



## Assessment of antiproliferative and genotoxicity effects of traditional medicinal plants *Plantago ovata* Forssk. and *Linum usitatissimum* L. by *Allium cepa* L. test root cells combining with Comet assay

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This study assessed the antiproliferative and genotoxic effects of *Linum usitatissimum* L. and *Plantago ovata* Forssk. plants used in Turkish traditional medicine for medicinal purposes. *P. ovata* and *L. usitatissimum*, which have a purgative effect, have been used extensively in recent years. The *P. ovata* plant has a large quantity of soluble fibre in the seed coat, while the seeds of the *L. usitatissimum* also have a large quantity of mucilage from the soluble fibres. In the present study, the genotoxic and antiproliferative effects of these species were examined using the *Allium cepa* L. test. As an experimental group, three different concentrations (for *P. ovata*-7.2 mg/mL, 14.4 mg/mL and 28.8 mg/mL; for *L. usitatissimum*- 20 mg/mL, 40 mg/mL and 80 mg/mL) were used (two different concentration series were used, taking into consideration the traditional usage quantities); purified water was used as a negative control, and methyl methane sulfonate (the DNA-alkylating agent MMS, 0.01 mg/mL) was used as a positive control. To evaluate the mitotic index (MI) and chromosome aberrations type (CA) of the *A. cepa* root-tip meristem cells, approximately 1000 cells per concentration group, including the controls, were numbered. For both extractions, it was observed that the root length decreased from the negative control to other concentrations, with the lowest length in the positive control. *P. ovata* and *L. usitatissimum* were found to cause alters in the rates of the MI relative to the negative control in *A. cepa* due to the increased concentration. The comet assay distinctly indicated that concentration-dependent DNA damage in the root-tip meristem cells of *A. cepa* were detected for the different concentrations of plant extracts. These results showed that *P. ovata* and *L. usitatissimum* aqueous extracts had increased concentrations that were above the recommended antiproliferative and genotoxic effects.

**Keywords:** Antiproliferative, Genotoxicity, Medicinal plants, Mitotic index, Traditional use

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One of the earliest records of the use of medicinal plants was written in 2600 BCE in Mesopotamia. All of the plants in these cuneiform inscriptions are now used for many ailments, including cold, cough, parasitic infections and inflammatory disorders. Another record of the use of medicinal plants dates to a similar time in Egypt<sup>1</sup>. The first date of collection and cultivation of these medicinal plants is unknown, but their use in humans probably resulted from the emergence of specific health problems within these societies. Today, such plants are often the raw material for the production of medicines<sup>2</sup>. Around 21,000 plants are used for therapeutic purposes worldwide, and developed countries, in particular, are especially oriented towards using botanical resources for treatment. A significant proportion of the

medicines used for treatment originate in natural sources<sup>3</sup>, and approximately 80% of the world's people rely on conventional medicinal plants for health-related issues<sup>4</sup>. In Turkey, approximately 500 out of 9000 plant species are used for therapeutic purposes. Most Anatolian people living in countryside areas (far from the city) traditionally employ plants for their nutritional and medicinal qualities<sup>5,6</sup>. However, excessive or prolonged use of medicinal plants may cause poisoning, and health problems may sometimes arise as a result of consuming plants that are not known to be toxic<sup>7</sup>. This kind of unaware or unconscious use of plants can lead to public health problems. Although plants are noted dependable because they are natural, natural products are not always reliable<sup>4</sup> or safe and some plant species are highly toxic to humans. It is, however, possible to determine the effect of a plant by using short-term

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genotoxicity tests<sup>8</sup>. The *A. cepa* test has been used by many research groups, primarily as a tool to detect a great variety of environmental pollutants<sup>9</sup>. But it can also be used to assess the genotoxic implications of conventional herbal medicines<sup>10</sup> because the *A. cepa* root test uses a design that is responsive enough to define the innumerable substances that bring about chromosomal aberrations. The test is generally susceptible, reliable and can detect clastogens, carcinogens and mutagens<sup>11</sup>.

