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Assessment of Cytogenotoxic Potential of Aqueous Fruit Extracts of *Pistacia lentiscus* L. Using the *Allium cepa* Bioassay

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تقييم السمية الخلوية الجينية للمستخلصات المائية لثمار البطوم (*Pistacia lentiscus* L.) باستخدام اختبار البصل (*Allium cepa*)

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Abstract:
Libya is characterized by a rich diversity of medicinal plants, among which *Pistacia lentiscus* L. is of considerable pharmacological importance due to its content of bioactive compounds with reported sedative and therapeutic properties. The present study aimed to evaluate the cytological effects of aqueous extracts prepared from fresh fruits of *P. lentiscus* at different concentrations (5, 10, 20, and 40g/200 ml) on the root meristem cells of *Allium cepa*. The roots were exposed to the extracts for 3, 6, 12, and 24h, while distilled water served as the control. Statistical analysis using two-way ANOVA revealed a highly significant interaction between extract concentration and exposure duration on the mitotic index (MI). Treatments with 10 g and 20g concentrations caused a significant reduction in the MI compared to the control, with the greatest decrease observed after 24 h of exposure. Analysis of mitotic abnormalities showed that the 5 g treatment differed significantly from the 20 g and 40 g concentrations at 24 h. Chromosomal stickiness at metaphase was the most frequently observed abnormality, suggesting possible disruption of proteins involved in chromatin organization. These findings demonstrate that aqueous extracts of fresh *P. lentiscus* fruits can interfere with normal mitotic activity and induce chromosomal abnormalities in A. cepa cells. Therefore, careful regulation of extract concentration and exposure duration is recommended to minimize potential cytotoxic and genotoxic effects.

Keywords: Pistacia Lentiscus L, Cell Division, Chromosomal Abnormalities

الملخص
تتميز ليبيا بتنوعها الغني من النباتات الطبية، ومن بينها نبات البطوم (Pistacia lentiscus L.) ذو الأهمية الدوائية الكبيرة لاحتوائه على مركبات حيوية فعالة ذات خصائص مهدئة وعلاجية. هدفت هذه الدراسة إلى تقييم التأثيرات الخلوية للمستخلصات المائية المُحضّرة من ثمار البطوم الطازجة بتراكيز مختلفة (5، 10، 20، و40 غ/200 مل) على خلايا المرستيم الجذري للبصل (*Allium cepa*). عُرّضت الجذور للمستخلصات لمدة 3، 6، 12، و24 ساعة، بينما استُخدم الماء المقطر كعينة ضابطة. كشف التحليل الإحصائي باستخدام تحليل التباين ثنائي الاتجاه (ANOVA) عن تفاعل ذي دلالة

إحصائية عالية بين تركيز المستخلص ومدة التعرض على مؤشر الانقسام الخلوي (MI). أدت المعالجات بتركيزي 10 غ و20 غ إلى انخفاض ملحوظ في مؤشر الانقسام الخلوي مقارنةً بالعينة الضابطة، مع ملاحظة أكبر انخفاض بعد 24 ساعة من التعرض. أظهر تحليل التشوهات الانقسامية أن المعالجة بجرعة 5 غرامات اختلفت اختلافًا كبيرًا عن تركيزي 20 غرامًا و40 غرامًا بعد 24 ساعة. وكان التصاق الكروموسومات في الطور الاستوائي أكثر التشوهات شيوعًا، مما يشير إلى احتمال حدوث خلل في البروتينات المشاركة في تنظيم الكروماتين. تُبين هذه النتائج أن المستخلصات المائية لثمار البطوم الطازجة قد تُؤثر على النشاط الانقسامي الطبيعي وتُسبب تشوهات كروموسومية في خلايا البصل. لذا، يُوصى بتنظيم دقيق لتركيز المستخلص ومدة التعرض لتقليل الآثار السامة للخلايا والجينات.

الكلمات المفتاحية: البطوم، انقسام الخلايا، التشوهات الكروموسومية.

