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Antimitotic effect of *Verbascum sinuatum* L. extracts on meristematic cells

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ABSTRACT

Verbascum sinuatum L. is a medicinal plant of the Scorphuliaceae family, widespread in Algeria. It is rich in bioactive molecules, which gives it various biological effects. This study targeted the fraction of alkaloids. We studied the effect of this fraction on the mitosis of meristematic cells. Different indices of genetic alterations were calculated. These results were compared to the negative and positive control. An ANOVA type statistical study ($p< 0.05$) was performed. The amount of alkaloid found in the studied fraction is 50.17µg/g of plant powder. This targeted fraction presents a significant antimitotic effect the cells of onion. This mitodepressive effect generated several abnormalities in chromosomes, nuclei and cells represented mainly by agglutinations and chromosomal fragmentations.

Keywords: *Allium cepa* L., *Verbascum sinuatum* L., alkaloids, antimitotic, genotoxic.

INTRODUCTION

Verbascum sinuatum L.,is also called the sinuous mullein. This biennial herbaceous plant of the family Scorphulariaceae is widespread in the Mediterranean area. They are 80 to 200cm tall plants with sinuous basal leaves and yellow flowers (Qureshi and Bhatti, 2008). Phytochemical studies of the genus *Verbascum* have isolated and characterized a large number of bioactive molecules like (Sarralheiro *et al.,*2020) in their study on the identification of metabolites of *Verbascum betonicifolium* L. identified mainly iridoids, glycosides and flavonoids. This richness in bioactive molecules makes this genus a reservoir of medicinal plants widely used in phytotherapy in many treatments. Priyanka and Ghosh (2016) established the relationship between plants for therapeutic use with physicochemical properties of soils. The biological effects of different species of Verbascum have been of great interest. The work of (Yagmur *et al.,* 2019) on leaf extracts of *Verbascum exuberans* L. revealed an anti-inflammatory effect by suppression of TNF α and interleukin 1- beta production. Furthermore, leaf extracts of *Verbascum siniatucum* L. have an effect on the viability of *Trypanosoma congolense* and may present a solution in pest control (Mergia, 2016). Many other biological effects such as antioxidant and hepatoprotective effects have been reported (Grygor *et al.,* 2013).

Alkaloids are nitrogenous compounds of very variable structure and essentially of plant origin. There is an interest in the therapeutic use of these bioactive molecules. Thus, in the face of managing the spread of the corona virus, studies have targeted the antiviral action of alkaloids (Yejin *et al.,*2021) have highlighted the action of gemcitabine, oxysophoridine and lycorine on the proliferation of the corona virus. The alkaloids derived from spermine are the most frequently met in the genus *Verbascum.* They present, in general, a macrocyclic skeleton constituted most often, by a hydroxystrilamine group, a typical amino acid and a â hydroxyamino acid. The biological effects are closely related to the structure of these biomolecules. They have mechanisms of action and molecular targets closely related to their structure (Sarralheiro *et al.,*2020). Their main targets are the microtubules of the mitotic spindle and topoisomerase I and II. As such, they are potential candidates in the development of new molecules in chemotherapeutics in the treatment of various cancers (Imperatore *et al*., 2014).

In this context, we were interested in evaluating the effect of *Verbascum sinuatum* L. leaf alkaloids

on the division of root cells of *Alluim cepa* L. harvested in northern Algeria (Bejaia). To our knowledge, the mitotoxic and genotoxic effects of leaf alkaloids *of Verbascum sinuatum* L. isolated in Algeria have not been reported. Our study is based on the *Allium cepa* test. It allows, on the one hand evaluating chromosome abnormalities in cells of onion and on the other hand to highlight disturbances of mitosis (Ma *et al*., 2005). This assay is widely used assessment of genotoxicity of natural substances in the environment. It is based on the use of meristematic cells of *Allium cepa* L., to view the damage and disruption of cell division (Olorunfemi *et al.*, 2012).

MATERIALS AND METHODS

Sampling

Verbascum sinuatum L., leaves were collected in the region of Addekar in Sif El Hammam in the region of Bejaia (Algeria). They were dried in a dry place and then reduced to powder. The vegetable powder obtained was kept at 4°C until use.

Preparation of the alkaloid fraction

The extraction of the alkaloid fraction is based on their solubility difference according to the pH, according to the protocol of (Harbonne,1998) with some modifications. 5g of *Verbascum sinuatum* L. plant powder was defatted with 10ml petroleum ether for 24h at 25°C. The defatted plant powder was soaked as in methanol and then filtered. After drying we proceed to the solubilization in chloroform acidified at pH 3. After decantation, the acid phase is added with 10 ml of chloroform then adjusted to pH 9 with Na_2CO_3. After evaporation at 60° C., the dry residue obtained is stored at 4° C. until use. During its use, this fraction is dissolved in physiological water.

