Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
22(3): 541-548, May/Jun. 2012

Evaluation of genotoxic and mutagenic effects of aqueous extract from aerial parts of *Linaria genistifolia* subsp. *genistifolia*

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Abstract: Genotoxic and mutagenic effects of aqueous extract from aerial parts *Linaria genistifolia* (L.) Mill. subsp. *genistifolia*, Plantaginaceae (*Lg*-ext) were investigated by using both Allium cepa root meristematic cells and bacterial reverse mutation assay in *Salmonella typhimurium* TA98 and TA100 with or without metabolic activation system (S9), respectively. In *Allium* root growth inhibition test, EC50 value was determined approximately 15 g/L and 0.5xEC50, EC50 and 2xEC50 concentrations of *Lg*-ext were introduced to onion tuber roots and distilled water and methyl methane sulfonate (MMS, 10 ppm) used as a negative and positive control, respectively. The characteristic effect caused by tested preparations was an increase of mitotic index (MI) in 7.5 g/L and 15 g/L (except 24 and 96 h) and simultaneous decrease of MI in 30 g/L and in MMS. While stickiness, bridges, chromosome laggards and disturbed anaphase-telophase were observed in anaphase-telophase cells, c-metaphase, pro-metaphase, polyploidy and binuclear cells were observed in other cells. *Lg*-ext was not found to be mutagenic on *S. typhimurium* TA 98 and TA100 with or without S9. The results were also analyzed statistically by using SPSS for Windows, and Duncan's multiple range tests were performed respectively. These results indicate that Lg-ext exhibits genotoxic activity in *A. cepa* root meristematic cells but not mutagenic activity in Ames test system.

Article

Received 27 Jul 2011
Accepted 16 Dec 2011
Available online 3 Feb 2012

Keywords:
Allium
Ames
chromosome aberration
Linaria genistifolia
Plantaginaceae

ISSN 0102-695X
http://dx.doi.org/10.1590/S0102-695X2012005000028

Introduction

The genus *Linaria*, a well known genus of the family Plantaginaceae, is widely distributed throughout the northern hemisphere with its centre of distribution in the Mediterranean basin and eastern Asia. It comprises currently around 200 species, twenty of which are in Turkey popularly known as "nevruz otu" and nine of them endemics (Davis, 1978; Seçmen et al., 2000). Some *Linaria* species have been used in folk remedies for various purposes such as hemorrhoid, skin eruptions, sores, ulcers, diuretic, laxative, tonic, antiscorbutic, antidiabetic and to treat some vascular disorders. Dosage is critical and it should not be given to pregnant women, since the plant might be slightly toxic (San Feliciano et al., 1993; Baytop, 1999). *Linaria* species are reported to contain alkaloids, iridoid glucosides, flavonoids, aurones and diterpenoids (Otsuka, 1992; Ilieva et al., 1993; Bianco et al., 1996; Hua et al., 2002; Ahmad et al., 2006; Tundis et al., 2008; Ferhat et al., 2010). They contains a poisonous glucoside that is reported to be mildly poisonous to cattle (Moroshita, 1991).

Vicia faba, Tradescantia paludosa, Pisum sativum, Hordeum vulgare, Crepis capillaris and *A. cepa* are used to study of cytogenetic and genotoxic effect of plant extract or chemical(s). Among them, *Allium* test is one of the best-established test systems in order to determine the toxicity in the laboratories (Fiskesjö, 1985; Grant, 1992; Rank, 2003; Saxena et al., 2005; Konuk et al., 2007; Liman et. al., 2011). Because onions are easy to store and to handle, and also macroscopic and microscopic parameters can be observed easily. Moreover this system is well correlated with the data obtained from eukaryotic and prokaryotic systems (Fiskesjö, 1988).

Examining the mutagenicity of plant extract or chemical(s), Ames test, or the so-called *Salmonella*/microsome test, is widely used (Konuk et al., 2008; Uysal et al., 2010; Liman et al., 2010). This test can be carried out rapidly and cheaply and it is also one of the most reliable short-term bacterial test systems (Maron & Ames, 1983; Mortelmans & Zeiger, 2000). In this system, *S. typhimurium* mutant strains, obtained from *S. typhimurium* LT2 parental line *in vitro*, are employed (Ames et al., 1975).