Introduction

Many plant species used in traditional medicine have been shown to contain bioactive compounds with therapeutic potential, which can be used either directly or in extracts for medicinal purposes. This practice remains particularly widespread in developing countries (Nannar and Ahire,2023). According to the World Health Organization, approximately 65–80% of the global population relies on traditional medicine to satisfy basic healthcare needs, often due to economic constraints and limited access to modern medical services (Omwenga *et al*., 2015). Moreover, increasing evidence suggests that many synthetic drugs primarily alleviate symptoms without addressing the underlying causes of diseases. Consequently, crude plant preparations such as decoctions, infusions, and tinctures are commonly used in traditional medicine for treating various ailments (Mine and Yusuf, 2023).

The family Anacardiaceae represents one of the important plant families in Libya and includes several genera, notably the genus *Pistacia*. Among its species, *Pistacia lentiscus* L. is widely distributed across several regions of Libya, including Garian, Benghazi, Al-Baida, El Marj, Tokra, Ras Al-Hilal, and the Green Mountain region (Jafri and El-Gadi, 1985). Beyond Libya, this species is also found in Morocco, Algeria, Tunisia, Iran, Turkey, Iraq, Europe, and the Middle East (Asma, 2019). Locally, *P. lentiscus* is known by various vernacular names such as Baattoom, Dhorru, and Buttoom (Jafri and El-Gadi, 1985).

The chemical composition of *P. lentiscus* includes fatty acids such as oleic, linoleic, palmitic, and stearic acids (Charef *et al*., 2008), as well as phenolic compounds, including flavonoids, anthocyanins, tannins, and glycosides (Bendifallah *et al*., 2014). The fruit oil of *P. lentiscus* has been traditionally used for the treatment of wounds and burns in Mediterranean folk medicine. Previous studies have demonstrated that this oil is rich in unsaturated fatty acids, tocopherols, carotenoids, and phenolic compounds, and exhibits antimicrobial, anti-proliferative, and wound-healing properties (Djerrou *et al*., 2010; Trabelsi *et al*., 2012; Mezni *et al*., 2014, 2016, 2018). Despite its extensive traditional use, most pharmacological studies on *P. lentiscus* have focused on its essential oils and resins, while limited attention has been given to the cytotoxic and genotoxic effects of its aqueous fruit extracts. Although crude plant products are generally considered less toxic than synthetic drugs (Koul *et al*., 2005), they may still exert cytotoxic or mutagenic effects on plant, microbial, or human cells (Lee *et al*., 2004). Therefore, evaluating the cytogenotoxic potential of medicinal plant extracts is essential to ensure their safe use.

The cytotoxicity and genotoxicity of plant extracts can be effectively assessed using the mitotic index and chromosomal aberration analysis in plant bioassays. Among these, the *Allium cepa* test is widely used due to its sensitivity, reliability, and high mitotic activity in root meristem cells (Grant, 1994; Leme, 2009; Firbas and Amon, 2017). Accordingly, the present study aimed to investigate the potential cytotoxic and genotoxic effects of aqueous extracts prepared from fresh fruits of *P. lentiscus* on *A. cepa* root meristem cells.

2. Materials and Methods

Plant Identification

The plant species *Pistacia lentiscus* L. was taxonomically identified and authenticated according to Jafri and El-Gadi (1985). Identification was confirmed at the Herbarium of the Department of Botany, Faculty of Science, Omar Al-Mukhtar University, El-Bayda, Libya.

Collection of Plant Material

Fresh fruits of *P. lentiscus* L. were collected in 2023 from El Marj city, northeastern Libya. The plant exhibits early flowering in May, early fruiting in August, and late fruiting in October**.**

Source of *Allium cepa* Seeds

Seeds of *Allium cepa* L. were obtained from local agricultural supply stores in Benghazi city, Libya.

