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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

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ABSTRACT

Medicinal plants are an inexhaustible source of secondary metabolites, namely polyphenols including flavonoids, alkaloids and terpenoids, which generate various biological activities. Keeping this in mind, we were interested in a spontaneous plant Peganum harmala L. to demonstrate the antimitotic and genotoxic effect of the alkaloid and flavonoid fractions (aqueous and butanolic) of the leaves of this species on root meristematic cells of Allium cepa L. Mitotic indices, phase indices, aberration indices as well as cytotoxicity limit values were calculated for our different samples and controls, namely the negative (distilled water) and positive controls (colchicine 1mg/ml and quercetin 1mg/ml). The results indicate a mitodepressant and sublethal effect, observed with the alkaloid and flavonoid fractions of the leaves of Peganum harmala L. Exposure of meristematic cells to the samples resulted in an antimitotic and genotoxic effect translated into a large number of chromosomal, nuclear and cellular aberrations.

***Keywords*:** Alkaloid fraction, *Allium cepa* L., antimitotic, flavonoid fractions, genotoxic effect, *Peganum harmala* L.

INTRODUCTION

Man has always relied on plants for their vital needs. This interest has increased due to the therapeutic benefits of medicinal plants (Nandi and Ghosh, 2016; Sabitha Rani *et al.,* 2019). In order to contribute to the valorization of local medicinal plants known for their therapeutic virtues, we were interested in the present work in the study of the antimitotic and genotoxic activity of the alkaloid and flavonoid fractions of the leaves of *Peganum harmala* L., harvested in Timzerth, Dayate Aiat in the wilaya of Laghouat in southern Algeria. *Peganum harmala* L., local name Harmel, is an endemic species of the family Zygophyllaceae, it grows in semi-arid areas on sandy, stony and nitrate soils. This plant is known for its richness in various secondary metabolites mainly alkaloids, coumarins and flavonoids (Al yahya, 1986). It has anti-bacterial, anti-fungal, anti-viral, anti-oxidant, anti-diabetic, anti-tumor, anti-leishmaniasis, insecticidal effect, cytotoxic activity, as well as hepato-protective effects (Jinous and Fereshteh, 2012). However, if mis-used it can be very toxic for animals and humans in particular. It is responsible for paralysis of the nervous system and causes death by respiratory arrest in vertebrates, and can cause pregnancy termination in women (Bellakhdar, 1997).

The Allium test is a standardized test for monitoring cytogenotoxicity, known for its simplicity and reliability (Fiskesjö, 1985). It combines two targets: toxicity and genotoxicity. In addition, it is important to note that its cost is low and that it correlates well with mammalian test systems (Fiskesjö, 1985; Cabrera and Rodriguez 1999; Jovtchev *et al*., 2002; Grant 1994;1999; Yi and Meng 2002).The method for assessing chromosomal aberrations at the meristematic cell level 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) as an effective test for the analysis and monitoring of the genotoxicity of natural substances (Cabrera and Rodriguez, 1999). Therefore, we used the *Allium cepa* L. test to evaluate the antimitotic and genotoxic effect of our samples.

MATERIALS AND METHODS

Plant material

The leaves of *Peganum harmala* L., were freshly harvested from ten healthy individuals 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).The samples were placed in paper bags and stored in a cooler and then refrigerated until they were used in the laboratory. The identification of the plant was carried out by Professor Smail-Saadoun Noria, Director of the Natural Resources Laboratory (LRN), University Mouloud Maameri, Tizi-Ouzou (Algeria).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, sealed and stored in the laboratory cabinet.

Extraction of alkaloids

To extract the total alkaloids, we used the conventional method of Harbone (1998), which we have optimized. A quantity of 5g of the powder from the leaves of *Peganum harmala* L. was degreased in 10ml of petroleum ether for 24 hours at room temperature then filtered with Wattman paper N° 1 paper and the filtrate was discarded. The powder thus degreased was macerated in methanol under the same conditions as before. The filtrate thus recovered was dry evaporated at 60°C, then taken up by chloroform and acidified by 5% HCl at pH=3. The acidic aqueous phase was recovered, to which chloroform was added, then basified with 5% Na_2CO_3 at pH=9. The chloroform phase is then evaporated. The dry residue which represents the total alkaloids is taken up by chloroform which will be evaporated dry at 60°C and the residue thus obtained is recovered by distilled water.

Extraction of flavonoids

For the extraction of flavonoids, we used the liquid method of Bekkara *et al*. (1998) where 1g of vegetable powder is brought into contact with 20ml of methanol at room temperature. After 24 hours incubation, the solution is filtered with Wattman paper N° 1, the filtrate obtained is evaporated at 60°C, the resulting residue is solubilized with a mixture of distilled water and ethyl acetate and then decanted. The aqueous phase is recovered to which 10 ml of n-butanol is added. After decantation, two distinct phases are obtained: the flavonoic butanolic phase and the aqueous flavonoic phase. The solvents are evaporated and the residues obtained are solubilised in distilled water, stored at +6 °C and protected from light in smoked bottles.