*Plantago* and *Linum* species are used in conventional medicine for the remedial treatment of ailment such as wound healing, gastric ulcer, anti-inflammatory, abscess, wound healing, diarrhea and gastrointestinal infections<sup>12</sup>. One of these species is *P. ovata*. It is an economically momentous medicinal plant of the Plantaginaceae. Its seed husk is produced widely for its use in the several areas such as cosmetic, food and pharmaceutical industries<sup>13</sup>. In particular, the seeds, which are also commonly known as psyllium and ispaghula, are extensively used as purgatives in powdered or granular preparations. In the Australia, USA and Canada, *P. ovata* seeds are also added to breakfast cereals supplement to enhance the intake of dietary grains and decrease serum cholesterol levels<sup>14</sup>. When the disorders caused by the use of this plant were first examined, an allergic reaction caused by *P. ovate* seeds was noted early as 1941<sup>15</sup>. Since then, many allergies (rhinitis, asthma) have been reported, as well as anaphylaxis and asymptomatic eosinophilia<sup>16</sup>. *L. usitatissimum* belongs to the family Linaceae and has a wide worldwide reputation for its various medical uses, as well as being employed in the production of textiles, food and chemical and pharmaceutical products<sup>17</sup>. In addition, the linseed meal that remains after oil extraction contains anti-nutritional factors, mainly CG (cyanogenic glycosides), which are an inseparable component of flax components<sup>18</sup>. The uptake of these compounds can form a complex with ferric ions of mCO (mitochondrial cytochrome oxidase), causing acute or chronic poisoning, resulting in abnormal breathing and irritability<sup>19</sup>. The fact that these plants also had negative effects and the absence of any studies involving the antiproliferative and genotoxic efficacies of these purgative plants in *A. cepa* test, demonstrates the necessity of the current research. The main purpose of the present research is to investigate the sensitivity of the *A. cepa* test as a warning system for evaluating *P. ovata* and

*L. usitatissimum*. The antiproliferative and genotoxic effects of these species were investigated using *A. cepa* root-meristem cells.

## Materials and Methods

### Plant materials and extractions

Seeds of *P. ovata* and *L. usitatissimum* were purchased from a herbal market in Batman. The seeds were first washed with distilled water. They were then dried for 3 h at 50°C. The seeds were allowed to stand for 5 min in 250 mL boiling water at the determined concentrations and then filtered<sup>20</sup>. In the experiments, three different concentrations were determined for each plant: for *P. ovata* Forssk., 7.2 mg/mL, 14.4 mg/mL and 28.8 mg/mL and for *L. usitatissimum* L., 20 mg/mL, 40 mg/mL and 80 mg/mL (Taking into consideration the traditional usage quantities, assay solutions of *P. ovata* and *L. usitatissimum* were prepared at two various concentration series and concentrated two and four times more).

### *Allium cepa* test

Equal-sized onion bulbs were taken as the assay plant and they were obtained locally (Batman, Turkey). The onion bulbs were transferred over the assay tubes stuffed with distilled water at RT (room temperature; 20±2°C)<sup>21</sup>. After two days, the roots-tips from these bulbs were cut and they were treated with previously prepared extractions and were exposed for 24 h. Distilled water was used as the NC (Negative control) group and MMS was used as the PC (positive control) group. Three bulbs were used for every concentration series, including the control groups in the experiment. Root-tips of the bulbs were cut after the experiment and directly transferred to a dark medium overnight in Farmer's fixative (3:1, EtOH and glacial acetic acid) at 4°C<sup>22</sup>, which had been newly arranged before use. The bulbs root-tips were washed with distilled water and then stored in tubes containing 70% alcohol until the experimental stage. In the preparation stage, the root-tips were washed with distilled water and hydrolyzed (1N HCl at 60°C for 10 min), as defined by Souguir *et al.*, (2008)<sup>23</sup>. The root cap cells was moved with a razor blade before crushing the root tissues, and exemplary were stained with aceto-carmin [2% (w/v)] for 24 h. To evaluate the MI and aberrations of the *A. cepa* root-tip meristem cells, approximately 1000 cells per group were counted, including the controls. The slides were assessed by using light microscopy to observe the cells in the four phases of cell division and any

chromosomal abnormalities. Mitotic stages are seen cytologically in meristematic cells. The mitotic activity (index) can be calculated with the data obtained as a result of the counting process.