Preparation of Aqueous Extracts

Fresh fruits of *P. lentiscus* were thoroughly washed with tap water, followed by distilled water. The fruits were then ground using a mortar and pestle. Four different concentrations (5, 10, 20, and 40g) of the fresh fruit material were prepared by soaking each amount in 200 mL of distilled water in separate 250 mL beakers. The mixtures were placed on a shaker for 24 h at room temperature (28 ± 2 °C). After extraction, the solutions were filtered using filter paper. Distilled water served as the control treatment (0% concentration).

Seed Germination and Treatment

Allium cepa seeds were surface-sterilized and germinated in Petri dishes lined with filter paper. Distilled water was renewed every 24 h for four days. Seedlings with root lengths of 0.5–1 cm were selected and treated with the prepared aqueous extracts at concentrations of 0, 5, 10, 20, and 40 g/200 mL for exposure periods of 3, 6, 12, and 24 h. After treatment, root tips were fixed in freshly prepared Carnoy's solution (glacial acetic acid: absolute ethanol, 1:3 v/v) for 24 h and subsequently preserved in 70% ethanol until analysis. The roots were hydrolyzed in 1 N HCl at 60 °C for 10 min, washed with distilled water, and stained with 2% acetocarmine for 24 h (Abdulkarim *et al*., 2021). One root tip was squashed per slide and examined under a light microscope (XSZ-107 BN) at 400× magnification. Six replicates were analyzed for each treatment to calculate the mitotic index (MI) and the percentage of chromosomal abnormalities in both dividing and non-dividing cells. The indices were calculated using the following formulas (Farizan *et al*., 2021):

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Number of total cells}} \times 100$$

$$\text{Phase index} = \frac{\text{Number of cells in each mitotic phase}}{\text{Total number of dividing cells}} \text{X100}$$

$$\%\ \text{Aberrant dividing cells} = \frac{\text{Number of aberrant dividing cells}}{\text{Total number of dividing cells}} \times 100$$

Statistical Analysis

Data were expressed as mean values ± standard deviation (SD). Statistical significance was evaluated using two-way analysis of variance (ANOVA) with IBM SPSS Statistics version 20. Differences among treatment means were assessed using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

3. Results

Effect of Aqueous Extracts on the Mitotic Index

The effects of different concentrations (5, 10, 20, and 40 g/200 mL) of aqueous extracts of fresh *Pistacia lentiscus* fruits on the mitotic index of *Allium cepa* root meristem cells at various exposure times (3, 6, 12, and 24 h) are presented in Table 1 and Figure 1. Distilled water was used as the control.

The control group exhibited the highest mitotic index values at 3 and 6 h, recording means of 52.66 and 52.90, respectively. In contrast, the lowest mitotic index values were observed following 24 h of exposure to the 10 g and 20 g concentrations, with mean values of 42.00 and 41.97, respectively. The 5 g and 40 g concentrations showed comparatively higher mitotic index values.

Table 1. Effect of different concentrations of the aqueous extract of *Pistacia lentiscus* fresh fruits at different exposure times on the sum of total cells (TC), sum of cell dividing (CD), mean of the mitotic index (xMI), and the percentage of mitotic phases (P, M, A, and T).

