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ANTIMITOTIC AND GENOTOXIC EFFECT OF METHANOLIC EXTRACTS OF LEAVES OF *Peganum harmala* L. ON THE MERISTEMATIC CELLS OF *Allium cepa* L.

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ABSTRACT

Medicinal plants are an inexhaustible source of molecules with several biological activities. The main objective of this research was the evaluation of the antimitotic and genotoxic effect by the *Allium cepa* test of the methanolic extract of the leaves of *Peganum harmala*, a spontaneous plant from the arid zones of Algeria (Timzerth, Laghouat). Different concentrations (1, 4, 10, and 16 mg·mL^{-1}) of the extract, as well as two standards (colchicine and quercetin) at 1 mg·mL^{-1} concentration were tested. Mitotic index (MI), limit value for cytotoxicity (LVC), phase index (PI), and aberration indexes (AI) were calculated, and different aberrations were identified in treated meristematic onion cells. Statistical analyses by ANOVA and Newman-Keuls test were performed. The results confirmed our previous findings that the methanolic extract has an anti-mitotic effect similar to that of the standards. The increase in extract concentrations was accompanied by a decrease in the various indexes calculated. The extracts blocked mitosis in prophase which was predominantly present, and caused increase in abnormalities, represented by dominant rates of agglutinations, chromosomal fragmentations and binuclear cells. confirmed that the effect was independent of concentrations, though to varying degrees.

Additional keywords: Arid zones, colchicine, medicinal plants, mitotic index, quercetin

RESUMEN

Efecto antimitótico y genotóxico de extractos metanólicos de hojas de *Peganum harmala* L. sobre células meristemáticas de *Allium cepa* L. en la región de Laghouat, Argelia

Las plantas medicinales son una fuente inagotable de moléculas con diversas actividades biológicas. El objetivo de este estudio fue la evaluación del efecto antimitótico y genotóxico mediante la prueba con *Allium cepa* del extracto metanólico de las hojas de *Peganum harmala*, una planta espontánea de las zonas áridas de Argelia (Timzerth, Laghouat). Se probaron diferentes concentraciones (1, 4, 10 y 16 mg·mL^{-1}) del extracto, así como dos estándares (colchicina y quercetina) a la concentración de 1 mg·mL^{-1}. Se calculó el índice mitótico, el valor límite de citotoxicidad, el índice de fase y el índice de aberración, el cual permitió identificar diferentes aberraciones en células meristemáticas de cebolla. Los resultados se compararon mediante ANOVA y prueba de Newman-Keuls, y confirmaron nuestros hallazgos previos de que el extracto metanólico tiene un efecto antimitótico similar al de los estándares. El aumento de las concentraciones del extracto se acompañó de una disminución de los diferentes índices calculados. Los extractos bloquearon la mitosis en la profase, la cual estuvo predominantemente presente, y provocaron un aumento de las anomalías, representadas por tasas dominantes de aglutinaciones, fragmentaciones cromosómicas y células binucleares. Además, se confirmó que el efecto fue independiente de las concentraciones, aunque de distinta magnitud.

Palabras clave adicionales: Colchicina, índice mitótico, plantas medicinales, quercitina, zonas áridas

INTRODUCCION

Medicinal plants are considered as an inexhaustible source of bioactive molecules that are responsible for several biological activities against disease processes in humans (Rhattas, 2016). Chemotherapy is based on the administration of substances that inhibit the growth of cancer cells, but those substances are generally highly toxic to healthy cells and have many undesirable effects.

The return to herbal medicine opens a promising avenue for the development of new natural formulas with no side effects and with biological activity (Sabitha et al*.,* 2019). *Peganum harmala*, also called "El Harmel" is common in sub-desert areas of North Africa and in parts of

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Europe, Asia and southern Russia (Bourogaa et al., 2015), and it is known to have antibacterial, antifungal, antiviral, antidiabetic, antitumor, antileishmania, insecticidal and cytotoxic activities (Jinous and Fereshteh, 2012), and, even, anticancer activity (Boeira et al., 2001; Adhami et al., 2011).