The *Allium cepa* L. test

The plants used for the *Allium cepa* L. test are individuals belonging to the species *Allium cepa* L. (family Amaryllidaceae or Alliaceae, depending on the chosen taxonomic treatment), commonly known as onion. The *Allium cepa* L. tests genotoxicity using chromosomes (Bonciu *et al.,* 2018).

The protocol followed for the *Allium cepa* L. test is that of Fiskesjö (1985) with modifications made by Shweta *et al*. (2012). Bulbs of *Allium cepa* L. onions of the same size are placed in beakers containing water for 72 hours at laboratory room temperature. After elongation, the 0.5 to 1 cm long root apexes were brought into contact with the alkaloid fraction as well as the flavonoid fractions (aqueous and butanolic) of the leaves of *Peganum harmala* L. The negative control is represented by distilled water, moreover, the positive control is represented by colchicine (1mg/ml) for the alkaloid fraction and quercetin (1mg/ml) for the flavonoid fractions. After 24 hours incubationat ambient temperature, 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). Fixation is intended to block any evolution of cell division and maintain the structural integrity of the chromosomes (Jahier, 1992).The roots thus fixed are colored by acetic carmine, prepared by boiling 55 ml of distilled water and 45 ml of acetic acid, add 0.5 g of carmine powder, keep boiling for 5 miutes. which is used for its double action as a fixative/stain and allows the observation of the cores. The treated root fragments were prepared between slides and lamellae. Cytogenenetic analysisis done with an optical microscope at magnification (×400).The blades are viewed from right to left and from top to bottom. The counting of the cells in mitotic division as well as the

anomalies generated by the action of our samples is performed on 350 cells of the different phases of mitosis, namely: Prophase (P), Metaphase (M), Anaphase (A) and Telophase (T).

Cytogenetic analysis is carried out by evaluating four biological parameters. For each sample, 5 tests were carried out (Fiskejo, 1993; Antosiewicz, *et al.,* 1990).The parameters evaluated were:

The mitotic index (MI): MI (%)= (Number of cells division / Number of cells examined (350 cells)) × 100

The limit value for cytotoxicity: LVC(%) = (MI of treated cells / MI of negative control cells) × 100

The phase index (PI): PI (%) = [(Number of cells in (P, M, A, T) /Number of cells examined (350 cells)] × 100

The aberration index (AI): AI (%) = [(Total chromosomal aberrations / Number of cells examined (350 cells)]× 100

Statistical analysis

ANOVA-type analyses of variance were carried out, in order to highlight significant differences between our extracts (alkaloid and flavonoid fraction) and the controls (negative and positive) used, with the STATISTICA Software at the threshold ($p<0.05$).

RESULTS AND DISCUSSION

Analysis of the meristematic cells of *Allium cepa* L. by the alkaloid fraction extracted from the leaves of *Peganum harmala* L. reveals a remarkable decrease in the mitotic index. The rate reached 36.60±1.47%, which is less than half the rate calculated for the negative control (92.20±1.34%). This index is close to the value of the mitotic index of cells treated with the positive control (colchicine) which is 35.00±1.16%, with the dominance of prophase whose phase index is equal to 11.94±6.87%. On the other hand, cells brought into contact with quercetin (positive flavonoid control), their mitotic index decreases to 44.40±0.72%, close to that obtained in the presence of the butanolic flavonoid fraction which reaches a value of 44.00±0.40%. On the other hand, for meristematic cells in contact with the aqueous flavonoid fraction, the mitotic index value is 62.60±0.82% (Fig.1). Meristematic cells treated with both the negative (distilled water) and positive controls (colchicine and quercetin), as well as our samples (alkaloid and flavonoid fractions) are mostly blocked in prophase. Note that the metaphase index is nil for the cells treated with the alkaloid and flavonoid fractions (Fig. 2).

The use of specific solvents of increasing polarity: methanol, ethyl acetate and n-butanol allowed the separation of flavonoids according to their degree of solubility in these extraction solvents and their structural complexity. Methanol removes non-phenolic compounds such as carotenoids, chlorophyll pigments and fats. In addition, ethyl acetate allows the extraction of monoglycosides. As for n-butanol, it allows the extraction of diglycosides and triglycosides (Sharaf *et al.*, 1997). According to Halim *et al.* (1995), the main flavonoids in the leaves of *Peganum harmala* are represented by: Acacetine-7-O-rhamnoside, Acacetine-7-O-[6 ''-O-glucosyl-2''-O-(3'''-acetylrhamnosyl)] glucoside and Acacetine-7-O-(2'''-O-rhamnosyl-2 O-glucosylglucoside). *Peganum harmala* contains alkaloids of the type β-carbolines, the most important of which are harmine, harmaline, harmol and harmalol and quinazolines which are responsible for toxicological and pharmacological effects (Pulpati *et al.*, 2008; Beyer *et al*., 2009; Herraiz *et al*., 2010).

