/mL) did not induce any change in MN frequencies compared to untreated group (control) ($P>0.01$).

There are many factors affecting the MN frequency in lymphocytes: age, gender, smoking and alcohol consumption, viral infection, X and gamma ray exposures (MÜLLER 1996). In this study, donors neither smoking nor drinking alcohol were included. They were not exposed to X and gamma ray and had no viral infection. An increase in MN may result from interactions of a great variety of cytotoxic and genotoxic agents with DNA. MN

Table 1 — Micronucleus (MN) (%) in human lymphocyte cultures exposed to methanol extracts of *Senecio trapezuntinus*.

Doses (mg/mL)	Female			Male			Mean MN ± SD (%)
	I	II	III	I	II	III	
Control	1.2	1.4	0.6	0.8	0.8	1.4	1.03 ± 0.34
0.002	1.0	1.8	1.2	1.2	1.0	2.0	1.36 ± 0.42
0.01	1.4	1.6	1.2	0.8	1.4	2.2	1.43 ± 0.46
0.05	2.2	2.8	2.2	1.6	2.4	3.0	2.36 ± 0.49 *
0.1	2.6	3.0	2.0	1.8	2.2	3.0	2.43 ± 0.51 *

ANOVA: * $P<0.01$
MN: Micronucleus
SD: Standard Deviation

is an extremely valuable and highly relevant endpoint for the detection of potential carcinogens. Our results show an increase in the percentage of MN (Table 1), suggesting a strong interaction between extracts (0.05 and 0.1 mg/mL) of *Senecio trapezuntinus* and DNA, which could be responsible for the observed genotoxicity.

When the potential genotoxicity of the methanol extract on lymphocyte cultures was analyzed through MI evaluation, a significant decrease was found for *Senecio trapezuntinus* (0.05 and 0.1 mg/mL - *P<0.01*). Concentration rates of 0.002 and 0.01 mg/mL were not affected MI (*P>0.01*) (Table 2).

When PRI was studied, no modifications could be detected for extract concentrations of 0.002 and 0.01 mg/mL (*P>0.01*). As shown in Table 3, changes in PRI were observed for 0.05 and 0.1 mg/mL extract concentrations of *Senecio trapezuntinus* (*P<0.01*).

MI frequencies and PRI values decreased with increasing concentration of *Senecio trapezuntinus*. This state can be explained with two different mechanisms: cellular death and decreasing of cell divisions. The results indicate the genotoxic effects as well as antiproliferative effects and suggest that methanol extract of *Senecio trapezuntinus* exhibits genotoxic properties as well as antimitotic

Table 2 — Mitotic index (MI) (%) in human lymphocyte cultures exposed to methanol extracts of *Senecio trapezuntinus.*

Donor	Doses (mg/mL)	Total counted cells	Total number: dividing cells	Mean MI ± SD (%)
Female	Control	6000	111	1.85 ± 0.78
	0.002	6000	109	1.81 ± 0.73
	0.01	6000	102	1.70 ± 0.90
	0.05	6000	22	0.36 ± 0.22 *
	0.1	6000	14	0.23 ± 0.02 *
Male	Control	6000	106	1.76 ± 0.36
	0.002	6000	100	1.66 ± 0.28
	0.01	6000	100	1.66 ± 0.36
	0.05	6000	32	0.53 ± 0.07 *
	0.1	6000	31	0.51 ± 0.10 *
Female + Male	Control	12000	217	1.80 ± 0.54
	0.002	12000	209	1.74 ± 0.50
	0.01	12000	202	1.68 ± 0.61
	0.05	12000	54	0.45 ± 0.17 *
	0.1	12000	45	0.37 ± 0.16 *

ANOVA: * *P<0.01*
MI: Mitotic Index
SD: Standard Deviation

and antiproliferative properties. A negative correlation was observed between MN induction and cell proliferation; namely the higher the MN frequency were detected in exposed individuals, the lower the values of nuclear division progression were expressed as PRI (Fig. 2). This may mean that cells with greater chromosomal damage may die before cell division or they may be less capable of entering this phase. Multiple MN as the result of the loss of large part of the chromosome impairs or even prevents the cell division (NATH and ONG 1990).