This study is part of a contribution to a better knowledge and valorization of *P. harmala* by the evaluation of the antimitotic and genotoxic effect on *Allium cepa*, meristematic cells of methanolic extracts of the plant leaves from the Laghouat region (Algeria).

MATERIALS AND METHODS

Plant material. Details of the plant material and the procedure followed were presented in our previous paper (Ouzid et al., 2021). Briefly, different concentrations of the methanolic extracts of *P. harmala* leaves were brought into contact with the root tips of onion.

The leaves were freshly harvested from ten healthy individuals, and cleaned of all impurities, in April 2015. Sampling was carried out randomly in Dayate Aiat, Timzerth region, wilaya of Laghouat (Algeria). Bioclimatically, the study area is located in the arid zone, with a dry season of 11 months per year (Limane et al., 2014). Samples were placed in paper bags, stored in a cooler, and refrigerated until use. The harvested leaves were dried in the shade at room temperature, lying on the laboratory bench for 10 days. They were ground to a fine powder, stored in smoked glass jars to protect from light in order to prevent oxidation, sealed and stored in the laboratory cabinet.

Preparation of the methanolic extract. Ten grams of the powdered leaves were put in an Erlenmeyer flask containing 100 mL of pure methanol and macerated for 24 hours at 25 °C, and then filtered. The filtrate was centrifuged at 2500 rpm for 10 minutes to recover the supernatant, which was the methanolic extract. This extract was evaporated using a rotavapor at 45 °C, then stored for later use.

Chromosome analysis test. The assessment of chromosomal aberrations in meristematic cells of *Allium cepa* L. roots is validated by the International Programme on Chemical Safety, World Health Organization (IPCS, WHO) and the United Nations Environment Programme (UNEP). This test is recognized as effective for the analysis and monitoring of genotoxicity of natural substances (Cabrera and Rodriguez, 1999).

The protocol followed for the *Allium cepa* L. test was that of Fiskesjö (1985) with modifications made by Shweta et al. (2012). Onion bulbs of *Allium cepa* L. of the same size were placed in beakers containing water for 72 hours at room temperature in the laboratory. After bulb sprouting, the root apices of 0.5 to 1 cm length were contacted with methanolic extracts of 1, 4, 10, and 16 $mg{\cdot}mL^{-1}$ of *P. harmala* leaves. The methanolic extract were analyzed by gas chromatography-mass spectrometry. Two standards were used for comparisons: colchicine, a plant alkaloid that blocks the formation of the mitotic spindle by binding to tubulin and prevents its polymerization into microtubules, thus blocking mitosis at the metaphase stage; and quercetin, a flavonol-type molecule, that acts directly by inhibiting protein kinase and inhibits lipogenesis (Lalaoui et al., 2004). Negative controls were the root apices in contact with only distilled water. After 24 hours of incubation, the roots were fixed in a freshly prepared mixture of one volume of acetic acid and three volumes of 95 % ethanol (1:3 v/v). The fixed roots were stained with acetic carmine, which acts as a fixative and dye, and allows the observation of the nuclei. Samples were prepared between slides and coverslips. The observation was done under the light microscope at magnification (×400). The counting of cells in mitotic division as well as the anomalies generated by the action of the samples, carried out on 350 cells of the different phases of mitosis, namely: prophase, metaphase, anaphase, and telophase (Jahier, 1992).

Cytogenetic analysis was performed by evaluating four biological parameters. For each sample, four tests were performed (Fiskesjö, 1985):

The mitotic index (MI):

$$MI(\%) = \frac{Number\ of\ cell\ divisions}{Number\ of\ cells\ examined}\ x\ 100$$

The limit value for cytotoxicity (LVC):

$$LVC(\%) = \frac{MI\ of\ treated\ cells}{MI\ of\ negative\ control\ cells}\ x\ 100$$

The phase index (PI):

$$PI\ (\%) = \frac{Number\ of\ cells\ in\ each\ phase}{Number\ of\ cells\ examined}\ x\ 100$$

The aberration index (AI):

$$AI\ (\%) = \frac{Total\ chromosomal\ aberrations}{Number\ of\ cells\ examined}\ x\ 100$$

Statistical analysis. Analyses of variance (ANOVA) were carried out in order to highlight significant differences between the methanolic extracts and the controls using the Stat Box 6.40 software at the threshold ($p<0.05$). A complementary multiple mean comparison test (Newman-Keuls) was performed to classify the antimitotic effect of extracts into homogeneous groups.