The standard ANOVA statistical analysis of mitotic indices revealed highly significant differences between the positive control (colchicine) and the negative control ($P=0.00$), between the negative control and the alkaloid fraction ($P=0.00$). Similarly, there were highly significant differences ($p=0.00$) between the mitotic index of the negative control, that of the positive control (quercetin) and that of the aqueous and butanolic flavonoid fraction. Highly significant differences were also observed between the mitotic index of the positive control (quercetin) and that of the aqueous flavonoic fraction. In addition, no significant difference ($p=0.67$) was observed between the mitotic index of the positive control (quercetin) and that of the butanolic flavonoic fraction. This confirms their action in inhibiting mitosis. However, non-significant differences were observed for both alkaloid and flavonoid fraction phase indices.

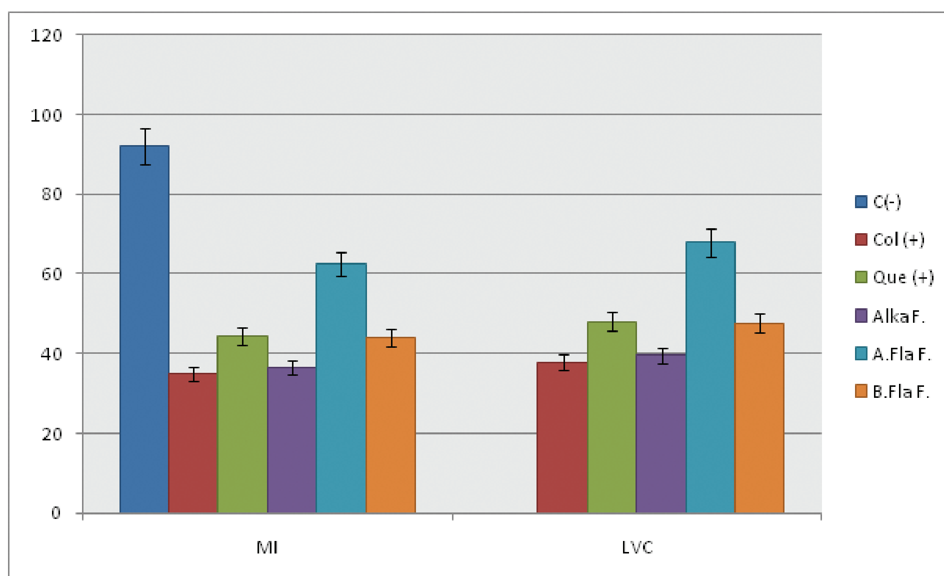

Fig. 1 : Mitotic index and limit value of cytotoxicity of the different fractions tested. C(-): Negative control, Col(+): Colchicine (Positive alkaloid control), Que (+): Quercetin (Positive flavonoid control), Alka F.: Alkaloid Fraction, A.Fla F.: Aqueous Flavonoid Fraction, B.Fla F: Butanolic Flavonoid Fraction, MI: Mitotic Index, LVC: Limit Value for Cytotoxicity.

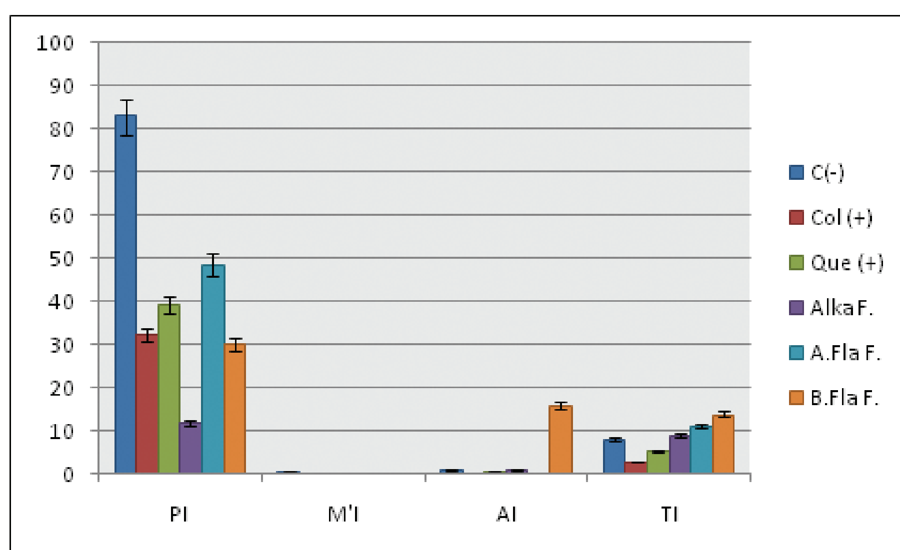

Fig. 2: Phase index of the different fractions tested. PI: Prophase Index, M'I: Metaphase Index, AI: Anaphase Index, TI: Telophase Index.