Antimitotic test

This test is based on the work of (Aashiq *et al*., 2016), onion bulbs (0.5 to 1 cm) are put in distilled water for 3 days at 37°C then root apices were contacted with the alkaloid fraction at 1mg/ml. After one day of incubation, roots apices are fixed with acetic acid and 95% methanol (1:3 V/V). The fixed roots are stained with acetic carmine. The samples are observed under an optical microscope at magnification (X400).We count the different stages of cell division. Aberrations are counted out of 350 cells

Alkaloid assay

Leaf alkaloid content is estimated according to the protocol of Patel et *al*. (2015). This assay is based on the ability of alkaloids to form a colored complex in the presence of ferric chloride ($FeCl_3$) with a maximum absorbance at 380nm. A standard curve of equation: y=0.007x; R2=0.988 is established using colchicine as a control. We express the results as µg quercitin equivalent/g vegetable powder (µg eq Q/g).

Cytogenetic analysis

The parameters evaluated are: Mitotic index (MI): dividing cells/total cells X100. Phase index (PI): cells in each phase/total cells X100. Cytotoxicity limit value (CLV): Mitotic index of treated cells/ Mitotic index of untreated cells X100. Aberration index (AI): cells with aberration/total observed aberrations X100.

Statistical analysis

An analysis of variance (ANOVA) was performed with SATISTICA software.

RESULTS AND DISCUSSION

Mitotic index is used to assess cell division. It allows estimating the number of cells actually able to divide (Siviková, 1996). *Allium cepa* L cells treated with distilled water (T-) showed the highest mitotic index reaching 92.20±1.34 (Table 1). These root cells exhibited all phases of cell division. Thick chromosomes located at the equatorial plate identify the prophase, characterized by a condensed nucleus and the metaphase. Anaphase can be recognized by the migration of the two groups of chromosomes towards the poles. Telophase represents the reconstitution of the nucleus in the two daughter cells (Figure 1). The analysis of Table 1 revealed a significant action of colchicine and the leaf alkaloid fraction of *Verbascum sinuatum* L on root cell division. They exhibited mitotic indices of 35.00±0.72 and 33.00±2.33 in contact with colchicine and alkaloid fraction, respectively.

Table 1: Mitotic parameters evaluated with our different samples.

	Negative control	Positive alkaloid control	Alkaloid fraction
Mitotic Index	92.20±1.34	35.00±0.72	33.00±2.33
Prophase Index	82.91±3.07	32.28±1.73	32.162.41
Metaphase Index	0.62±0.39	0.11±0.05	0.05±0.04
Anaphase Index	0.96±0.34	0.28±0.13	0.00±0.00
Telophase Index,	7.98±3.52	2.85±0.70	0.91±0.47
Chromosomal agglutinations	-	05.60±1.42	21.49±1.05
Binucleated cells	-	0.74±0.66	0.05±0.04
Chromosomal bridges	-	0.00±0.00	0.05±0.04
Chromosomal fragmentations	-	1.89±0.55	2.06±0.5
Disorganization of the equatorial plate	-	0.23±0.14	0.00±0.00
Cells without a nucleus	-	2.8±1.61	1.94±0.20
Cell elongations	-	0.86±0.37	0.29±0.25

Cell cycle checkpoints allow genome integrity. DNA damaging agents activate cell cycle checkpoints that block entry into mitosis. The mechanisms of checkpoint activation by alkaloids have been explored in the development of antiproliferative molecules to treat many cancers. Some alkaloids activate these checkpoints. Following oxidative stress, xylophene induces cycle arrest in G2/M leading to cell death. As for piperine, another alkaloid, induces apoptosis by blocking the p13K/Akt/Gsk3â signal transduction pathway in OVACAR-13 ovarian cancer cells (Chen *et al*., 2020).

The antimitotic effect of some alkaloids is related to their structural properties. This is the case for pyrizidine alkaloids. They contain functional groups capable of binding directly to the DNA molecule. The DNA-alkaloid complex leads to cross-linking of the DNA fragment and a blockage of mitosis. Alkaloids can also block mitosis by binding to DNA and induce inhibition of topoisomerase II (Sung *et al*., 1999).

Topoisomerases I are involved in the regulation of DNA supercoiling during its transcription and replication. They represent a privileged target of antimitotic agents. Some alkaloids by binding to the TOP I-DNA complex cause irreversible DNA breaks, which induce a slowing down of the replication fork progression. Recently, Pourquier and Lansiaux (2011) developed clinical derivatives of alkaloids to better target their binding Site.

Alkaloids can also block mitosis by preventing mitotic spindle formation. Colchicine is an alkaloid known for its high affinity to tubulin. It binds irreversibly to microtubules. Its binding domain is located between the two subunits of the same dimer to form a complex that is unfavorable to the polymerization of microtubules and thus interferes with the formation of the mitotic spindle. Also other alkaloids act as mitotic spindle poisons. They interact directly with tubulin, induce structural changes, and thus inhibit its depolymerization. This leads to a blockage of mitosis in prophase by preventing the formation of the mitotic spindle (DeLuca *et al*., 2020).