RESULTS AND DISCUSSION

The study revealed that meristematic cells of *A. cepa* that were not treated (negative control) had an average mitotic index of 87.74 ± 1.37 %. All phases of mitosis were clearly observed, similar to the results of Siddiqui et al. (2007).

Analysis of cells treated with colchicine (1 mg·mL^{-1}) and quercetin (1 mg·mL^{-1}) reveals a remarkable decrease in the average mitotic index (Table 1), which reached 33.88 ± 1.70 % for colchicine and 43.88 ± 0.46 % for quercetin, which corresponds to more than half of that calculated for the negative control, reflecting the antimitotic effect of these agents.

Table 1. Mitotic indexes and limit values for cytotoxicity under different extracts of leaves of *Peganum harmala*

	MI ± SE (%)	LVC ± SE (%)
Negative control	87,74 ± 1,37 f	--
Colchicine (1 mg·mL^{-1})	33,88 ± 1,70 cd	38,61 ± 1,94 cd
Quercetin (1 mg·mL^{-1})	43,80 ± 0,46 e	50,00 ± 0,52 e
Meth.extr (16 mg·mL^{-1})	1,25 ± 0,20 a	1,21 ± 0,25 a
Meth.extr (10 mg·mL^{-1})	14,11 ± 0,32 b	16,07±0,37 b
Meth.extr (4 mg·mL^{-1})	29,76 ± 0,50 c	33,92 ± 1,24 c
Meth.extr (1 mg·mL^{-1})	35,93 ± 0,62 d	40,95 ± 0,70 d

Meth.extr: Methanolic extract; MI: Mitotic Index; LVC: Limit value for cytotoxicity; SE: standard error. In each column, means followed by distinct letters are statistically different according to Newman-Keuls test ($P \leq 0.05$)

Table 1 shows that the mitotic indexes were 1.25± 0.20 % for the 16 mg·mL^{-1} concentration, and 14.11 ± 0.32, 29.76 ± 0.50, and 35.93 ± 0.62 % from 10 to 1 mg·mL^{-1} concentrations, respectively. This reflects a sharp decrease in the mitotic index following the increase in the concentrations of leaf methanolic extracts.

It was found a highly significant difference (P= 0.00) among the mitotic indexes of the methanolic extracts of the leaves of *P. harmala*, the negative control and the two standards, which demonstrates an antimitotic effect of the extracts at different concentrations, as well as that of colchicine and quercetin. In addition, multiple comparisons of means revealed the formation of six groups: a, b, c, d, e and cd (Table 1). This even revealed that the methanolic extract at the concentration 1 mg·mL^{-1} had a higher effect than quercetin at the same concentration.

The decrease of the mitotic index following the increase in the concentrations of extracts indicates a mitodepressive effect of the methanolic extract of the plant leaves. A similar study, performed with the crude fungal extracts of endophytic foliar fungi of *P. harmala*, using ethyl acetate as extraction solvent, revealed comparable results (Ouzid et al., 2019). The plant is known for its richness in alkaloids (harmines, harmalines), phenols, terpenes and saponosides with cytotoxic activity; according to El-ghamery et al. (2003), the components contained in certain plant extracts should interfere with the normal development of mitosis, preventing a number of cells from entering prophase and thus blocking the mitotic

cycle during interphase.