The mitotic index is considered a parameter for estimating the frequency of cell division (Marcano *et al*., 2006). Decreased mitotic activity in root meristematic cells of *Allium cepa* L. indicates a mitosuppressive effect of the fraction and aqueous extracts of *Peganum harmala* L. Similar mitosuppressive effects were observed in meristematic cells of *Allium cepa* L. treated with aqueous extracts of five medicinal plants used in the Nigerian pharmacopoeia: *Azadirachta indica* A. JUSS; *Morinda ludica* Benth; *Cymbopogon citratus* DC. Stapf, *Mangifera indica* L. and *Carica papaya* L. at the following concentrations: 1, 2.5, 10, 20% (w/v) (Akinboro and Bakare, 2007). Several *in vitro* studies have shown the anticancer effect of flavonoids against many cancer cell lines as well as *in vivo*. Bosetti *et al*. (2005) and Fink *et al*. (2007) have shown that the consumption of

Table 1: Aberration indices assessed at the level of cells treated with positive controls and alkaloid and flavonoid fractions of *Peganum harmala* L. leaves.

Traitement	CA±SE	BC±SE	CE±SE	CB±SE	CF±SE	DEP±SE	GC±SE	CWN±SE	AB±SE	AC±SE	Total±SE
Col (+)	5.60±1.42	0.74±0.66	0.86±0.48	0.00±0.00	1.89±0.55	0.23±0.14	1.94±1.13	0.86±0.37	0.17±0.15	0.0±0,00	12.29±4.90
Que (+)	8.97±1.76	0.00±0.00	1.71±0.56	0.00±0.00	6.74±2.54	0.17±0.10	1.08±0.59	0.00±0.00	0.00±0.00	3.54±1.71	22.21±7.26
Alka F.	12.62±0.77	0.22±0.10	0.00±0.00	0.00±0.00	0.17±0.07	0.11±0.05	1.31±0.28	0.00±0.00	0.17±0.07	0.00±0.00	14.60±1.34
A. Fla F.	4.14±0.60	0.00±0.00	0.80± 0.54	0.06±0.04	0.85±0.34	0.00±0.00	0.00±0.00	0.62±0.56	0.11±0.09	0.00±0.00	6.58±2.20
B. Fla F.	10.57±0.67	0.51±0.25	4.40±2.16	0.00±0.00	3.94±1.75	0.17±0.01	0.34±0.03	0.68±0.06	0.06±0.02	7.31±1.39	27.97±7.28

C(-): Negative control, Col(+): Colchicine (Positive alkaloid control), Que (+): Quercetin (Positive flavonoid control), Alka F.: Alkaloid Fraction, A.Fla F.: Aqueous Flavonoid Fraction, B.Fla F: Butanolic Flavonoid Fraction.

SE: standard error; CA: chromosomal agglutinations; BC: binucleated cells; CE: cell elongations; CB: chromosomal bridges; CF: chromosomal fragmentations; DEP: Disorganization of the equatorial plate; GC: gigantic cells; CWN: cells without a nucleus (ghost cells); AB: apoptotic bodies; AC: absence of cytodieresis.Aberration ± Standard error (%).

flavonoids reduces the risk and incidence of several types of cancers, namely breast and lung cancer. The reduction in mitotic activity could be due to inhibition of DNA and nucleoprotein synthesis in the biological system (Chauhan *et al*., 1998) and modification or alteration in the expression of certain genes (Siddiqui *et al*., 2007; Sultan and Celik, 2009). Sadaf *et al.* (2021) demonstrated the anticancer effect of methanolic extracts of *Peganum harmala* L. seeds and roots on prostate cancer cell lines (PC3) as well as breast cancer cell line (MCF7). These significant antitumour and cytotoxic effects could be due to the presence of phytochemicals, including flavonoids and phenolic compounds.

The increase in the number of prophases in *A. cepa* cells treated with the aqueous extracts and leaf fraction of *Peganum harmala* L., suggests, according to Damato (1954), that this is due either to too long a duration of treatment or to the use of too high doses, resulting in slower entry into the other stages of mitosis, notably, metaphase, anaphase and telophase. Firbas and Amon (2014) noted that both anaphase, telophase and metaphase assays are suitable for the detection of genotoxic effects of ionizing radiation. Overall, the test of *Allium cepa* is proved to be a very convenient, highly sensitive and informative cytogenetic tool for rapid screening of ionizing radiation and radionuclide pollution (Firbas and Amon, 2017).

Fig. 1 also shows the limit value for cytotoxicity (LVC %) of meristematic cells of *Allium cepa* L. treated with the alkaloid fraction and flavonoid fraction of the leaves of *Peganum harmala* L. compared to positive controls. The ANOVA standard statistical analysis showed significant differences between the limit value for cytotoxicity of cells treated with the alkaloid fraction and those treated with the flavonoid fractions ($p=0.03$). On the other hand, no significant difference was observed between the limit value of cytotoxicity of quercetin and the butanolic flavonoid fraction ($p=0.76$).