MI (%) = Number of Dividing Cells / Total Number of Acquired Cells × 100.

As given in the formula above, mitotic activity expresses the ratio between dividing cells and non-dividing meristematic cells. Mitotic index rate is determined in order to determine the level of effect of a substance acting on the cell on mitotic division.

#### Comet assay

This experiment method is a visual fluorescent technique used in many studies. The Comet technique was used by making some modifications to the study by Tice *et al.*, (2000)<sup>24</sup>. *Allium* bulbs treated with various concentrations of plants extracts (POE; *P. ovata* extract and LUE; *L. usitatissimum* extract) were used for the comet assays. The roots were transferred in a glass petri dish containing 450 µL of precooled Tris-buffer, pH 7.5. The onion roots were quickly cut and separated root nuclei were gathered in the buffer. Every microscope slide was covered prior to use in the assay with 1% NMPA (Normal melting point agarose) layer and properly dried at RT (Room temperature). These slides can be stored in moist boxes until 2<sup>nd</sup> and 3<sup>rd</sup> agarose layers are poured<sup>25</sup>. After that, 30 µL of 1% LMPA (Low melting point agarose) at 37°C was mixed together with 30 µL of the nuclear suspension and added onto the first layer to each slide. The gel was kept in the refrigerator for a while to solidify. Finally, the preparation of the slides was completed by preparing 0.5% LMPA (80 µL) in phosphate-mixed saline and placing it as a thin layer on the second layer. The slide was kept in the refrigerator for a while to solidify. Prior to execution in electrophoresis, the slides were gently moved in a gel electrophoresis tank and left to separate the DNA strands by incubation for 20-30 min in electrophoretic buffer solutions (30 mM NaOH and 1 mM EDTA at pH 13). After the incubation is completed in the alkaline electrophoresis buffer, the DNAs were put into in this buffer solution in an electrical field of 300 mA 20 V (by keeping the tank in ice) for 30 min. In order to prevent additional DNA damage, all these steps were carried out in an environment where the light was as low as possible. After completing the process of electrophoresis, the slides are washed for 3 min and 3 times with 5 mL/slide (0.4 M Tris HCl buffer pH 7.5) cold neutralization buffer to remove

the alkaline buffer solution from the slides. After neutralization is complete, the slides were fluorescent dyed and DNA was stained using 60 µL of EtBr solution (Ethidium bromide 20 µg/mL)<sup>26</sup>. 100 comets/slides indiscriminately selected nuclei were analyzed using a fluorescence microscope. DNA images were classified in various sub-categories according to the degree of damage caused and scored. Unimpairment → 0, impairment DNAs were scored from 1 to 4 (1; low impairment, 2; medium impairment, 3; elevated impairment, 4; significant impairment) according to the degree of DNA impairment and the results were determined as an AU (Arbitrary unit). Ni = number of cells in i degree, i = degree of impairment (0, 1, 2, 3, 4).

$$\text{Arbitrary Unit (AU)} = \sum_{i=0}^4 Nixi$$

#### Statistical analysis

In each assay, all samples data registered was from triplicate ( $n = 3$ ) and all the results are summarized as mean ± SD. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to show the alterations among the parameters means.  $p < 0.05$  is accepted statistically significant. All analysis were done using SPSS version 15.0.

