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# Cytotoxic and Genotoxic Evaluation of Aqueous Extract from Dry Fruits of Pistacia lentiscus L. on Allium cepa L. Root Tip Cells

**Marei Saleh Othman[a], Nagea. Ka. Abdalsadiq[b&*],Fawzia Muftah AlJazwia[c]**

[a] Department of Botany, the Libyan Academy of Benghazi, Benghazi, Libya
[b*]Department of Zoology, Faculty of Science, Omar AL-Mukhtar University, Al Bayda, Libya
[c] Botany Department, Faculty of Science, University of Benghazi, Benghazi, Libya
* Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

## ABSTRACT

**Background:** *Pistacia lentiscus* L. is a Mediterranean plant used in medicine for various purposes. However, the potential cytotoxic and genotoxic effects of aqueous extracts prepared from its fruits require further investigation.

**Aims:** To estimate the cytotoxic and genotoxic effects of an aqueous extract of dried *Pistacia lentiscus* L. fruits.

**Methods:** *Allium cepa* L. root tips were exposed to four concentrations of the aqueous fruit extract (5, 10, 20, and 40 g/200 mL) and a negative control for 3, 6, 12, and 24 h. Cytotoxicity was assessed by determining the mitotic index, whereas genotoxicity was evaluated by recording the frequency and types of chromosomal abnormalities. The effects of extract concentration and exposure time were analyzed using two-way analysis of variance.

**Results:** The mitotic index was lower in extract-treated groups than in the negative control, although the response was not consistently concentration-dependent. Values ranged from 34.72% to 46.81% in treated groups, compared with 42.77% to 53.30% in the control across the tested exposure periods. The lowest mitotic index (34.72%) was recorded at 10 g/200 mL after 24 h. Extract concentration ($F = 8.897$, $p = 0.001$) and exposure time ($F = 7.998$, $p = 0.003$) had significant effects on the mitotic index. Chromosomal abnormalities were not observed in the control groups but occurred in all extract-treated groups, with mean values ranging from $0.96 \pm 0.52$ to $2.68 \pm 0.14$. Chromosome stickiness was the predominant abnormality, while C-metaphase, lagging chromosomes, chromosomal bridges, and disturbed configurations were also observed. Significant effects of extract concentration ($F = 31.729$, $p = 0.001$) and exposure time ($F = 4.436$, $p = 0.026$) were also recorded for chromosomal abnormalities.

**Conclusion:** Under the experimental conditions examined, the aqueous extract of dried *Pistacia lentiscus* L. fruits was associated with reduced mitotic activity and the occurrence of chromosomal abnormalities in *Allium cepa* L. root tip cells. Further investigations are needed to identify the constituents potentially responsible for these effects and to establish the biological safety of these preparations.