The objective of this study was to investigate the genotoxic and mutagenic effects of *Lg*-ext by employing both *A. cepa* anaphase-telophase test and bacterial reverse mutation assay in *S. typhimurium* TA98 and TA100 strains

with or without S9, respectively.

Materials and Methods

Organisms

The *S. typhimurium* test strains TA98 and TA 100 were obtained from Nuran Diril, Hacettepe University, Turkey. While TA98 were used for determining the frame shift, TA100 was used to determine the base pair exchange type of mutations. *Allium cepa* (2n=16) onion bulbs, 25-30 mm diameter, without any treatment, were purchased from a local supermarket.

Chemicals

S9 from Liver of rat (Sprague-Dawley), Bacto agar, nutrient broth no.2 (Oxoid), 2-aminoanthracene (2AA), β-nicotinamide-adenine dinucleotide phosphate (β-NADP), glucose-6-phosphate (G6P), mitomycin-C (MMC), ampicillin and histidine were obtained from Sigma-Aldrich. Sodium azide (SA), citric acid monohydrate, sodium hydroxide, potassium chloride, sodium chloride, and dimethyl sulphoxide (DMSO) were purchased from Riedel. 4-Nitro-*O*-phenylenediamine (NPD) and 2-aminofluorene (2AF) were purchased from Fluka. Magnesium chloride, crystal violet, potassium phosphate and sodium ammonium phosphate were obtained from Merck.

Plant collection and extraction

The aerial parts of *Linaria genistifolia* (L.) Mill. subsp. *genistifolia*, Plantaginaceae, were collected from Başören Village, Afyonkarahisar, Turkey, in late-May 2010. The taxonomic identification of plant materials was confirmed by Dr Mehmet Temel, Department of Biology, Afyon Kocatepe University, Turkey. A voucher specimen was deposited at the Herbarium of Afyon Kocatepe University (number Kala 1410). Air dried and powdered aerial parts of the *L. genistifolia* subsp. *genistifolia* (80 g) were extracted with 1 L boiling water for 10 min (Sofowora, 1999). Both the decoctions and squeezed extracts were filtered with a 2.5-µm filter (Whatman® no. 42) to remove the suspended particles and stored at 4 °C until usage. These were considered as the stock solutions and freshly prepared extracts were applied daily.

Allium cepa *anaphase-telophase test*

Prior to initiating the test, the outer scales of the bulbs and the dry bottom plate were removed without destroying the root primordia. In order to determine effective concentration (EC50), for each water sample, a series of six bulbs were placed in distilled water for 24 h and afterwards the best growing five bulbs exposed for four days (d) to the *Lg*-ext solutions (10, 20, 40, 60 and 80 g/L, respectively) at room temperature (~21±4 °C). The test concentrations were renewed at every 24 h during the experiments. On the 5th day, root lengths (lengths of ten roots from each bulb) were measured from both *Lg*-ext exposed bulbs, and control group. EC50 value was considered as the concentration which retards the growth of root 50% when compared to the control.

In the determination of application doses, 2xEC50, EC50 and 0.5x EC5, positive (MMS, 10 ppm) and negative control group were used for 12, 24, 48, 72 and 96 h. Fixation and staining of the root tip cells were carried out as reported earlier (Yıldız et al., 2009; Liman et al., 2010). The MI and the frequencies of chromosomal aberrations (CA) were carried out according to Saxena et al. (2005). For each test group, five slides (1 root tip/slide) were prepared by squashing root tips with 45% acetic acid. Slides were randomly coded and scored blindly. For MI, the different stages of mitosis were counted in a total of 5000-6000 cells (1000 cells/slide) per concentration, and expressed as a percentage. In chromosome aberration test, 100 cells in anaphase or telophase were examined for aberrations per slide if it is possible.

Ames plate incorporation test

Cytotoxic doses of *Lg*-ext (3000, 1500, 750, 375, 187.5 and 93.75 mg/plate) were determined by following the method of Dean et al. (1985).