#### Results

The study investigated the cytotoxic effects of *P. ovata* and *L. usitatissimum* in *A. cepa* root-tip cells. The efficacy of different quantity of the application of plant extracts on root length is shown in Table 1. As it can be shown in Table 1, when *P. ovata* extracts were applied to the *A. cepa* roots, the highest root length was acquired from the control group (2.6 ± 0.05), while the lowest root length was acquired at 28.8 mg/mL (1.5 ± 0.02) concentration. In

Table 1 — Efficacy of aqueous extract of *P. ovata* on root lengths of *A. cepa*

Plant sample	Concentration (mg/mL)	Mean root length ± SD (cm)
	Negative control	2.6 (±0.05) <sup>a</sup>
POE	7.2	2.4 (±0.03) <sup>b</sup>
	14.4	2.3 (±0.04) <sup>b</sup>
	28.8	1.5 (±0.02) <sup>c</sup>
	Positive control (MMS) 0.01	0.6 (±0.02) <sup>d</sup>

\*The difference between values represented by the same letter in each parameter column is insignificant ( $p < 0.05$ )

\*\*a,b,c,d The differences between group averages containing different letters in the same column are important

the positive control (MMS), this value decreased to 0.6±0.02 cm. The control was compared with the application groups using SPSS 15.0 software, and no statistically significant change was found between the 7.2 mg/mL and 14.4 mg/mL concentration group, while a significant change was found between other concentration groups. In Table 2, when the effect of *L. usitatissimum* extract on onion root length was examined, the highest root length was acquired in the control group (3.3±0.05), while the lowest root length was acquired at 80 mg/mL (1.8±0.01) concentration. In the positive control (MMS), this value decreased to 0.9±0.02 cm. There was statistically significant dependence between all application rates. The statistical evaluation of the results showed that these plant extracts caused a decrease in root length. This reduction in root length is thought to result from damaged cell formation, because some of the structural and numerical changes occurring in chromosomes can lead to the death of the cells that cause the roots to grow, and the roots do not continue to extend until the end of the experiment and can not therefore be evaluated.

Table 2 — Efficacy of aqueous extract of *L. usitatissimum* on root lengths of *A. cepa*

Plant sample	Concentration (mg/mL)	Mean root length ± SD (cm)
LUE	Negative control	3.3 (±0.05) <sup>a</sup>
	20	2.8 (±0.03) <sup>b</sup>
	40	2.1 (±0.02) <sup>c</sup>
	80	1.8 (±0.01) <sup>d</sup>
	Positive control (MMS) 0.01	0.9 (±0.02) <sup>e</sup>

\*The difference between values represented by the same letter in each parameter column is insignificant (p<0.05)

\*\*a,b,c,d,e The differences between group averages containing different letters in the same column are important

Cytogenetic analysis is shown as one of the most efficacious methods used to determine species-specific effects at the DNA level. Occurring as a result of mutagenesis, it offers researchers the opportunity to examine cytogenetic changes. The mitotic index is a cytogenetic parameter that helps measure the proliferation (M phase) of mitotic cells in the cell cycle and inhibition of the MI is considered cell death<sup>27</sup>. In the literature review, no studies on the efficacy of these plants on MI have been found in plant test systems. The aberrations detected in the mitotic phases of the root meristem cells of the germinated *A. cepa* plant at various application rates of *P. ovata* and *L. usitatissimum* plants are given in Table 3 and 4, are compared to the control group. The statistical data obtained by comparing the values in the groups show the differences among the parameters, and revealed that the difference between mitotic index values was important. Accordingly, as a result of the application of plant extracts of different doses to *A. cepa* roots, it was determined that the MI diminished as the concentration increased. Table 3 shows the MI, type and number of mitotic aberrations in the root-tips of *A. Cepa* revealed to *P. ovata* extracts. A statistically significant meaningful change was detected between the concentrations of *P. ovata* extracts. While the MI value was monitored to be highest in the negative control (8.74±0.05), this value reached a lower level at 28.8 mg/mL concentration (3.89±0.02). In the positive control, the MI value was determined to be 2.63±0.01. However, in Table 4, no statistically significant difference between the concentrations of *L. usitatissimum* extract at 20 mg/mL and 40 mg/mL, there was a significant difference between the other concentrations. In the positive control, the MI value was determined as

Table 3 — MI, type and number of mitotic abnormalities in the root-tips of *A. cepa* revealed to *P. ovata* aqueous extracts