Time (h)	Concentration (g/200ml)	sum TC	sum CD	×MI±SD	P%	M%	A%	T%
3	**Control**	2410	1269	52.66±0.729701	90.06	3.65	3.25	3.05
	5	2674	1362	50.94±1.161288	91.73	4.29	2.03	1.95
	10	3093	1386	44.82±0.528715	91.34	4.15	2.37	2.15
	20	3501	1662	47.47±0.549053	91.08	4.22	2.44	2.25
	40	4024	1886	46.87±0.217808	92.18	4.03	1.99	1.80
6	**Control**	2794	1478	52.90±0.653266	92.23	2.66	2.34	2.77
	5	3475	1586	45.64±0.653710	90.88	4.37	2.35	2.40
	10	3603	1758	48.80±0.248697	92.04	4.14	2.15	1.68
	20	3988	1874	46.99±0.261478	92.44	3.74	1.78	2.04
	40	4347	2018	46.42±0.381126	92.98	3.62	2.02	1.37
12	**Control**	3391	1754	51.73±3.230448	93.52	2.31	2.35	1.82
	5	3852	1734	45.02±0.411619	92.53	3.87	1.87	1.73
	10	4012	1787	44.54±0.241757	92.00	4.70	1.72	1.58
	20	4141	1861	44.94±0.606623	92.32	3.69	1.95	2.03
	40	4826	2286	47.37±0.119173	91.77	4.24	2.06	1.93
24	**Control**	4390	1871	42.62±0.863311	93.41	2.76	2.18	1.65
	5	4637	2152	46.41±0.256221	92.03	4.17	1.95	1.85
	10	4670	1962	42.00±0.508959	91.65	4.28	2.17	1.90
	20	5125	2151	41.97±0.241618	91.84	4.25	2.03	1.87
	40	5207	2375	45.61±0.404602	91.09	4.67	2.09	2.14

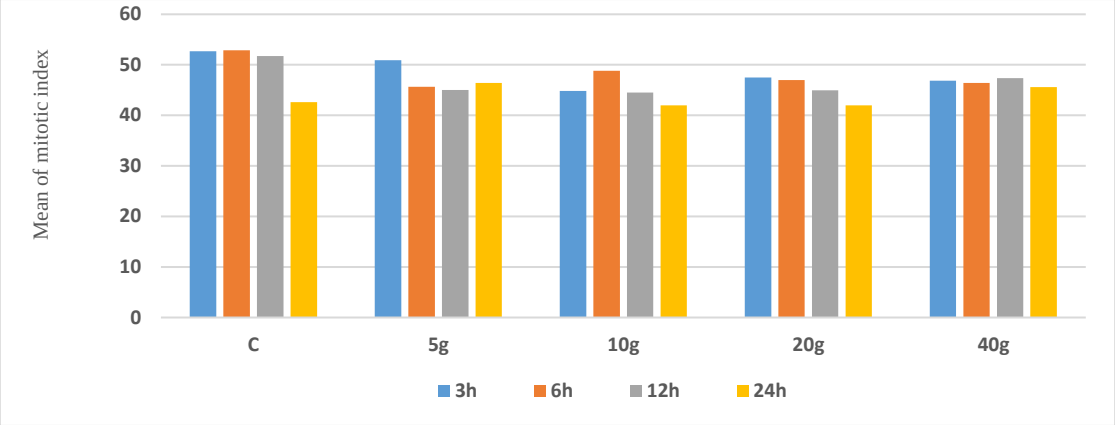

Figure 1. Effect of different concentrations of the aqueous extract of *Pistacia lentiscus* fresh fruits at different exposure times on the mean of the mitotic index compared to the control (C).

Two-way ANOVA analysis (Table 2) revealed a highly significant interaction between extract concentration and exposure duration on the mitotic index (P = 0.001). Exposure time alone had a significant effect on the mitotic index (P = 0.024), whereas concentration alone did not show a statistically significant effect (P = 0.076). Additionally, treatments with 10 g and 20 g concentrations significantly reduced the mitotic index compared with the control (P = 0.0012 and P = 0.016, respectively). Shorter exposure periods (3 and 6 h) differed significantly from the 24 h exposure across all tested concentrations. Microscopic examination further indicated that prophase constituted the highest proportion of mitotic stages, followed by metaphase, anaphase, and telophase (Table 1).

Table 2. Univariate Analysis of Variance.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	118.905[a]	7	16.986	3.533	.027
Intercept	43663.578	1	43663.578	9080.765	**.001**
Concentrations	53.557	4	13.389	2.785	**.076**
Time	65.349	3	21.783	4.530	**.024**
Error	57.700	12	4.808		
Total	43840.184	20			
Corrected Total	176.606	19			
a. R Squared = .673 (Adjusted R Squared = .483)					

Effect of Aqueous Extracts on Abnormal Cell Division

Statistical analysis of abnormal cell division (Table 3) showed that the combined effects of extract concentration and exposure duration resulted in a highly significant increase in chromosomal abnormalities (P = 0.001). Significant differences were observed among the different concentrations (P = 0.001) and exposure times (P = 0.012).