Ames test was performed as a standard plate incorporation assay with *S. typhimurium* strains TA98 and TA100 with or without S9 (Maron & Ames, 1983). Selection of the strains was based on the testing and strain selection strategies of Mortelmans & Zeiger (2000). These strains were tested on the basis of associated genetic markers. For each tester strain, a specific positive control was always used to test the experimental flaws, if any. While 4-nitro-*O*-phenylenediamine (NPD) for TA 98 and sodium azide (SA) for TA100 were used as positive controls without S9, 2-aminofluorene (2AF) and 2-aminoanthracene (2AA) were used as positive controls with S9, respectively.

S9 mix (500 µL) (or 500 µL phosphate buffer), the test solution (100 µL) for each concentration and a cell suspension (100 µL) from an overnight culture (1-2x10^9 cells/mL) were added to 2 mL top agar (kept at 45 °C) and vortexed for 3 s. The entire mixture was overlaid on the minimal agar plate. The plates were incubated at 37 °C for 72 h and then the revertant bacterial colonies on each plate were counted. Both the positive and negative controls (distilled water) were maintained concurrently. Samples were tested on triplicate plates in two independent parallel experiments.

Statistical analysis

The data of root length, MI, mitotic phases, CA expressed as percentages, and the levels of significance in different treatment groups were analyzed statistically. For these, Duncan multiple range tests were performed by using one-way analysis of variance (ANOVA) on SPSS 15.0 version for Windows software both *A. cepa* anaphase-telophase and Ames test.

Results and Discussion

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, an increase in the usage of these plants in the developed world has been observed for a few years (Parekh et al., 2005). Therefore, knowledge of mutagenic and toxic effects of these plants become very important. Because many plants synthesize toxic substances for defense against viruses, bacteria and fungi etc. and these compounds could have potentially deleterious effects in humans. Neither phytochemical nor biological studies of *L. genistifolia* subsp. *genistifolia* have been previously reported on the topic studied.

Test systems to determine the genotoxicity and/or mutagenicity can be divided into groups based on the biological systems employed and their genetic endpoint detected. Bioassays with prokaryotes enable the detection of agents that induce gene mutation and primary DNA damages. On the other hand, analyses with eukaryotes enable the detection of a greater damage extent, varying from gene mutations to chromosome damages and aneuploidies (Houk, 1992; Leme & Marin-Morales, 2009). Using both pro- and eukaryotic test system make the results both strengthen and correlate to verify if the chemical(s) has/have really any bad effects on the genes.

Allium root growth test results are shown in Table 1. The EC50 was found to be approximately 15 g/L. It can be easily seen that the effect of *Lg*-ext in Allium root growth was dose-dependent. Root growth decreased at all concentrations tested and yielded with statistically significant results ($p<0.05$). In the meantime over 40 g/L concentration, roots became dark colored, thicker and gel like formations. The inhibition of root growth generally related to apical meristematic activity (Webster & Macleod, 1996), and to cell elongation during differentiation (Fusconi et al., 2006) and the appearance of stunted roots indicate the retardation of growth and cytotoxicity (Yıldız et al., 2009). As reported earlier, neo-clerodane diterpenoids and flavonoids were isolated from *Linaria saxatilis* var. *glutinosa* by showing cytotoxic activity in different neoplastic cell cultures (Gordaliza et al., 1997). Tundis et al. (2005) determined the antiproliferative action of several flavones isolated from *Linaria reflexa* Desf., Plantaginaceae, against the large cell lung carcinoma cell line COR-L23, hepatocellular carcinoma cell line HepG-2, renal adenocarcinoma cell line ACHN, amelanotic melanoma cell line C32 and colorectal adenocarcinoma cell line Caco-2, and reported that pectolinarigenin and some flavonoid glycosides like pectolinarin exhibited strong cytotoxic activity on COR-L23 cell line with an IC50 value of 5.03 and 4.07 μM, respectively. Akkol & Elçi (2009) suggested that the extracts of *Linaria* species (*L. grandiflora, L. genistifolia* subsp. *confertiflora* and *L. aucheri*) had analgesic and anti-inflammatory effects without toxicity.

Table 1. Results of the *Allium* root growth inhibition test.