Plant sample	Concentration (mg/mL)	P	M	A	T	PSM	PSA	PST	F	C-M	S	AB	LC	TCN	NDC	MI (± SD)
POE	Negative control	35	18	19	6	3	1	1	3	6	2	1	5	1030	89	8.74(±0.05) <sup>a</sup>
	7.2	20	19	17	8	1	2	1	2	7	2	1	5	1160	75	6.46(±0.04) <sup>b</sup>
	14.4	13	10	17	3	5	2	1	--	7	2	1	9	1023	55	5.36(±0.02) <sup>c</sup>
	28.8	32	3	--	--	--	1	--	3	8	3	1	1	1132	45	3.89(±0.02) <sup>d</sup>
	Positive control (MMS) 0.01	9	3	--	--	--	--	1	1	6	3	--	2	1105	29	2.63(±0.01) <sup>e</sup>

\*P- Prophase; M- Metaphase; A-Anaphase; T- Telophase; PSM- Polar shifting in metaphase; PSA- Polar shifting in anaphase; PST- Polar shifting in telophase; F- Fragment; C-M- C-mitosis; S- Stickiness; AB- Anaphase Bridges; LC- Laggard chromosome; TCN- Total cell number; NDC- Number of divided cells; TCA- Total chromosome aberration; MI- Mitotic index; SD- Standart Deviation; not determined

\*\*a,b,c,d,e The differences between group averages containing different letters in the same column are important

\*\*\*The difference between values represented by the same letter in each parameter column is insignificant (p<0.05)

3.87±0.02. It was clearly seen from the results obtained that the MI significantly reduced in all treatments compared to the controls. It has been found that the dose of 28.8 mg / mL for *P. ovata* and 80 mg/mL for *L. usitatissimum* causes a genotoxic effect that causes changes in chromosome structure and function by creating a mitodepressive effect on onion root-tip meristematic cells. Eight types of aberration were identified due to the decrease in mitotic index. These were PSA, PSM, PST, fragment, c-mitosis, stickiness, bridges and laggard chromosome. C-mitosis was the most common abnormality. C-mitosis occurs due to the effect of some environmental factors on spindle threads and, accordingly, the occurrence of polyploidy and aneuploidy. As seen in Table 3 and 4, *A. cepa* root meristem cells presented a sensitive and efficient response to the tests applied after exposure to the DNA-alkylating agent Methyl Methane Sulfonate (MMS). According to the data obtained, it was determined that both plants caused alterations in the

MI and chromosome aberration rates in *A. cepa* check against the negative control, depending on the application rate.

The substances given to the plant for growth, development or toxicity tests are perceived as stress in the plant in the first place and this effect is suppressed by DNA synthesis or inhibition of mitosis in the form of mitodepressive effects<sup>28</sup>. This study is the first to determine the clastogenic effects on the onion root-tip meristems of these plants, which are frequently used by humans. The genotoxic efficacy of aqueous extracts of *P. ovata* and *L. usitatissimum* on *A. cepa* roots were determined on the basis of chromosomal abnormalities. Abnormalities were observed in almost every concentration; in particular, increasing the concentration induced the formation of chromosomal abnormalities. Among the observed chromosome abnormalities were polar shifting in metaphase, PSA, PST, fragment, C-mitosis, stickiness, bridges, and laggard chromosome (Fig. 1).

Table 4 — MI, type and number of mitotic abnormalities in the root-tips of *A. cepa* revealed to *L. usitatissimum* aqueous extracts

Plant sample	Concentration (mg/mL)	P	M	A	T	PSM	PSA	PST	F	C-M	S	AB	LC	TCN	NDC	MI (± SD)
LUE	Negative control	39	19	16	6	3	2	1	2	8	1	--	5	1025	85	8.26(±0.05) <sup>a</sup>
	20	25	17	18	8	2	3	1	1	8	1	1	4	1152	70	6.05(±0.04) <sup>b</sup>
	40	13	15	18	2	2	2	--	--	8	1	1	8	1022	61	5.93(±0.03) <sup>b</sup>
	80	10	3	1	--	1	1	--	3	6	3	--	1	1135	50	4.43(±0.03) <sup>c</sup>
	Positive control (MMS) 0.01	8	2	--	--	1	1	1	--	7	3	--	1	1110	43	3.87(±0.02) <sup>d</sup>