According to Siddiqui et al. (2007), the decrease in the mitotic index is probably due to a modification or alteration in the expression of certain genes, each action occurring separately in the interphase nucleus through the ultimate influence of the structure. This index is used to evaluate the antimitotic and genotoxic effects of various natural extracts; when it decreases below 22 % of the negative control, it causes a lethal effect on the test organisms. A decrease in the latter by 50 % compared to the control is considered a sublethal effect and constitutes the limit value for cytotoxicity (Marcano et al., 2006). According to the above definitions, it can be deduced that the methanolic extract of *P. harmala* leaves is considered lethal to *A. cepa* meristematic cells treated with the concentrations 16 and 10 $mg{\cdot}mL^{-1}$, with a LVC of 33.92 ± 1.24 and 40.95±0.70 % respectively. The 4 and 1 $mg{\cdot}mL^{-1}$ extracts, and both standards were considered sublethal. There was a highly significant difference (P= 0.00) between the cytotoxicity cut-off values of the methanolic extracts of *P. harmala* leaves, negative control and the two standards (colchicine and quercetin). This shows a cytotoxic effect of the extracts at different concentrations (1, 4, 10, and 16 $mg{\cdot}mL^{-1}$), as well as colchicine and quercetin (1 $mg{\cdot}mL^{-1}$).

The percentages of the different phases of mitosis calculated on all the examined cells are reported in Table 2; at the 16 $mg{\cdot}mL^{-1}$ concentration prophase index was 1.13 ± 0.17 % and telophase index 0.11 ± 0.10 %. It is noted that the prophase index was relatively the highest, and decreases with increasing concentrations of the prepared methanolic extracts, followed by the telophase index. On the other hand, the metaphase and anaphase indexes were only marginally present, and sometimes even absent.

Table 2. Phase indexes at the four phases of mitosis under different extracts of leaves of *Peganum harmala*

	PI ± SE (%)	M'I ± SE(%)	AI ± SE (%)	TI ± SE (%)
Negative control	59,60 ± 3,38 f	1,42 ± 0,39	2,05 ± 0,56	24,57 ± 4,83 a
Colchicine (1 $mg{\cdot}mL^{-1}$)	32,05 ± 1,58 d	0,00 ± 0,00 a	0,00 ± 0,00 a	1,82 ± 0,40 a
Quercetin (1 $mg{\cdot}mL^{-1}$)	40,57 ± 0,90 e	0,00 ± 0,00 a	0,00 ± 0,00 a	3,26 ± 1,07 a
Meth.extr (16 $mg{\cdot}mL^{-1}$)	1,13 ± 0,17 a	0,00 ± 0,00 a	0,00 ± 0,00 a	0,11 ± 0,10 a
Meth.extr (10 $mg{\cdot}mL^{-1}$)	12,51 ± 0,62 b	0,00 ± 0,00 a	0,00 ± 0,00 a	1,60 ± 0,33 a
Meth.extr (4 $mg{\cdot}mL^{-1}$)	20,28 ± 0,84 bc	0,00 ± 0,00 a	0,00 ± 0,00 a	9,48 ± 0,54 a
Meth.extr (1 $mg{\cdot}mL^{-1}$)	26,85 ± 0,99 cd	0,00 ± 0,00 a	0,06 ± 0,04 a	9,02 ± 0,91 a

PI: Prophase index; M'I: Metaphase index; AI: Anaphase index, TI: Telophase index; Meth. extr: Methanolic extract; SE: Standard error. In each column, means followed by distinct letters are statistically different according to Newman-Keuls test ($P \leq 0.05$)

The ANOVA revealed a highly significant difference between the phase index values of the methanolic extracts of the plant leaves, negative control and the two standards (colchicine and quercetin) (P= 0.00). This shows an antimitotic effect of the extracts at different concentrations (1, 4, 10 and 16 $mg{\cdot}mL^{-1}$) as well as colchicine and quercetin (1 $mg{\cdot}mL^{-1}$), at the level of the four mitotic phases. In addition, multiple comparisons of means revealed the formation of six groups for the prophase index, and only one group for that of metaphase, anaphase and telophase (Table 2). This phenomenon is probably related to an intense alteration of microtubules preventing chromosome assembly at the metaphase stage. Some natural compounds such as certain saponins and flavonoids could lead to a progressive disappearance of metaphases, anaphases and telophases with a relative increase in the number of prophases, which then gradually disappear by returning to the interphase state (Fusconi et al., 2006 and Roger, 2007). Firbas and Amon (2014) noted

that anaphase, telophase and metaphase assays were suitable for the detection of genotoxic effects of ionizing radiation. Overall, the *Allium cepa* test is a sensitive and informative cytogenetic tool for rapid screening for ionizing radiation and radionuclide pollution (Firbas and Amon, 2017).