Doses (g/L)	Average length (cm)±SD*	Growth (%)	Decrease (-) in growth (%)
Control	2.71±0.31a	100	-
10	1.86±0.18b	68.71	31.29
20	1.09±0.17c	40.26	59.74
40	0.87±0.10d	32.21	67.79
60	0.70±0.17e	25.94	74.06
80	0.51±0.12f	18.93	81.07

*Means with the same letter do not differ statistically at the level of 0.05. SD: Standard deviation.

Table 2 summarizes the effect of *Lg*-ext on MI and mitotic phase in the root meristematic cells of *A. cepa* treated for 12, 24, 48, 72 and 96 h. While at all concentrations used in the incubations of root in 7.5 g/L and 15 g/L (except 24 and 96 h) increased MI, applications of 30 g/L and MMS decreased MI compared to negative control at each exposure time. The highest values were obtained from 12 h examination of 7.5 g/L concentrations of *Lg*-ext (45.18±0.75), and the lowest one in 96 h applications of MMS (20.14±0.62). The increased and decreased MI showed statistically significant results ($p<0.05$). As stated by Fernandes et al. (2007), the cytotoxicity levels of an agent can be determined by the increase or decrease in the MI. MI lower than the negative control may indicate that the growth and development of exposed organisms have been affected by test compounds. On the other hand, MIs above those of the negative control are result of the induction of increased cell division, which may characterize an event detrimental to cells, leading to uncontrolled proliferation and even tumor formation (Hoshina et al., 2002). The increased cell proliferation activity can be the consequence of a reduction of the time necessary for DNA repair (Evseeva et al., 2003).

All concentrations of *Lg*-ext used in the experiment caused changes in the percentage of particular phases' distribution in comparison to the control. The characteristic effect caused by tested preparations was an increase of prophase index except in 12 h at 30 g/L

Table 2. The effects of *Lg*-ext on MI and mitotic phase of *A. cepa* root meristem cells.

Concentration (g/L)	Counting cell number	MI±SE*	Mitotic Phases (%)±SE*			
			Prophase	Metaphase	Anaphase	Telophase
Control- 12 h	5102	30.04±0.59a	88.92±1.31a	1.49±0.28a	3±0.68a	6.59±1.05ab
MMS-10 ppm	5255	27.08±1.56b	75.26±2.44b	4.75±0.5b	10.48±1.45b	9.51±1.27bc
7.5	5148	45.18±0.75c	91.49±1.36a	1.73±0.39a	1.4±0.23a	5.38±1.65a
15	5150	33.34±0.47d	93.29±0.54a	1.4±0.09a	1.24±0.36a	4.07±0.81a
30	5136	24.31±0.6e	84.55±0.53c	3.35±0.38c	1.58±0.41a	10.52±1.11c
Control- 24 h	5365	32.76±1.07a	87.99±1.7ab	4.72±1.48a	1.14±0.3a	6.15±0.83a
MMS-10 ppm	5136	26.67±0.63b	86.89±1.27a	3.57±0.31a	3.24±0.42b	6.3±1.08a
7.5	5257	38.29±0.99c	95.76±0.9d	0.56±0.19b	0.78±0.21a	2.9±0.8b
15	5210	28.49±0.53b	91.71±1.55bc	1.45±0.38b	0.75±0.17a	6.09±1.17a
30	5355	28.12±0.69b	93.16±0.49cd	0.4±0.09b	0.88±0.37a	5.56±0.62ab
Control- 48 h	5229	29.14±1.04a	84.43±0.67a	3.25±0.56a	4.69±0.5a	7.63±0.42a
MMS-10 ppm	5177	24.27±0.83b	86.91±0.93a	3.45±0.46a	3.86±0.36a	5.78±0.69ab
7.5	5043	32.71±0.63c	92.79±1.06b	3.52±0.95a	2.24±0.93b	1.45±0.72c
15	5183	33.96±1.03c	97.05±1.3c	1.17±0.37b	0.51±0.23c	1.27±0.86c
30	5204	25.56±1.23b	95.78±0.69bc	-	0.13±0.03c	4.09±0.73b
Control- 72 h	5288	29.37±1.52a	84.44±0.64a	3.27±0.15ab	2.98±0.56a	9.31±0.47a
MMS-10 ppm	5064	25.18±1.37b	82.79±0.49a	4.71±0.12b	3.21±0.67a	9.29±0.44a
7.5	5151	37.75±0.36c	89.4±0.61b	4.14±0.49ab	4.72±0.25b	1.74±0.22b
15	5088	33.01±0.81c	93.67±0.97c	2.55±0.58a	0.67±0.22c	3.11±0.26b
30	5066	22.54±1.19b	93.56±1.86c	2.73±1.11a	1.32±0.37c	2.39±1.06b
Control- 96 h	5142	31.58±1.04a	85.45±1.15ab	2.76±0.42a	3.01±0.46a	8.77±1.1a
MMS-10 ppm	5159	20.14±0.62b	82.92±1.62a	4.61±0.32b	3.53±0.51ab	8.92±1.16a
7.5	5168	35.45±1.38c	88.69±0.77b	2.79±0.46a	4.85±0.92b	3.65±0.66b
15	5200	26.27±0.63d	94.54±1.1c	1.03±0.34c	0.66±0.27c	3.76±0.96b
30	5170	24.82±0.85d	96.61±0.53c	-	0.24±0.16c	3.13±0.39b