When the mitotic index decreases below 22% of the control it causes what is called the "lethal effect" on the test organisms (Antosiewicz, 1990). A decrease in the mitotic index of 50% relative to the control is usually a sublethal effect (Panda and

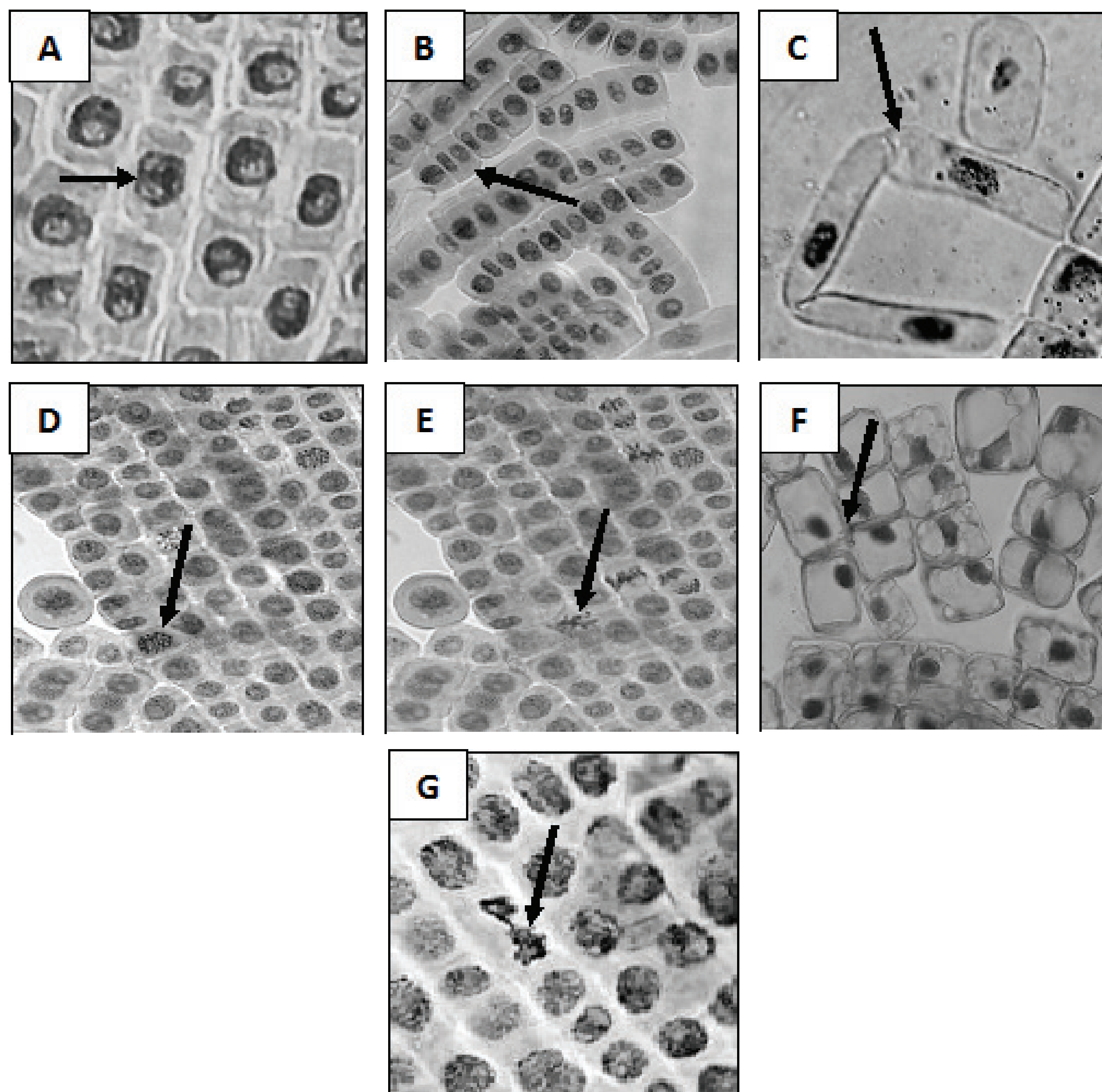

Fig. 3: the different aberrations observed in the meristematic cells of *Allium cepa* L., seen under an optical microscope (X400).
A: chromosomal agglutinations. B: binucleated cells, C: cell elongations.
D: chromosomal fragmentations. E: disorganization of the equatorial plate in metaphase. F: gigantic cells.
G: apoptotic bodies.

Sahu, 1985) and is referred to as the limit value for cytotoxicity (Sharma, 1983). According to these two definitions it can be deduced that the alkaloid fraction and the flavonoic butanolic fraction of the leaves of *Peganum harmala* L. are considered to be sublethal for the meristematic cells of *Allium cepa* L. in the same way as colchicine and quercetin.