The observation of aberrations is an indicator of alterations in the genetic material and the cell. These abnormalities can lead to cellular damage including apoptosis. They may be due to blockage of DNA synthesis or inhibition of mitotic spindle formation (Dimitry et al., 2013).

The aberrations observed when treating onion roots with the different concentrations of methanolic extracts of *P. harmala* leaves (Figure 1) differed from one extract to another, and the percentage increased relatively with concentration. The maximum reached 57.90 % at the 16 mg·mL^{-1} concentration and 11 % at the 1 mg·mL^{-1} concentration. For the positive controls, the total anomalies encountered were high for colchicine (30.30 %) compared to quercetin (25.40 %) (Table 3).

Table 3. Aberration indexes with different methanolic extracts of leaves of *Peganum harmala*

	CA±SE (%)	BC±SE (%)	CE±SE (%)	CB± SE (%)	CF± SE (%)	DE± SE (%)	GC± SE (%)	CWN±SE (%)	AB± SE (%)	AC± SE (%)	Total (%)
Colchicine (1 mg·mL^{-1})	26,45 ±2,56	0,11±0,09	0,00±0,00	0,00±0,00	3,59±0,32	0,00±0,00	0,17±0,09	0,00±0,00	0,00±0,00	0,00±0,00	30,30
Quercetin (1 mg·mL^{-1})	17,08±1,22	0,51±0,20	0,39±0,20	0,00±0,00	1,14±0,65	0,22±0,09	2,51±0,39	0,34±0,17	2,85±0,93	0,45±0,12	25,40
Meth.extr (16 mg·mL^{-1})	49,53±1,69	0,17±0,10	1,42±0,22	0,00±0,00	0,22±0,09	0,00±0,00	2,10±0,32	0,85±0,29	0,28±0,1	3,36±0,43	57,90
Meth.extr (10 mg·mL^{-1})	41,93±1,27	0,11±0,05	0,74±0,35	0,00±0,00	1,14±0,27	0,22±0,09	1,36±0,24	1,36±0,31	2,73±0,60	3,25±0,26	52,80
Meth.extr (4 mg·mL^{-1})	20,68±1,66	0,51±0,27	1,19±0,20	0,00±0,00	0,74±0,11	0,34±0,09	0,51±0,23	0,28±0,12	0,45±0,17	2,68±0,43	27,30
Meth.extr (1 mg·mL^{-1})	6,39±0,62	0,00±0,00	1,42±0,25	0,00±0,00	0,00±0,00	0,39±0,12	0,45±0,12	0,00±0,00	0,28±0,10	2,11±0,33	11,00

CA: chromosome agglutination; CB: chromosome bridge; CF: chromosome fragmentation; DE: disorganization of the equatorial plate; GC: gigantic cell; CWN: cell without nucleus; CE: cell elongation; AB: apoptotic body; BC: binuclear cell; AC: absence of cytodieresis; Meth. extr: Methanolic extract; SE: standard error

There were not significant differences (P= 0.88) between the mean aberration indexes of the methanolic extracts of *P. harmala* leaves and the two standards (colchicine and quercetin). This demonstrates the antimitotic effect of the extracts at different concentrations as well as colchicine and quercetin (1 mg·mL^{-1}). The multiple comparisons of means revealed the formation of a single group (Table 3). The mean of the outliers of the methanolic extract of the plant leaves at the concentration of 4 mg·mL^{-1} (2.74 %) ranked between the mean of colchicine 1 mg·mL^{-1} (3.03 %) and quercetin 1 mg·mL^{-1} (2.55 %). Therefore, the methanolic extract at the concentration of 4 mg·mL^{-1}, had a higher antimitotic effect than quercetin (1 mg·mL^{-1}), used as a standard flavonoid, and lower than colchicine (1 mg·mL^{-1}), used as a standard alkaloid.

The chromosomal aberrations observed in this study were obviously caused by the bioactive molecules present in the methanolic extracts of *P. harmala* leaves, since no aberrations were observed in the negative control.