All tested concentrations (5, 10, 20, and 40 g/200 mL) caused highly significant increases in abnormal dividing cells compared with the control (P = 0.001). Notably, the 10 g concentration differed significantly from the 20 g and 40 g concentrations after 24 h of exposure (Table 4). The most frequently observed chromosomal abnormality was chromosome stickiness during metaphase (Figures 4a and 4b).

Table 3. Univariate Analysis of Variance.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	6.108[a]	7	.873	89.492	.001
Intercept	23.371	1	23.371	2396.843	**.001**
Conce	5.942	4	1.486	152.352	**.001**
Time	.166	3	.055	5.680	**.012**
Error	.117	12	.010		
Total	29.597	20			
Corrected Total	6.225	19			
a. R Squared = .981 (Adjusted R Squared = .970)					

Table 4. Effect of different aqueous extract concentrations of *Pistacia lentiscus* L. fresh fruits on the abnormality of dividing cells

Time (h)	Concentrations (g/200ml)	sum CDs	sum ADCs	Mean ADCs±SD	Sticky in Metaphase %
3	C	1269	0	0±0	0
	5	1362	17	1.25±0.200425	100
	10	1386	18	1.30±0.339845	100
	20	1662	22	1.32±0.169861	100
	40	1886	20	1.06±0.548566	100
6	C	1478	0	0±0	0
	5	1586	23	1.45±0.391812	100
	10	1758	23	1.31±0.103216	100
	20	1874	26	1.39±0.334938	100
	40	2018	24	1.19±0.084921	100
12	C	1754	0	0±0	0
	5	1734	28	1.61±0.084561	100
	10	1787	26	1.45±0.107757	100
	20	1861	24	1.29±0.074596	100
	40	2286	28	1.22±0.326748	100
24	C	1871	0	0±0	0
	5	2152	34	1.58±0.095599	100
	10	1962	32	1.63±0.144164	100
	20	2151	29	1.35±0.164927	100
	40	2375	29	1.22±0.059018	100

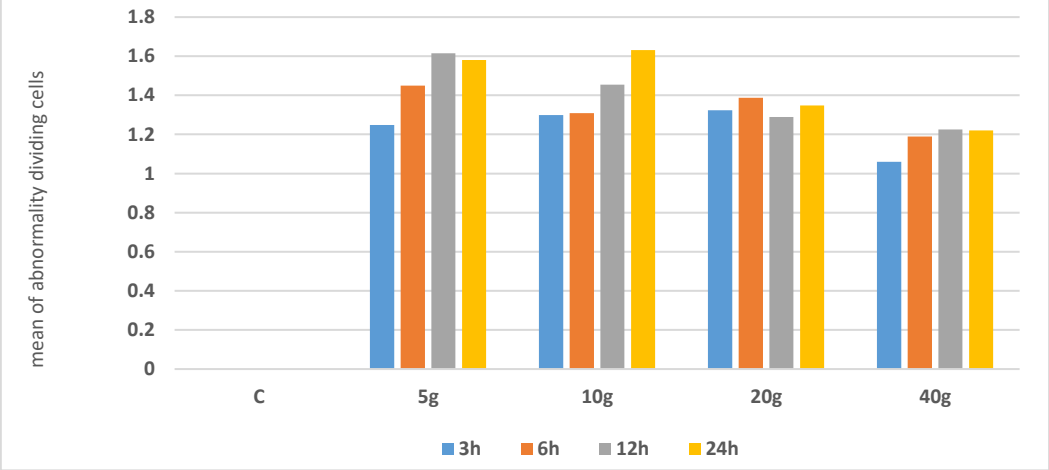

Figure 3. Effect of different aqueous extract concentrations of *Pistacia lentiscus* L. fresh fruits on the mean of the abnormality of dividing cells.