*Means with the same letter do not differ statistically at the level of 0.05 in each group. SE: Standard Error.

and simultaneous decrease of anaphase index except in 72 and 96 h at 7.5 g/L and telophase index except in 12 h at 30 g/L. This might be an indication of the blockage of chfr point (control point between prophase/metaphase). Scolnic & Halazonetis (2000) reported that chrf defines a checkpoint that delays entry into metaphase in response to mitotic stress. Most of the increased and decreased phase index showed statistically significant results ($p<0.05$).

In the *A. cepa* anaphase-telophase chromosome aberration test conducted with root meristematic cells of *A. cepa* as shown in Table 3. The most frequent abnormalities were stickiness, anaphase bridges, chromosome laggards and disturbed anaphase-telophase in anaphase-telophase cells. The effect of *Lg*-ext concentration on CA was significantly different ($p<0.05$) except in 12 h at 7.5 g/L compared to the negative control. Analysis of the chromosomes showed that 7.5 g/L of *Lg*-ext concentration except in 12 and 24 h and 15 g/L of *Lg*-ext concentration in 12 and 96 h induced chromosomal aberrations but other *Lg*-ext concentrations decreased chromosomal aberrations. No aberration was recorded in the chromosome of *A. cepa* exposed to the 30 g/L of *Lg*-ext concentration in 72 and 96 h. Stickiness (especially at 7.5 g/L in 48 h) indicates highly irreversible type toxic effect of *Lg*-ext, and its occurrences during the study could be because of sub-chromatid linkage between chromosomes (Mc-Gill et al., 1974; Chauhan et al., 1986; Kovalchuk et al., 1998; Ajay & Sarbhoy, 1988). Anaphase bridges could happen during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence or due to the breakage and fusion of chromosomes and chromatids. This bridges cause structural chromosome mutations (El-Ghamery et al., 2000; Luo et al., 2004). Disturbed anaphase-telophase (especially at 15 g/L in 12 h and at 7.5 g/L in 72 h) and chromosome laggards could occur by the effect of *Lg*-ext on microtubule formations (Amer & Ali, 1986; Kumari et al., 2009). Such spindle malfunctioning may arise due to inhibition of tubulin polymerization (Kuriyama & Sakai, 1974). The occurrence of chromosome laggards at anaphase was due to the failure of the chromosomes or acentric chromosome fragments to move to either of the pole.

In addition to these anomalies, others (c-metaphase, pro-metaphase, polyploidy and binuclear

Table 3. Percentage of chromosome aberrations of *Lg*-ext in different times and concentrations obtained for the *A. cepa* anaphase-telophase test.