Most of these abnormalities were defined by the presence of chromosomal agglutinations averaging 49.53 ± 1.69 % at the concentration of 16 mg·mL^{-1} in methanolic extract (Fig. 1), they present the relatively high rate compared to other abnormalities in all extracts. According to Bass et al. (2000), this tendency to agglutination could be the result of a decrease in viscosity transforming elongated molecules into globular ones. It is

probably caused by an aggregation of chromosomes that lose their mobility and become unable to migrate. It could also result from the depolymerization of DNA and the dissolution of nucleoproteins following a stress, which leads to cell death.

Compared to binuclear cells (0.51 ± 0.20 %) in cells treated with quercetin and methanolic extract (4 mg·mL^{-1}), their presence suggests that they are probably due to the eruption of the cytokinesis process at some checkpoint of the cell cycle. This acts on the formation of the phragmoplast, and prevents the formation of daughter cells, leading to the appearance of polyploid cells (Grant, 1976). As for chromosome fragmentations, their presence was in the majority in cells treated with colchicine, with an average of 3.59 ± 0.32 %. Cells without nuclei were also present, with a rate of 1.36 ± 0.31 % at the 10 mg·mL^{-1} concentration, their presence was nil at the 1 mg·mL^{-1} concentration and following colchicine treatment. According to Roger (2007), they are the result of karyolysis followed by nucleoprotein modifications with DNA depolymerization and hydrolysis by cellular enzymes. In addition, apoptotic bodies were also present, reaching 2.73 ± 0.6 % at 10 mg·mL^{-1}, 0.45 ± 0.17 % at 4 mg·mL^{-1} and 0.28 ± 0.1 % for 16 and 1 mg·mL^{-1}. According to Wyllie et al. (1984), they are due to cleavage of chromatin into irregular fragments or budding of the plasma membrane. The apoptotic bodies contain part of the cell's cytoplasm.

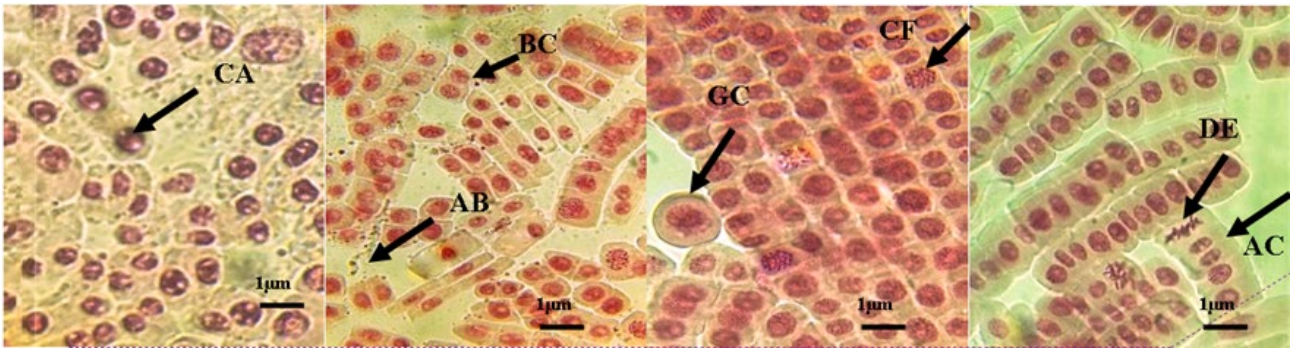

Figure 1. Anomalies observed in *Allium cepa* L. meristematic cells under light microscope (x400). CA: chromosome agglutination; CF: chromosome fragmentation; BC: binuclear cell; GC: gigantic cell; DE: disorganization of the equatorial plate; AC: absence of cytodieresis; AB: Apoptotic body.