\*P- Prophase; M- Metaphase; A-Anaphase; T- Telophase; PSM- Polar shifting in metaphase; PSA-Polar shifting in anaphase; PST- Polar shifting in telophase; F- Fragment; C-M- C-mitosis; S- Stickiness; AB- Anaphase Bridges; LC- Laggard chromosome; TCN- Total cell number; NDC- Number of divided cells; TCA-Total chromosome aberration; MI- Mitotic index; SD- Standart Deviation,- not determined

\*\* a,b,c,d The differences between group averages containing different letters in the same column are important

\*\*\* The difference between values represented by the same letter in each parameter column is insignificant (p<0.05)

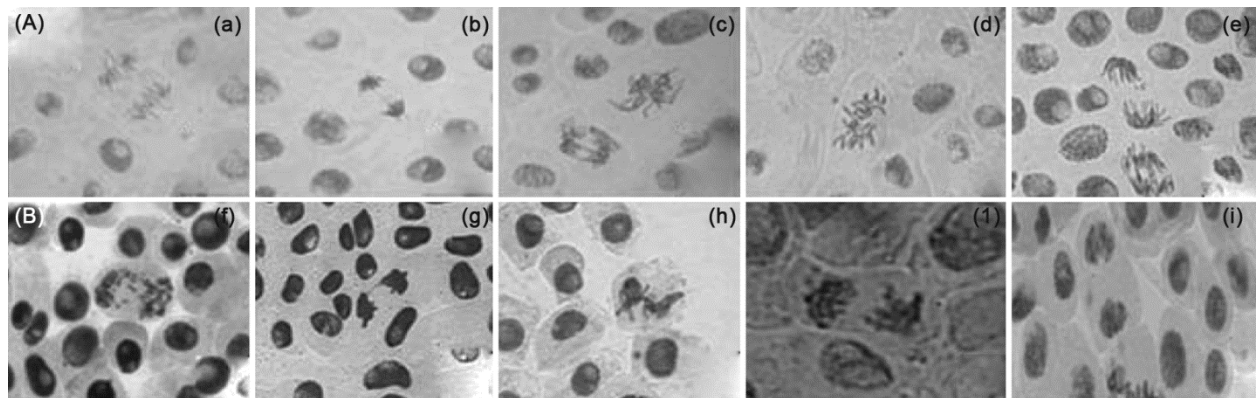


Fig. 1 — Chromosome aberration type in the root-tips of *A. cepa* revealed to *P. ovata* and *L. usitatissimum* extracts. A-series) Effects of *P. ovata* extracts a) Laggard chromosome b) Stickiness c) Anaphase bridge d) C-mitosis e) Polar shifting in telophase; B-series) Effects of *L. usitatissimum* extracts f) Fragment g) Anaphase bridge h) Stickiness i) Polar shifting in metaphase j) Polar shifting in telophase

Table 5 — Determination of DNA impairment of *A. cepa* revealed to aqueous *P. ovata* extract to using the Comet assay

Plant sample	Treatment (mg/mL)	DNA damage scores (mean $\pm$ SD)
POE	Negative control	95 ( $\pm 4.00$ ) <sup>a</sup>
	7.2	151 ( $\pm 1.25$ ) <sup>b</sup>
	14.4	165 ( $\pm 2.56$ ) <sup>b</sup>
	28.8	200 ( $\pm 5.01$ ) <sup>c</sup>
	Positive control (MMS) 0.01	223 ( $\pm 12.25$ ) <sup>d</sup>

\*  $p < 0.05$ , level of significance of mitotic index compared with the untreated control

\*\* a,b,c,d The differences between group averages containing different letters in the same column are important