Concentration (g/L)	Anaphase-telophase anomalies %						Other Anomalies %					
	CCN	S	AB	CL	DAT	TA± SE*	CCN	CM	PM	P	BNC	TA± SE*
Control- 12 h	400	3.84	11.69	14.21	1.4	31.14±2.5a	5102	0.06	0.06	-	0.1	0.21±0.06a
MMS-10 ppm	500	3.6	3.8	10.6	7.6	25.6±1.28a	5255	0.17	0.4	0.04	0.02	0.63±0.12b
7.5	371	10.84	2.51	2.65	14	30±2.55a	5148	0.08	0.17	-	0.02	0.27±0.07a
15	429	2.52	2.04	10.2	26	40.76±1.48b	5150	0.07	0.06	0.02	-	0.15±0.03a
30	291	1.93	2.36	4.55	7.4	16.24±1.99c	5136	0.04	0.07	-	-	0.11±0.03a
Control- 24 h	500	10.26	4.64	17.24	14.4	46.55±0.7a	5365	0.5	0.63	-	-	1.13±0.15a
MMS-10 ppm	500	19	3	15	19.2	56.2±1.82b	5136	0.07	0.02	-	0.22	0.31±0.08b
7.5	252	8.27	0.77	-	4.94	13.97±1.11c	5257	-	0.06	-	0.05	0.11±0.05b
15	152	1.32	0.71	2.26	5.68	9.98±0.73d	5210	0.03	0.04	-	-	0.07±0.03b
30	137	1.82	0.42	0.8	5.77	8.82±0.86d	5355	-	0.03	-	0.04	0.07±0.04b
Control- 48 h	500	10.99	5.7	19.59	9.99	46.25±1.46a	5229	0.08	0.03	-	-	0.11±0.04a
MMS-10 ppm	500	7.82	8.65	13.49	26.27	56.22±1.61b	5177	0.2	0.02	-	0.32	0.54±0.11a
7.5	389	15.78	14.83	12.2	19.19	62.01±3.31b	5043	0.1	0.6	0.02	-	0.72±0.46a
15	289	0.4	1.6	1	2.4	5.4±0.97c	5183	0.12	0.32	0.02	-	0.46±0.16a
30	196	0.38	-	-	1.82	2.20±1.76c	5204	-	-	-	-	-
Control- 72 h	500	14.6	4	10.6	10.2	39.4±3.14a	5288	0.09	-	-	-	0.09±0.05a
MMS-10 ppm	500	11.69	5.55	7.42	19.16	43.82±1.22b	5064	0.14	-	0.01	-	0.15±0.03a
7.5	352	7.91	2.82	5.87	36.28	59.87±2.94c	5151	0.23	0.41	0.04	-	0.68±0.1b
15	301	3.47	11.13	4.23	9.75	28.58±1.84d	5088	-	0.49	-	-	0.48±0.08b
30	-	-	-	-	-	-	5066	0.08	0.01	-	-	0.09±0.02a
Control- 96 h	500	3.82	2.02	6.63	6.67	19.13±2.37a	5142	0.1	0.03	-	-	0.13±0.03a
MMS-10 ppm	500	22.2	1.4	5.4	20.2	49.2±2.26b	5159	0.08	0.01	-	-	0.09±0.02a
7.5	170	2.68	10.09	1.03	17.1	30.9±4.07c	5168	0.1	0.21	0.02	0.02	0.35±0.08b
15	144	4.16	3.54	-	19.48	27.18±0.98c	5200	-	-	-	0.04	0.04±0.02a
30	-	-	-	-	-	-	5170	-	-	-	-	-

*Means with the same letter do not differ statistically at the level of 0.05 in each group. SE: Standard Error, CCN: Counting Cell Numbers, S: Stickiness, AB: Anaphase Bridge, CL: Chromosome Laggards, DAT: Disturbed Anaphase-Telophase, TA: Total Anomalies, PM: Pro-metaphase, CM: c-metaphase, P: Polyploidy, BNC: Binuclear cell.

cell) were also observed. While the lowest anomalies were observed 0.04±0.02% at the 15 in 12 h, the highest ones were observed 0.72±0.46% at 7.5 g/L in 48 h. Statistically significant (p<0.05) frequencies of other anomalies were recorded in 12 h at 7.5 g/L, all 24 h applications, in 72 h at 15 and 30 g/L, and also in 96 h at 7.5 g/L. It was not observed any anomalies in 48 and 96 h at 30 g/L. C-metaphase, a possibly reversible effect, might occur due to disturbed microtubules by *Lg*-ext and could be induced aneuploidies (Fiskesjö, 1988; Shahin & El-Amodi, 1991; Odeigah et al., 1997). Polyploidy cells can occur due to the lack of fragmoplast that prevents the formation of the daughter cells (Vidakovivé-Cifrek et al., 2002; Fernandes et al., 2007). Binuclear cells are accepted as the inhibition of cytokinesis in any control points of the cellular cycle (Ateeq et al., 2002).