Giant cells are considered a kind of adaptation to abiotic stress. It is an alternative to the classical cell cycle where DNA is duplicated without cell division, mitosis has not taken place and the cell cycle is reset. As for the disorganizations of the equatorial plate, their presence was with a rate of 0.39 ± 0.12, 0.34 ± 0.09 and 0.22 ± 0.09 % respectively, for the concentrations of 1, 4, and 10 mg·mL^{-1}, and were absent at the concentration 16 mg·mL^{-1}. According to Roger (2007), microtubules could be the target of certain flavonoids and triterpenes, their action would prevent the formation of cell plaque. This phenomenon was observed using the extract of *P. harmala* flowers and *Lantana camara* L. leaves (Turkuglo, 2008). Furthermore, Sadaf et al. (2021) demonstrated the effect of methanolic extracts of *P. harmala* seeds and roots on prostate and breast cancer cell lines. These significant antitumour and cytotoxic effects could be due to the presence of phytochemicals, including flavonoids and phenolic compounds.

CONCLUSIONS

This study revealed that methanolic extracts of *Peganum harmala* leaves affect mitosis and exert an antimitotic and genotoxic effect on meristematic cells of *Allium cepa*. This is reflected by a decrease in mitotic indexes and phase indexes compared to untreated cells (negative control) for all concentrations tested (1, 4, 10, and 16 mg·mL^{-1}). Microscopic observations revealed different types of abnormalities such as chromosome agglutination, binuclear cells, absence of cytodieresis and chromosome fragmentations. These results are comparable to those found in

meristematic cells treated with colchicine and quercetin (both at 1 $mg \cdot mL^{-1}$) used as standards. The 1 $mg \cdot mL^{-1}$ concentration of the extract even showed a better effect than quercetin, thus reflecting the high antimitotic effect of the methanolic leaf extract of *P. harmala*. This species constitutes a promising source of antimitotic substances. It would be interesting to carry out further studies such as cell cultures to understand the molecular and cellular mechanism of the secondary metabolites from the leaves of this plant.

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LITERATURE CITED

1. Adhami, H.R., H. Farsam, and L. Krenn. 2011. Screening of medicinal plants from Iranian traditional medicine for acetyl-cholinesterase inhibition. Phyto-therapy Research25: 1148.
2. Bass, H.W., S. Nagar, L. Hanley-Bowdoin, and D. Roberston. 2000. Chromosome condensation induced by giminivirus infection of mature plant cells. Journal of Cell Science 113: 1149-1155.
3. Boeira, J.M., J. Dasilva, B. Erdtmann, and J.A.P. Henriques. 2001. Genotoxic effects of the alkaloids harman and harmine assessed by comet assay and chromosome aberration test in mammalian cells *in vitro*. Pharmacology and Toxicology 89: 287.
4. Bourogaa, E., R.M. Jarraya, M. Damak, and A. Elfeki. 2015. Hepatoprotective activity of *Peganum harmala* against ethanol-induced liver damages in rats. Archives of Physiology and Biochemistry 121(2): 62-67.
5. Cabrera, G.L., and D.M.G. Rodriguez. 1999. Genotoxicity of soil from farmland irrigated with wastewater using three plant bioassays. Mutation Research 426(2): 211-214.
6. Dimitry, S., A., Pesnya, and V. Romanovsky. 2013. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the *Allium cepa* test. Mutation Research 750: 27-33.
7. El-ghamery, A.A., M.A. El- kholy, and A. Yousser. 2003. Evaluation of cytological effect of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. Mutation Research 537(1): 29-41.
8. Firbas, P., and T. Amon. 2014. Chromo-some damage studies in the onion plant *Allium cepa* L. Caryologia 67(1):25-35.
9. Firbas, P., and T. Amon. 2017. Combined of chemical analysis, fish micronuclei and onion chromosome damage for assessing cleaning effect in the WWTP central Domžale-Kamnik and quality of Kamniška Bistrica River. Cepal Review 121: 2825-2842.
10. Fiskesjö, G. 1985. The *Allium* test in wastewater monitoring. Environmental Toxicology and Water Quality 8: 291-298.
11. Fusconi, A., O. Repetto, E. Bona, N. Massa, C. Gallo, E. Dumas-Gaudot, and G. Berta. 2006. Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv. Frisson seedlings. Environ-mental and Experimental Botany 58: 253-260.
12. Grant, W.F. 1976. Chromosome aberrations in plant as monitoring system. Environmental Health Perspective, 27: 37-43.
13. Jahier, J. 1992. Techniques de cytogénétique végétale. INRA, ed., Paris. p. 181.
14. Jinous, A., and R. Fereshteh. 2012. Chemistry, pharmacology and medicinal properties of *Peganum harmala* L. African Journal of Pharmacy and Pharmacology 6: 1573-1580.
15. Lalaoui, K., L. Mzibri, M. Laget, H. Guiraud-Dauriac, G. Dumenil, and H. Hmamouchi. 2004. Antiproliferative effect of medicinal plants on a cervical cancer line. Maroc Medical 26(2): 90-94.
16. Limane, A., N. Smail-Saadoun, A. Belkebir-Boukais, and K. Kissoum-Hamdini. 2014. Root architecture adaptation of *Pistacia atlantica* subsp. *atlantica* according to an increasing climatic and edaphic gradient: case of a north-south transect in Algeria. Turkish Journal of Botany 38: 536-549.
17. Marcano, L., I. Carruyo, Y. Fernández, X. Montiel, and Z. Torrealba. 2006. Determination of vanadium accumulation in

