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Cytological Effects of Certain Active Constituents of *Peganum harmala* L. 1. Effect of Harmol and Harmine Alkaloids on Mitosis of *Allium cepa*

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Abstract. The present study was carried out to investigate the effects of harmol and harmine alkaloids extracted from the medicinal plant *Peganum harmala* L. on the root tips of *Allium cepa* L.

The cytological studies included mitotic index, total percentage of abnormalities and percentage of each type of abnormalities.

The alkaloids used caused an increase in the mitotic index in *Allium capa* roots and induced a high percentage of abnormalities.

Introduction

Many alkaloids which are of plant origin have proved to cause cytological abnormalities. Since the discovery of colchicine as a polyploidizing agent [1], numerous alkaloids have been tested cytologically by several investigators. Among the alkaloids tested, veratrine, vinblastine, and vinblastine and yohimbine were found to have substantial cytological effects [2-4].

In this study, an attempt was made to investigate the cytological effect of two alkaloids separated from *Peganum harmala* L., namely, harmol and harmine. *Peganum harmala* L. is a herbaceous plant belonging to the family zygophyllaceae and is common in the coastal belt of the Mediterranean sea. This plant has proved to have antibacterial [5], antirheumatic and analgesic activities [6] due to its alkaloidal content. The oil extracted from seeds of this plant was mentioned by Charnot to have a therapeutic value in some eye infections [7].

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Materials and Methods

Peganum harmala L. naturally growing in Mersa Mattrouh, Egypt was used. The pure alkaloids harmol and harmine were isolated from the chloroform extract of the plants of *Peganum harmala* L. using the thin layer chromatographic method [8].

Cytological experiments

Allium cepa L. (var.- Giza 6 Mohassan) was used for the cytological experiments. *Allium cepa* roots were treated with 20 and 40 µg/ml of each alkaloid for a duration of 2, 4 and 8 hours. Tap water was used for the control experiments. The roots were then cut off, fixed in Carnoy's fixative (acetic acid, alcohol 1:3) for 24 hours; leuco basic Fuchsin preparations were used for scoring. 1000 – 2000 cells were counted from 5 different root tips for each treatment. The mitotic indices of the treated roots and their control were determined using the following formula:

$$\text{Mitotic index} = \frac{\text{No. of dividing cells}}{\text{Total No. of the cells}} \times 100$$

Results and Discussion

The alkaloids harmol and harmine caused, in general, an increase of the mitotic index in *Allium cepa* root tip meristems (Table 1). The mitotic index increased with increasing time of treatment in most cases. On the other hand, the mitotic index decreased on increasing harmol concentration from 20 to 40 µg/ml, but for harmine, a notable increase in the mitotic index was observed by increasing its concentration.

The increasing effect of harmol and harmine on the mitotic index may be due to shortening the duration of the mitotic cycle in cells that were in the interphase stage at the time of treatment since there was a concomitant increase in metaphases and anaphases [9].

Table 1 shows also that the treatment of *Allium cepa* roots with harmol and harmine alkaloids resulted in a remarkable increase in the percentage of abnormalities reaching more than 95% for all treatments. A similar high increase in the percentage of total abnormalities in *Vicia faba* and *Allium cepa* after treatment with Vinca alkaloids was reported by Abd-El-Tawab [10].

Table 1. Mitotic index and percentage of total abnormalities in *Allium cepa* L. roots treated with harmol and harmine

Alkaloid	Time of treatment (hr.)	Mitotic index alkaloid conc. (μg/ml)			% of total abnormalities alkaloid conc. (μg/ml)		
		–(cont.)	20	40	–(cont.)	20	40
	0	15.42			12.37		
	2		27.91	18.97		97.14	94.38
	4		30.87	24.58		99.03	96.76
	8		40.27	36.26		100.00	100.00
	0	15.42			12.37		
Harmine	2		24.98	34.17		97.61	98.02
	4		30.19	30.33		99.34	98.92
	8		34.74	38.75		100.00	100.00

Different types of abnormalities were observed in *Allium cepa* roots treated with the two alkaloids (Table 2).