Table 6 — Determination of DNA impairment of *A. cepa* revealed to aqueous *L. usitatissimum* extract to using the Comet assay

Plant sample	Treatment (mg/mL)	DNA damage scores (mean $\pm$ SD)
LUE	Negative control	94 ( $\pm 5.00$ ) <sup>a</sup>
	20	141 ( $\pm 1.05$ ) <sup>b</sup>
	40	163 ( $\pm 2.09$ ) <sup>b</sup>
	80	185 ( $\pm 6.08$ ) <sup>c</sup>
	Positive control (MMS) 0.01	230 ( $\pm 13.02$ ) <sup>d</sup>

\*  $p < 0.05$ , level of significance of mitotic index compared with the untreated control

\*\* a,b,c,d The differences between group averages containing different letters in the same column are important

Table 5 and 6 show the data of the measured comet parameters. In the current research, DNA impairment values for the negative control at POE and LUE were 95 and 94 AU. The elevated genotoxic activity was positive control at POE and LUE found as 223 AU and 230 AU, respectively. Generally, DNA damage appears to increase with each increasing concentration. In comparisons to the negative control groups (the revealed of *Plantago* and *Linum* extracts) significantly stimulated DNA impairment to the root of *Allium* bulbs in each concentration ( $p < 0.05$ ). The result acquired indicate no significant difference in the genotoxic activity between 7.2 and 14.4 mg/mL for *Plantago* extract; 20 and 40 mg/mL for *Linum* extracts treatment groups. In Table 5, it was clearly seen that the amount of DNA damage significantly increased in 28.8 mg/mL *Plantago* extracts compared to other *Plantago* extract concentration. *Plantago* extract (28.8 mg/mL) and the positive control has parallel efficacy genotoxic activity on the *A. cepa* root cells. Likewise, Table 6 shows, the concentration of DNA impairment increased in *Linum* extracts was determined as 80 mg/mL.

## Discussion

The potential antiproliferative and genotoxic efficacies of aqueous extracts of *P. ovata* and *L. usitatissimum* on *A. cepa* were assessed. As it was used in previous studies, tap water was used because it gave good results. The result of our study show that extract concentrations with the lowest root length were detected as 28.8 mg/mL for POE and 80 mg/mL for LUE. (Table 1 and 2). With the statistical evaluation of the results, it was noticed that these extracts caused a decrease in the number of roots depend on the application rate. The statistical evaluation of the results showed that these plant extracts caused a decrease in root length. In the *A. cepa* test, when there is inhibition of root growth, there is usually a decrease in the quantity of dividing cells<sup>29,30</sup>. This reduction in root length is thought to result from damaged cell formation, because some of the structural and numerical changes occurring in chromosomes can lead to the death of the cells that cause the roots to grow, and the roots do not continue to extend until the end of the experiment and can not therefore be evaluated. Similar results have also been reported by Akinboro and Bakare (2007)<sup>28</sup> in their study on some medicinal plants (*Morinda lucida* Benth. *A. indica* A. Juss *C. citratus* DC. Stapf., *C. papaya* Linn. and *M. indica* Linn.) and they found that there was a significant inhibition of root growth compared to the control, and that this was linked on the concentration of the extracts. They reported that inhibition of root growth in onion may be due to the entity of some heavy metals [different rates of Cu (Copper), Zn (Zinc), Mn (Manganese), Cd (Cadmium), Fe (Iron) and Pb (Lead)] in the extracts. These metals have been found to play a role in plants such as cucumber, lettuce and millet and inhibition of root growth in *A. cepa*<sup>31,32</sup>. Soykan and Koca (2014)<sup>33</sup> have determined that when the root lengths of the implementation groups were compared with the control groups, the length generally decreased depending on the dose and duration.