Alkaloids of plant origin are of growing interest for their antiproliferative effect. This biological effect is closely related to concentration. Recently, Israel *et al*. (2015) optimized the extraction conditions of alkaloids. We obtained in this study 50µg eq Q /g of plant powder. The rate of molecule that can be extracted is closely related to the extraction conditions (Chaudhary *et al*., 2008).

Microscopic observations showed that the alkaloid fraction of *Verbascum sinuatum* L leaves as well as colchicine at 1mg /ml (positive control) induced a large number of chromosomal abnormalities (Table1). Mostly, agglutinations were observed in the presence of the leaf alkaloid fraction with an aberration index of 21.49±1.05% against 05.60±1.42% for the positive controls. Binucleated cells, chromosomal fragmentations,

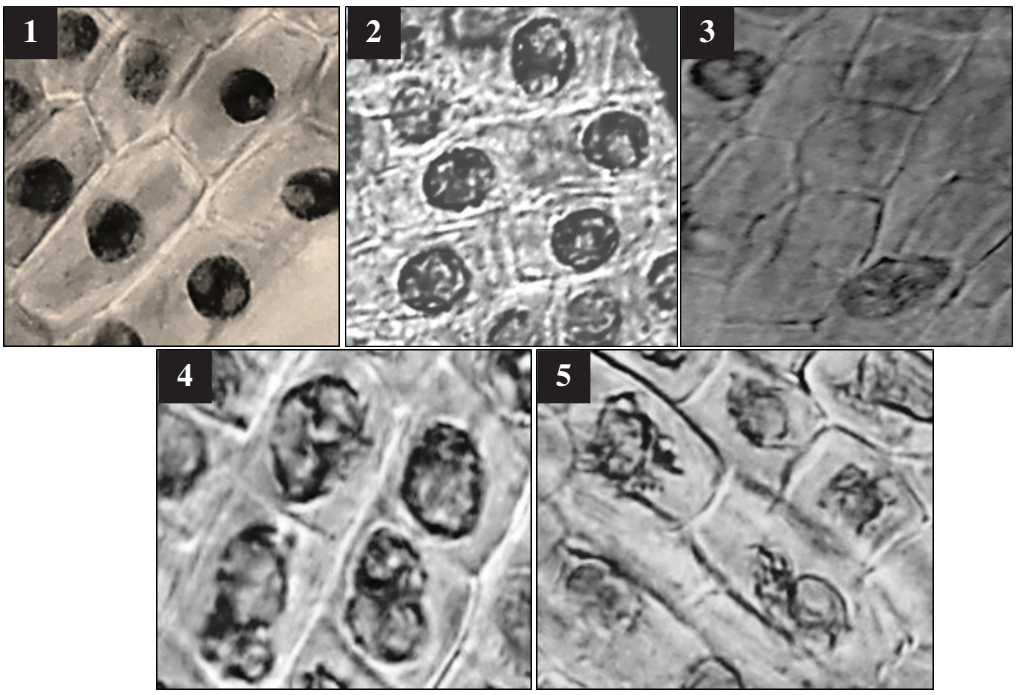

Fig.1 : **Abnormalities identified in onion cells (X400 magnification)**
1 : **Cell elongations**; **2**: **Chromosomal agglutinations; 3: Cells without a nucleus;**
4 : **Binucleated cells**; **5** : **Chromosomal fragmentations.**

and cells without nuclei were very weakly observed in contact with both samples. No disorganization of the equatorial plate was observed upon contact with the alkaloid fraction. However, chromosome bridges were observed only in contact with the latter (05±0.04%).

Disorganizations of the equatorial plate of *Allium cepa* meristematic cells upon contact with *Verbascum sinuatum* L. leaf alkaloids may be due to an aneugenic effect. This effect is called numerical damage of chromosomes. It is related to the ability to induce poor chromosome separation resulting from disruption of kinetochore and mitotic spindle regulation. Taxol is selected as a reference indicator of this effect in the development of software to determine the molecular mechanism of genotoxic agents. Nucleus-less cells and binucleated cells is an anagenic effect that may be related to the activity of Aurora kinases. These enzymes are key enzymes in the regulation of the centromere cycle and are therefore biomarkers in the determination of chromosome mis-segregation.

Structural damage of chromosomes is at the origin of the formation of chromosome bridges and fragmentations observed in contact with *Verbascum sinuatum* L. leaf alkaloids. This clastrogenic effect may be the result of DNA damage. Modeling genotoxic effects is the subject of a large number of studies (Dertinger *et al*., 2019).

CONCLUSION

Our work revealed a mitodepressive effect of the alkaloid fraction of *Verbascum sinuatum* L. leaves on cells of onion. The genotoxic action of this fraction causes a large number of cell division abnormalities. Agglutinations represent the majority of this dysfunction of mitosis. We also noted with variable frequencies, gigantic cells, binucleated cells, disorganization of the equatorial plate and chromosomal fragmentations. It would be very interesting to optimize the extraction conditions and to elucidate the molecular basis of the aneugenic and clastrogenic effect of *Verbascum sinuatum* L. leaf alkaloids.

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