Despiralization is one of the major anomalies, reaching 32–46% of the total percentage of abnormalities observed. Despiralization was found also to increase with increasing harmol and harmine concentrations. This was observed clearly in the prophase stage (Fig. 1). The appearance of this phenomenon might be due to that the two alkaloids might cause partial or complete dissolution of the matrix substance that belonging to the chromosomes or they might interfere with the process of synthesis between RNA and proteins as Hakeem and Shehab have suggested [11].

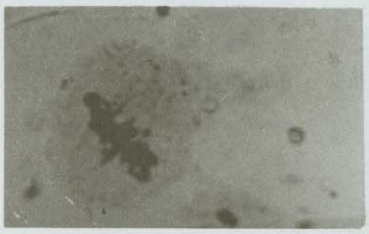

Fig. 1. Sticky metaphase I in *Vicia faba* PMCs after spraying with Gesal insecticide

Abnormal prophase is a common abnormality in all treatments with the two alkaloids in a relatively high percentage of cells. In these abnormal prophases, the arrangement of chromatin threads was abnormal (Fig. 2). Similar abnormal prophases were observed by Adam and Rashed after treatment of *Vicia faba* with the extract of *Ammi majus* [12].

Table 2. Percentage* of each type of abnormality in *Allium cepa* roots treated with harmol and harmine alkaloids

Alkaloid conc. µg/ml	Time of treat. (hr.)	Harmol							Harmine							
		Desp.	Abnor. proph.	Spind. dist.	Stick-iness	Lagg-ing	Rough surface	Bridges	Desp.	Abnor. proph.	Spind. dist.	Stick-iness	Lagg-ing	Rough surface	Bridges	Contrac-tion
20	2	42.08	35.69	6.54	5.07	5.92	3.97	0.73	41.31	28.44	13.69	4.76	5.82	4.75	1.15	0.08
	4	38.25	30.73	10.30	6.09	8.57	4.76	1.30	32.88	27.40	20.55	4.84	5.42	5.66	0.52	2.37
	8	35.26	29.23	14.91	6.58	9.64	4.01	0.37	38.44	28.73	18.02	2.86	3.54	6.73	0.17	1.51
40	2	46.56	32.02	7.46	6.00	5.32	1.74	0.90	43.75	29.92	10.03	4.52	7.55	4.11	0.12	–
	4	44.08	35.74	6.95	4.47	4.38	3.79	0.59	39.08	30.81	13.30	4.56	6.71	4.57	0.97	–
	8	43.52	30.67	10.15	5.54	0.04	3.80	0.28	45.10	30.32	12.77	3.25	5.00	3.03	0.26	

*The percentages of the types of abnormalities were calculated relative to the number of abnormal cells.

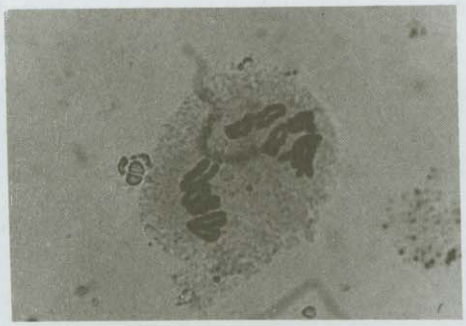

Fig. 2. Disturbed anaphase I in PMCs of *Vicia faba* plant after spraying with Gesal insecticide

Spindle disturbance was another anomaly observed on treatment of *Allium cepa* roots with harmol and harmine alkaloids but in relatively low percentages compared with the two previous anomalies (Table 2). Spindle disturbance included the following forms:

1) Prophase metaphase, where the metaphase chromosomes retained their prophase positions (Fig. 3).