Cytogenetic analysis of meristematic cells of *Allium cepa* L. treated with the alkaloid fraction and flavonoid fraction of *Peganum harmala* L. leaves indicates the presence of different types of cell division abnormalities. These abnormalities are expressed by varying levels of aberration indices relative to the positive controls (colchicine and quercetin) (Table 1). The main abnormalities observed are shown in Fig.3 and are as follows: Chromosomal agglutinations (Fig. 3A), binucleated cells (Fig.3B), cell elongations (Fig.3C),

chromosomal fragmentations (Fig.3D), disorganization of the equatorial plate in metaphase (Fig.3E), gigantic cells (Fig.3F) and apoptotic bodies (Fig.3G).

The statistical analysis ANOVA showed non-significant differences between the aberration indices for our different extracts, reflecting a similar genotoxic effect of our extracts with colchicine and quercetin (positive controls).

Chromosomal aberrations are changes in the structure of chromosomes resulting from the breakage or exchange of chromosomal material (Tülay and Özlem, 2010; Firbas and Amon, 2014). These observed aberrations indicate the effect of the aqueous extract and alkaloid fraction of the leaves of *Peganum harmala* L. on the organization of chromatin. This may be related to disorders in the quantity of histones, or other proteins, responsible for controlling the structure of nuclear chromatin (Stryer, 1997). Chromatin condensation can be produced as a result of stress conditions (Fusconi *et al*., 2006).

The frequent appearance of chromosomal agglutination in *Allium cepa* L. cells is probably due to an inhibition of the entry of prophases and a blockage of those in progress (Deysson, 1956). The presence of chromosomal bridges is a complex phenomenon related to fragmentation, stickiness of chromosomes, breaks and reunion of broken ends (Kabarity *et al*., 1974). Chromosomal fragmentations thus indicate the clastogenic potential of certain chemical compounds from plants; they can also be a consequence of anaphase/ telophase bridges (Chuhan and Gupta, 2005). Their action on chromosomes is generally localized at the DNA level (Chuhan and Sandararaman, 1990). Dose-response analyses are worth pursuing as they reflect the shift in genotoxicity from a simple yes/ no binary characteristic to a quantitative measure that can better inform risk assessments, since the margin of exposure and other toxicological principles can be taken into account (Dearfield, 2017; Dertinger, 2019).

CONCLUSION

Our work on the study of the antimitotic and genotoxic effect of the alkaloid and flavonoid fractions of the leaves of *Peganum harmala* L. on the meristematic cells of *Allium cepa* L. showed the accumulation of cells at the prophase stage, the presence of chromosomal fragmentation and agglutination, a cytotoxic effect on the cells resulting in gigantic cells, and the absence of cytodieresis. A mitodepressive effect on the root meristematic cells of *Allium cepa* L., was also observed in cells treated with the alkaloid fraction and those of flavonoids from the leaves of *Peganum harmala* L. considering them sublethal on the cells. The effect of *Peganum harmala* L. leaf extracts on other eukaryotic systems and by other experimental approaches should be investigated to obtain additional information on the antimitotic and genotoxic effect of the secondary metabolites of these extracts, which should contribute to the elucidation of their mechanisms of action.

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REFERENCES :

Akinboro, A. and Bakare, AA. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *J. Ethnopharmacol.,* **112**:470-475.

Al yahya, MA.1986. Phytochemical studies of the plants used in traditional medicine of Saudi Arabia. *Fitoterapia,* **52** (3):179-182.

Antosiewicz, D. 1990. Analysis of the cell in the root meristem of *Allium cepa* L. under the influence of Ledakrin. *Folia Hi stochem.Cytobiol.,* **26**:79-96

Bekkara, F., Jay, M., Viricel, M.R. and Rome, S. 1998. Distribution of phenolic compounds within seed and seedlings of two *Vicia faba* cvs differing in their seed tannin content, and study of their seed and root phenolic exudations. *Plant and Soil*, **203** (1):27-36.

Bellakhdar, J. 1997. La pharmacopée marocaine traditionnelle; Médecine arabe et ancienne et savoirs populaires. [Traditional Moroccan Pharmacopoeia; Arabic and Ancient Medicine and Popular Knowledge]. Ibis Press. pp 247. French.

Beyer, J., Drummer, O.H. and Maurer, H.H. 2009. Analysis of toxic alkaloids in body samples. *Forensic Sci. Int.,* **185**: 1-9.

Bonciu, E., Firbas, P., Fontanetti, C.S., Wusheng, J., Karaismailoðlu, M.C., Liu, D., Menicucci, F., Pesnya, D.S., Popescu, A., Romanovsky, A.V., Schiff, S., Œlusarczyk, J., de Souza, C.P., Srivastava, A., Sutanh, A. and Papini, A. 2018. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia,* **71**(3):191-209.