onion root cells (*Allium cepa* L.) and its correlation with toxicity. Biocell 30: 259-267.

18. Ouzid, Y., N. Smail-Saadoun, and K. Houali. 2019. Antimitotic and antiproliferative activities of crude fungal extracts of endophytic foliar fungi of *Peganum harmala* L. from Dayate Aiat (Laghouat, Algeria). Journal of Fundamental and Applied Sciences 11(2): 587-604.
19. Ouzid, Y., G. Aiche-Iratni, L. Harchaoui, L. Moussaoui, L. Chabane, N. Smail-Saadoun, and K. Houali. 2021. Antimitotic and genotoxic effect on the meristematic cells of *Allium cepa* L. of the alkaloid and flavonoid fractions of the leaves of *Peganum harmala* L. from the Laghouat region, Algeria. International Journal of Minor Fruits, Medicinal and Aromatic Plants 7(2): 79- 88.
20. Rhattas, M. 2016. Ethnobotanical study of medicinal plants in the Talassemtane National Park (Western Rif of Morocco). Journal of Applied Biosciences 97: 36-37.
21. Roger, P. 2007. Anatomical and clinical semiology of vascular and circulatory lesions: Pathological anatomy. Vascular and circulatory lesions. Faculty of Medicine Montpellier-Nimes. France. p. 58.
22. Sabitha-Rani, A., G. Hajera-Sana, E. Sulakshana, P. Shravya, and M. Keerti. 2019. *Spilanthes acmella*-an important medicinal plant. International Journal of Minor Fruits, Medicinal and Aromatic Plant 5(2): 15-26.
23. Sadaf, H.M., Y. Bibi, M. Arshad, A. Razzaq, S. Ahmad, M. Iriti, and A. Qayyum. 2021. Analysis of *Peganum harmala*, *Melia azedarach* and *Morus alba* extracts against six lethal human cancer cells and oxidative stress along with chemical characterization through advance fourier transform and nuclear magnetic resonance spectroscopic methods towards green chemotherapeutic agents. Saudi Pharmeutical Journal 26(6): 552-565.
24. Shweta, S., S. Khadabadi, and T.G. Ganesh. 2012. *In vitro* antimitotic, antiproliferative, DNA fragmentation and anticancer activity of chloroform and ethanol extract of *Revia hypocrateriformis*. Asian Pacific Journal of Tropical Disease S: 503-508.
25. Siddiqui, S., M.K. Meghvansi, and Z. Hasan. 2007. Cytogenetic changes induced by sodium azide (NaN3) on *Trigonella foenum-graecum* L. seeds. South African Journal of Botany 73: 632-635.
26. Turkuglo, S. 2008. Molecular controls of growth arrest and apoptosis: dependent and independent pathways. Oncogene 11:199-200.
27. Wyllie, A.H, A.L. Smith, and D. Dunlop. 1984. Chromo-some cleavage in apoptosis association with condensed chromatin morphology and dependence on macro-molecular synthesis. Journal of Pathology 142: 67-77.