The results from the Ames test are shown in Table 4. The interpretation of the Ames test results for genotoxicity testing of chemicals United States Environmental Protection Agency (USEPA, 1996) methods were followed. According to the guideline, a mutagenic potential is assumed, if the revertant frequency is 2.0 or higher over the solvent control or dose-related increase in the number of revertant colonies in one or more strains (Mortelmans & Zeiger, 2000). Noncytotoxic doses of the *Lg*-ext tested were determined firstly. The results obtained suggested that all tested concentrations were not determined as cytotoxic. The average revertant colony numbers in negative control were 34.8±1.48 for TA98 and 147.6±12.05 for TA100 with S9 and 32±3.31 and 105±7.21 without S9 respectively. Spontaneous revertants were within the normal values for the four strains examined. While application of S9 in TA98 was decreased revertant colony numbers, application of S9 in TA100 was increased revertant colony numbers. On the contrary, the plates with the positive control mutagens

(SA, 2AF, 2AA and NPD) showed significant increases relative to the spontaneous mutation rate in the two tested strains. While the highest value observed was in the TA100 with S9 at 750 µg/plate concentration of Lg-ext (216.4±11.78), and the lowest value was in the TA98 with S9 at 750 µg/plate concentration of *Lg*-ext (25.6±1.14). Most of the results, increasing or decreasing relative to negative control group, were not statistically significant at $p<0.05$ (Duncan test) in examined strains. In order to establish a dose-response relationship, 5 different concentrations of *Lg*-ext were tested, and no induced revertants were observed along the dose range tested in either with or without S9 with two strains. The results of the present study showed that all applications of *Lg*-ext were not found to be mutagenic *S. typhimurium* TA98 and TA100 with or without S9.

In conclusion, our results indicate that *Lg*-ext was found cytotoxic and genotoxic in *Allium* test but not found mutagenic in Ames test. Although some *Linaria* species have been used in folk remedies for various purposes, they should be used with caution, always following the traditional methods of preparation exactly. Further studies are necessary to determine the active ingredient or compound(s) of *Linaria genistifolia* subsp. *genistifolia* and should be tested in other biological test systems to obtain definitive conclusions on their cytotoxic, genotoxic and mutagenic potential.

Acknowledgements

The authors wish to thank Afyon Kocatepe University Scientific Research Committee for supporting this study financially (Project No: 11.FEN.BIL.16) and Dr. M. Temel for the identification of the plant.

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Table 4. Mutagenicity of *Lg*-ext towards *S. typhimurium* TA98 and TA100 strain with or without S9.

Agent	Amount (µg/plate)	No of His^+ Revertants/plate Mean±SD*			
		TA98		TA100	
		- S9	+ S9	- S9	+ S9
Lg-ext	3000	30.2±3.42a	28.6±1.81a	110.6±8.56a	214±10.41a
	1500	31±3.67a	28±1.58a	111.4±4.33a	184±7.44a
	750	30±2.73a	25.6±1.14a	105,2±2.38a	216,4±11.78a
	375	31.6±1.81a	27.2±0.83a	109±8.09a	177,8±6.3a
	187.5	29.4±3.2a	27±1.58a	108±6.72a	183,6±21a
	93.75	31.2±2.38a	26.2±3.56a	102.2±4.43a	112,4±10.6a
Control		32±3.31a	34.8±1.48a	105±7.21a	147.6±12.05a
SA	10			1646.2±112.34b	
2AA	5				2279.2±108.72b
2AF	200		885.6±81.15b		
NPD	200	1380.4±114.61b			

*Means with the same letter do not differ statistically at the level of 0.05. SD Standard deviation, SA: Sodium azide, 2AA: 2-aminoanthracene, 2AF: 2-aminofluorene, NPD: 4-nitro-*O*-phenylenediamine.

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