Bosetti, C., Spertini, L., Parpinel, M., Gnagnarella, P., Lagiuo, P., Nergri, E., Franceschi, S., Montella, M., Peterson, J. and Dwyer, J. 2005. Flavonoïds and breast cancer risk in Italy. *Cancer Epidemiol. Biomarkers Prev.,* **14:**805-808.

Cabrera, G.L. and Rodriguez, D.M.G.1999. Genotoxicity of soil from farmland irrigated with waste water using three plant bioassays. *Mutat. Res.,* **426**(2):211-214.

Chauhan, L.K.S., Saxena, P.N. and Upta, S.K. 1998. Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa*. *Environ. Exp. Bot.,* **42**:181-189.

Chuhan, L.K.S. and Gupta, S.K. 2005. Combined cytogenetic and ultrastructural effects of substituted urea herbicides and synthetic pyrethroid insecticide on the root meristem cells of *Allium cepa*. Pestic. *Biochem. Phys. J.,* **82**(1):27-35.

Chuhan, L.K.S. and Sundararaman, V.1990. Effects of substituted ureas on plant cells : Cytoligical effects of isopruturon on the root meristem cells of *Allium cepa* L. *Cytologie,* **55** (11):91-98.

Damato, F. 1954. Action des facteurs physiques et chimiques sur la mitose.[Action of physical and chemical factors on mitosis]. *Int. Bot.,* **9** : 1-9. French.

Dearfield, K.L., Gollapudi, B.B., Bemis, J.C., Benz, R.D., Douglas, G.R., Elespuru, R.K., Johnson, G.E., Kirkland, D.J., Lebaron, M.J., Li, A.P., Marchetti, F., Pottenger, L.H., Rorije, E., Tanir, J.Y., Thybaud, V., Van Benthem, J., Yauk, C.L., Zeiger, E. and Luijten, M. 2017. Next generation testing strategy for assessment of genomic damage: a conceptual framework and considerations. *Environ. Mol. Mutagen.,* **58** (5):264-283.

Dertinger, S.D., Kraynak, A.R., Wheeldon, R.P., Bernacki, D.T., Bryce, S.M., Hall, N., Bemis, J.C., Galloway, S.M., Escobar, P.A. and Johnson, G.E. 2019. Predictions of genotoxic potential, mode of action, molecular targets, and potency via a tiered multiflow® assay data analysis strategy. *Environ. Mol. Mutagen*., **60**(6): 513-533.

Deysson, G. 1956. Les facteurs de la mito-inhibition végétale.[Factors of plant mito-inhibition].Volume VI. Springer-Verlag, Berlin. pp 241-274. French.

Duke, J.A. 1929. Handbook of Biological Active Phytochemicals and their Active Phytochemicals and their Activities. CRC Press, Boca Raton, FL.

Farnsworth, N.R. 1968. Hallucinogenic plants. *Science,* **162**:1086-1092.

Fink, B.N., Steck, S.E., Wolff, M.S., Birtton, J.A., Kabat, G.C., Gaudet, M.M., Abrahmson, P.E., Schroeder, J.C., Neugout, A. and Gommon, M.D. 2007. Dietary flavonoïd intake and breast cancer survival among women on Long. Island. *Cancer Epidemiol. Biomarkers Prev*.,**16**:2285-2292.

Firbas, P. and Amon, T. 2014. Chromosome damage studies in the onion plant *Allium cepa* L. *Caryologia,* **67**(1):25-35.

Firbas, P. and Amon, T. 2014.Chromosome damage studies in the onion plant *Allium cepa* L..*Caryologia,* **67**(1):25-35.

Firbas, P. and Amon, T. 2017. Combined of chemical analysis, fish micronuclei and onion chromosome damage for assessing cleaning effect in the WWTP central Dom•ale-Kamnik and quality of Kamniška Bistrica River. *Cepal Review*, **121**:2825-2842.

Fiskesjö, G. 1985. The *Allium* test as a standard in environmental monitoring. *Hereditas,* **102**: 99-112.

Fiskesjö, G. 1993. The *Allium* test in wastewater monitoring. *Environ. Toxicol. Water Qual.,* **8**:291-298.

Fusconi, A., Repetto, O., Bona, E., Massa, N., Gallo, C., Dumas-Gaudot, E. and Berta, G. 2006. Effects of cadmium on meristem activity and nucleusploidy in roots of *Pisums ativum* L. cv. Frisson seedlings. *Environ. Exp. Bot.,* **58**:253-260.

Grant, W.F. 1994. The present status of higher plant bioassays for the detection of environmental mutagens. *Mutat. Res*., **310**:175-185.

Grant, W.F. 1999. Municipal Land filllechate induces cytogenetic damage in root tips of Hordeum vulgare. *Ecotoxicol. Environ. Saf.,* **63**: 469-473.