In the present study, we found that *Senecio trapezuntinus* induced MN, decreased MI and PRI in human lymphocytes (Fig. 2). While the decreases of MI and PRI in the peripheral blood lymhocytes added to the different concentrations of methanol extract of *Senecio trapezuntinus* indicate that *Senecio trapezuntinus* may also act as an antiproliferative and antimitotic agent, the rise of MN shows that *Senecio trapezuntinus* at high concentrations may become carcinogenic and genotoxic. The rates of MI, PRI and MN could be affected by pyrrolizidine alkaloids. Because 13

Table 3 — Proliferation index (PRI) in human lymphocyte cultures exposed to methanol extracts of *Senecio trapezuntinus*.

Doses (mg/mL)	Female			Male			Mean PRI ± SD
	I	II	III	I	II	III	
Control	1.658	1.246	1.238	1.372	1.672	1.604	1.465 ± 0.203
0.002	1.644	1.212	1.248	1.350	1.714	1.596	1.460 ± 0.216
0.01	1.650	1.204	1.196	1.192	1.570	1.574	1.397 ± 0.221
0.05	1.152	1.118	1.052	1.084	1.252	1.104	1.127 ± 0.069 *
0.1	1.092	1.082	1.060	1.066	1.146	1.080	1.087 ± 0.030 *

ANOVA: * *P<0.01*.
PRI: Proliferation Index.
SD: Standard Deviation.
PRI = (1xM1 + 2xM2 + 3xM3)/500.
M1: The number of cells in first metaphase.
M2: The number of cells in second metaphase.
M3: The number of cells in third or more metaphase.

Micronucleus, mitotic and proliferation indexes changes of *Senecio trapezuntinus*

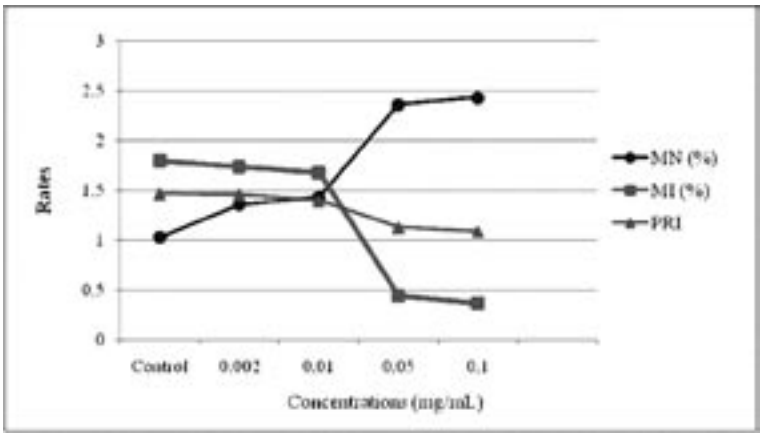

Fig. 2 — Micronucleus, mitotic and proliferation indexes changes of *Senecio trapezuntinus*. *Senecio trapezuntinus* induced MN, decreased MI and PRI in human lymphocytes. These increases and decreases were dose-dependent. Increase of MN was clear for 0.05 and 0.1 mg/mL concentrations. Decreases of MI and PRI were 0.05 and 0.1 mg/mL concentrations. A negative correlation was observed between MN induction and cell proliferation; namely the higher the MN frequency were detected in exposed individuals, the lower the values of nuclear division progression were expressed as PRI.

families of the flowering plants contain pyrrolizidine alkaloids (FURUYA *et al*. 1987). The principal families involved are the Asteraceae, Boraginaceae and Fabaceae, while the main genera are *Senecio* (Asteraceae), *Crotalaria* L. (Fabaceae), *Heliotroprium* L., *Trichodesma* R.Br. and *Symphytum* L. (*Boraginaceae*) (SEAWRIGHT 1989). The earliest studies of the alkaloids began with the isolation of two compounds from *Senecio latifolius* DC. by Watt in 1909 (SEAWRIGHT 1989). In present, it is reported that antimitotic and toxic effects of pyrrolizidine alkaloids extracted from *Senecio* (WILLMOT and ROBERTSON 1920; MCLEAN 1970; SANTOS-MELLO *et al*. 2002).

Furthermore, there are a lot of compounds at contents of plant species. 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 *Senecio* compounds may result in synergistic effects. A further study will be needed to determine the effects of different compounds isolated from *Senecio trapezuntinus* and evaluate the synergistic effects on MI, PRI and MN.

Acknowledgement — We are indebted to TÜBİTAK (Project no. TBAG-106T240) and Erciyes University Research Fund (Project no. FBA-05-21) for financial support.

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Received February 24th 2009; accepted June 17th 2009