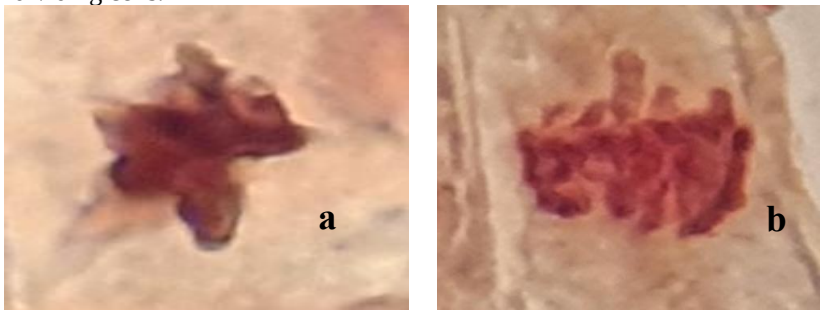

Figure 4. Effect of different aqueous extract concentrations of *Pistacia lentiscus* L. fresh fruits on metaphase. **a.** and **b.** sticky chromosomes in metaphase.

4. Discussion

The mitotic index, distribution of mitotic phases, and occurrence of chromosomal aberrations are essential cytogenetic parameters for evaluating the effects of chemical and biological agents on plant growth and genome stability. Cytogenetic assays using *Allium cepa* and *Vicia faba* have been widely employed to assess the mutagenic potential of environmental and natural compounds (Seth *et al*., 2008; Andrade *et al*., 2008). The *A.*

cepa assay is particularly valuable as an in vivo model due to the direct exposure of root meristem cells to test substances, allowing for the detection of DNA damage with potential relevance to higher organisms.

In the present study, aqueous extracts prepared from fresh fruits of *P. lentiscus* L. significantly reduced the mitotic index and induced chromosomal abnormalities in *A. cepa* root meristem cells. The greatest reduction in mitotic activity was observed after 24 h of exposure, particularly at concentrations of 10 g and 20 g. Similar inhibitory effects on mitotic index have been reported in *A. cepa* roots exposed to various chemical agents, including sulphite compounds (Türkoğlu, 2009) and plant-derived extracts such as cinnamon (El-Ghamery and Basuoni, 2015), indicating dose- and time-dependent cytotoxic effects. Previous studies have also demonstrated that aqueous plant extracts can inhibit seed germination and seedling growth in species such as *Raphanus sativus* and *Trigonella foenum-graecum*, largely due to the presence of phenolic compounds that interfere with cellular metabolism and division (Williams and Hoagland, 1982; Hamad, 2019). The observed reduction in mitotic index in the present study may therefore be attributed to phytotoxic constituents present in the aqueous extracts of *P. lentiscus* fruits.

Chromosome stickiness was the predominant abnormality observed, particularly at longer exposure times and higher concentrations. This phenomenon has been associated with disturbances in chromatin organization caused by alterations in nuclear proteins. Gaulden (1987) suggested that chromosome stickiness results from defects in non-histone proteins responsible for chromosome separation, while other studies have linked this abnormality to excessive nucleoprotein formation and improper protein–protein interactions (Nefic *et al*., 2013; El-Ghamery *et al*., 2003; Kuraś *et al*., 2006). The consistent occurrence of sticky chromosomes in the present study indicates that components of the aqueous extracts primarily target nuclear proteins, leading to impaired chromosomal behavior during mitosis.

Overall, the findings demonstrate that aqueous extracts of fresh *Pistacia lentiscus* fruits exert both cytotoxic and genotoxic effects on plant cells, particularly at higher concentrations and longer exposure durations. These results highlight the importance of evaluating the safety of medicinal plant extracts prior to their widespread use.

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