Halim, A.F., Saad, A.A. and Hashish, N.E .1995.Flavonol glycosides from *Nitraria Retusa*. *Phytochem.,* **40**:349-351.

Harbone, J.B. 1998. A guide to modern techniques of plant analysis. *Pytochemical methods, 3rd edition. Published by Chapman and Hall, London*.168pp.

Herraiz, T., González, D., Ancín-Azpilicueta, C., Arán, V. J. and Guillén, H. 2010. Beta-Carbolinealkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO). *Food.Chem. Toxicol.,***48**(3): 839-845.

Jahier, J. 1992.Techniques de cytogénétique végétale. INRA, ed., Paris.181pp.

Jinous, A. and Fereshteh, R. 2012. Chemistry, pharmacology and medicinal properties of *Peganum harmala* L. *Afr. J. Pharm. Pharmaco*., **6**(22): 1573-1580.

Jovtchev, G., Stergios, M. and Schubert, I. 2002. A comparison of N- methyl- N nitrosourea-induced chromatid aberrations and micronuclei in barley meristems using FISH techniques. *Mutat. Res.,* **517**:47-51.

Kabaraty, A., El-bayoumi, A.S. and Habib, A.A. 1974. Effects of morphine sulphate on mitosis of *Allium cepa* root tips. *Biol. Plantarum*., **16:**275-282.

Limane, A., Smail-Saadoun, N., Belkebir-Boukais, A. and Kissoum-Hamdini, K. 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. *Turk. J. of Bot.*, **38**:536-549.

Marcano, L., Carruyo, I., Fernandez,Y., Montiel, X. and Torrealba, Z. 2006. Determination of vanadium accumulation in onion root cells (*Allium cepa* L.) and its correlation with toxicity. *Biocell*., **30**:259-267.

Nandi, P. and Ghosh, S.N. 2016. Effect of medicinal plants as intercrop on plant and soil of Mosambi sweet orange gown in laterite oil. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **2**(2):11-13.

Panda, B.B. and Sahu, U.K. 1985. Induction of abnormal spindle function and cytokenisis inhibition in mitotic cells of *Allium cepa* by the organophosphorus incecticide fensulfotion. *Cytobios*, **42**:147-155.

Pulpati, H., Biradar, Y.S. and Raiani, M. 2008. High performance thin layer chromatography-densitometric method for the quantification of harmine, harmaline, vasicine, and vasicinone in *Peganum harmala*. *J. AOAC. Int.,***91**:1179-1185.

Quezel, P. and Santa, S. 1963.Nouvelle flore de l'Algérie et des régions désertiques méridionales.[New flora of Algeria and southern desert regions].CNRS, ed., T2.French. 59pp.

Sabitha Rani, A., Hajera Sana, G., Sulakshana, E., Shravya P. and Keerti, M. 2019. Spilanthes acmella-an important medicinal plant. *International Journal of Minor Fruits , Medicinal and Aromatic Plant.***5** (2): 15-26.

Sadaf, H.M., Bibi, Y., Arshad, M., Razzaq, A., Ahmad, S., Iriti, M. and Qayyum, A. 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 Pharm. J.***xxx** (xxxx) : xxx.

Sharaf, M., El –ansari, M.A., Matin, S.A. and Saleh, N.A. 1997. Four flavonoids glycosides from *Peganum harmala* L . *Phytochem.,* 44: 533-536.

Sharma,C.B. 1983. Plant meristems as monitors of genetic toxicity of environmental chemicals. *Curr. Sci.,* **52**:1000-1002.

Shweta, S., Khadabadi, S. and Ganesh, T.G. 2012. *In vitro* antimitotic, antiproliferative, DNA fragmentation and anticancer activity of chloroform and ethanolextract of *Revia hypocrateriformis. Asian Pac. J.Trop. Dis.* Suppl: 503-506.

Siddiqui, S., Meghvansi, M.K. and Hasan, Z. 2007. Cytogenetic changes induced by sodium azide (NaN_3) on Trigonella foenum-graecum L. seeds. *S. Afr. J. Bot.,* **73**:632-635.

Stryer, L. 1997. Biochemia. Wydawnictwo Naukowe PWN, Warszawa.1032-1069.

Sultan, A.O. and Çelik, T.A. 2009. Genotoxic and antimutagenic effects of *Capparis spinosa* L. on the *Allium cepa* L. root tip meristem cells. *Caryologia,* **62**(2):114-123.

Tülay, A.Ç. and Özlem, S.A. 2010. Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* Leaf extracts with *Allium* Test. *J. Biomed. Biotechnol.,* 1-8.

Yi, H.L. and Meng, Z.Q. 2002. Acta Phytoecol. Sin. 26: 303-307. In: Sang, N., Li, G. and Xin, X. 2006.*Municipal landfill lechate induces cytogenetic damage in root tips of Hordeum vulgare. Ecotoxicol. Environ. Saf.,* **63**:469-473.