In this study, it was clearly seen from the results obtained that the MI significantly reduced in all treatments compared with the controls. However, it has been observed that POE comparatively led to a reduction in the MI. Accordingly, various aberrations have occurred in the chromosome structure. (Table 3 and 4, Fig. 1). It is stated by Sudhakar *et al.*, (2001)<sup>34</sup> that one of the reasons for the reduce in the MI may be the blockage of DNA synthesis in the S-phase. It is

estimated that these plants with laxative effects slow down mitotic division when taken at higher than recommended concentrations. In the present study, the most commonly observed aberrations were polar shifting, C-mitosis and laggard chromosome. The antiproliferative and genotoxic efficacy of aq (aqueous) extracts of five medicinal plants were explored and it was found that these extracts had a mitodepressive efficacy on cell division; they also caused mitotic spindle disorder<sup>28</sup>. This suggests that substances such as alkaloids, tannins, saponins, and anthraquinones contained in the extracts of the plants studied may cause chromosomal abnormalities<sup>35</sup>. In parallel with our results, it has been reported that some medicinal plants cause various chromosomal aberration in mitotic cell divisions and decrease the periodicity of mitotic cell division depending on the treatment concentrations<sup>27,28,30</sup>. When this study is analyzed together with previous studies on this subject, the decrease in mitotic activity may be due to disruption of spindle threads and inhibition of DNA synthesis. Water extracts of plants caused many mitotic deviations, especially C-mitosis. C-mitosis occurs due to the effect of various factors (pesticides, herbal extracts, physical or chemical agents etc.) on spindle threads, and accordingly, the formation of polyploidy and aneuploidy<sup>36</sup>. In addition, in the current study stickiness, bridges and fragments also occurred (Fig. 1). Stickiness occurs in the metaphase plane of the chromatids in the metaphase through clustering; they are defined as not being separated from each other. Some investigators recommend that increased stickiness is due to chromosomal proteins being affected<sup>37</sup>. Stickiness is considered as a chromatid type aberration<sup>37</sup>, while bridges and fragments on chromosomes are considered as structural changes<sup>38</sup>, which suggests that some of the clastogenic agents do not directly affect DNA, but only indirectly due to chromosome stickiness. The fact that the water extracts of the plants produced a small amount of stickiness on the chromosomes shows that they could be clastogenic agents. However, Teixeira *et al.*, (2003)<sup>39</sup> studies in which two medicinal plant extracts prepared two different concentrations showed that in the higher *P. guajava* L. infusion concentration group, it caused a statistically meaningful level of inhibition of cellular division in onion root-tips, while no changes were acquired in onion root tips cured with *A. millefolium* L. The current study is very similar in terms of the way the medicinal plant concentrations used in the

study of Teixeira *et al.*, (2003)<sup>39</sup> were applied and the results obtained.

Comet test was performed to measure DNA damage in cells acquired from root cells of *A. cepa* where POE and LUE extracts were applied (Table 5 and 6). In the present study, it is clear from the comet assay that both extracts have a mutagenic effect in increasing doses of *Allium* root meristem cells. Moreover, DNA damage statistically significant for POE and LUE extracts at two higher concentrations (28.8 mg/mL and 80 mg/mL) when compared to negative control. Genotoxic activity of plant extracts, human lymphocytes<sup>40</sup>, onion root-tip and rat bone-marrow cells<sup>41</sup>, C2C12 cell culture<sup>42</sup> comet assay in *A. cepa*<sup>43</sup> has been studied and successfully presented in many biological systems. In support of the presented study, Cigerci *et al.*, (2016)<sup>43</sup> reported that *Thermopsis turcica* extracts have an anti-proliferative effect and concentration-dependent single chain DNA breaks in onion root cells are detected by comet analysis for treatment concentrations.

### Conclusion

Medical aromatic plants have been used as drug since the beginning of recorded human history to avoid and treat diseases and protect good health. However, lack of awareness and extensive use can reversely influence human health. Although plants are often imagined to be dependable since they are natural, nature does not always provide safe products. As is widely known, what distinguishes a medicine from a poison is often only its dosage. According to the study findings show that the negative effects of these plants, which are widely used by people in Turkey for purgative objectives, especially in rural areas, increase as the dose taken increases.

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### Conflict of Interest

The author declares that there is no conflict of interest.

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