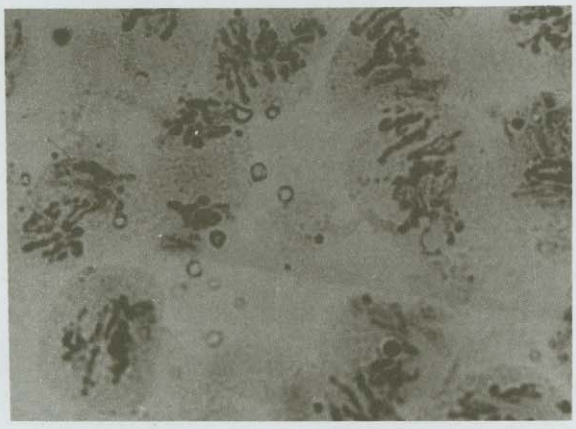

Fig. 3. Disturbed metaphase II in *Vicia faba* PMCs after spraying with Gesal insecticide and recovery for 4 days

2) Disturbed metaphases, in which the chromosomes were spread irregularly in the cell. This is also known as colchicine-metaphase or C-metaphase (Fig. 4).

Fig. 4. Multipolarity in anaphase I in *Vicia faba* PMCs after spraying with Gesal insecticide

3) Disturbed anaphase.
4) Multipolarity.
5) Polyploidy; this might be a consequence of earlier spindle irregularities.

The prophase metaphase abnormality observed in this study was similar to that observed by other authors as a result of treatment with sevin [13] and cyanine dyes [14].

Disturbed metaphases and anaphases were also noted in most treatments as a small percentage. This might be due to a disturbance in the formation and function of the mechanism responsible for chromosome movement; this abnormality has also been reported by Shehab [15] and Mohamed [16].

Other types of anomalies observed in the roots treated with the alkaloids harmol and harmine but as a small percentage included stickiness, lagging, rough surfaced chromosomes and bridges. The rough surfaced appearance of the chromosomes may be a result of depletion of proteins surrounding the chromosomes. Contraction of chromosomes was observed in *Allium cepa* roots only after treatment with 20 μg/ml harmol

Micro, macro nuclei and giant nuclei were observed in the interphase cells in *Allium cepa* roots treated with the alkaloids used.

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التأثير السيتولوجي لمواد فعّالة معيّنة لبعض النباتات الطبية على نبات البصل

تأثير قلويدات الهارمول والهارمين على الانقسام الميتوزي للبصل

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. (استلم في ٢١ شوال ١٤١٠هـ، قبل للنشر في ٢١ ذي القعدة ١٤١١هـ).

ملخص البحث. تناولت هذه الدراسة تأثير بعض قلويدات الهارمول والهارمين المستخلصة من نبات البيجانم (الحرمل) على خلايا القمم النامية لجذور البصل.

ونبات البيجانم (الحرمل) نبات عشبي ينتمي إلى العائلة الرطراطية وينمو بمحاذاة شاطىء البحر الأبيض المتوسط، وله بعض الفوائد الطبية، منها أنه يستعمل كمضاد لبعض أنواع البكتيريا والالتهابات الروماتيزمية ومسكن لبعض الآلام.

وقد تم استخلاص قلويدات الهارمول والهارمين من نبات البيجانم بواسطة كروماتوجرافيا الطبقة الدقيقة باستعمال الكلوروفورم والميثانول كمذيبات عضوية.

وقد عرضت جذور البصل لمحاليل هاتين المادتين كل على حدة بتركيزات ٢٠، ٤٠ مكجم/ مل لفترات زمنية ٣، ٤، ٨ ساعات، وذلك لدراسة التأثير السيتولوجي لهما.

وتضمنت الدراسة السيتولوجية معدّل الانقسام الميتوزي والنسبة المئوية للشذوذ الكروموسومي والنسب المئوية لكل نوع من أنواع الشذوذ الكروموسومي.

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وقد أوضحت الدراسة أن هاتين المادتين سببا زيادة في معدّل الانقسام الميتوزي لخلايا جذور البصل. كما أوجدنا نسبة عالية من الشذوذ الكروموسومي، وكانت أهم أنواع الشذوذ الكروموسومي السائدة الانفكاك الحلزوني، شذوذ الطور التمهيدي، اضطراب المغزل، لزوجة الكروموسومات بالإضافة إلى أنواع أخرى من الشذوذ الكروموسومي ولكن بنسب أقل.