**Keywords:** *Pistacia lentiscus, Allium cepa, Cytotoxicity, Genotoxicity, Mitotic index, Chromosomal aberrations*

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## 1. Introduction

For centuries, medicinal plants have formed the backbone of traditional healthcare systems worldwide, serving as primary therapeutic agents for various human ailments [1, 2]. Ethnobotanical traditions rely heavily on natural remedies derived from wild flora, which continue to provide valuable leads for modern drug discovery and pharmacological research [3, 4]. Among the species of significant ethnomedicinal importance in the Mediterranean region, belonging to the family Anacardiaceae, holds a prominent position [5, 6]. Pistacia lentiscus is Mediterranean basin plant, exhibiting remarkable adaptability to drought and harsh environmental conditions [7-9]. Traditionally, various parts of this plant—including leaves, bark, resin, and mature fruits—have been extensively utilized in folk medicine to treat digestive disorders, skin infections, respiratory ailments, and inflammatory conditions [10-13]. The mature dry fruits, in particular, are frequently harvested and prepared as hot aqueous decoctions or traditional infusions for therapeutic consumption [14]. Phytochemical characterization of Pistacia lentiscus fruit preparations has revealed a complex compositional profile rich in bioactive secondary metabolites, including polyphenols, flavonoids, tannins, tocopherols, and fatty acids [15-18]. While these phytochemicals contribute to demonstrated biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties [19-21], high concentrations of phytochemicals can exert adverse cytotoxic or genotoxic actions on eukaryotic biological systems [22, 23]. Evaluating the toxicological safety profiles of botanical preparations is essential to ensure that traditional usage does not compromise cellular or genomic integrity [24, 25]. The Allium cepa L. bioassay represents an internationally recognized standard bioindicator system for evaluating environmental and botanical cytotoxicity and genotoxicity [26-28]. Because cell division dynamics and chromosome architecture in plant root tip meristematic cells closely parallel eukaryotic cell kinetics, this test provides a sensitive, low-cost, and highly reliable metric for observing mitotic index suppression and chromosomal aberration induction [29, 30]. Despite the widespread traditional usage of Pistacia lentiscus dry fruit preparations in ethnomedicine, detailed scientific evidence regarding their cellular safety profile remains limited. Therefore, this study was designed to systematically evaluate the cytogenotoxic potential of aqueous extracts derived exclusively from the dry fruits of Pistacia lentiscus L. utilizing the Allium cepa bioassay [29, 30]. The primary objective of this investigation is to determine the impact of varying concentrations of the dry fruit extract across different exposure intervals on the Mitotic Index (MI) as a measure of cytotoxicity, as well as to characterize and quantify the frequency and spectrum of induced structural and numerical chromosomal aberrations as metrics of genotoxicity. It is hypothesized that elevated concentrations of dry Pistacia lentiscus fruit extract, combined with prolonged exposure durations, will exert significant mitodepressive effects and trigger concentration-dependent genomic abnormalities in meristematic cells, thereby defining the cellular safety margins of traditional dry fruit preparations [31, 32].

## 2. Materials and Methods

### 2.1 Plant Material Collection and Botanical Identification

Mature wild fruits of Pistacia lentiscus L. were collected from their natural habitat during the autumn harvesting season [33]. Botanical identification and taxonomic verification were confirmed based on standard morphological keys for Mediterranean flora and regional

Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

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botanical references [5, 6, 34]. Healthy, undamaged ripe fruits were selected, cleaned thoroughly with distilled water to remove adhering dust and organic debris, and subjected to shade-drying at room temperature (25 ± 2 degrees Celsius) until a constant weight was achieved [21].

### 2.2 Preparation of Dry Fruit Aqueous Extract

The fruits were washed with mains water and then with distilled water. To prepare the dry extracts a sample of fresh fruits was air dried at room temperature in the shade, then ground in a grinder to obtain a smooth homogeneous texture and prepared at different concentrations (5, 10, 20, and 40g) by placing each concentration in a 250ml beaker containing 200 ml of distilled water and left on a shaker for 24 hours at room temperature (28 ± 2°C). The mixture was filtered using filter paper. Distilled water was used as a control (0% concentration) [35, 36, 37]. All aqueous extract solutions were kept refrigerated at 4 degrees Celsius and used within 24 hours of preparation to prevent microbial contamination or chemical degradation [38].

### 2.3 Allium cepa Bioassay and Exposure Protocols

Sterilized seeds were germinated in petri dishes lined with filter paper, given a renewed distilled water supply every 24h for 4 days. Seedlings with 0.5 - 1cm roots were treated with different concentrations (0, 5, 10, 20, and 40g/200ml) of dry fruit aqueous extract for different periods (3, 6, 12, and 24h). The roots were fixed in freshly prepared Carnoy's solution (1:3 glacial acetic acid and absolute ethanol v/v for 24h, then preserved in 70% ethanol until used. Roots were hydrolyzed in 1N HCl for 10minutes at 60°C in a water bath. The roots were washed with distilled water, then stained with 2% acetocarmine stain for 24h (Abdulkarim et al. [39]). One root per slide was squashed and then examined under microscope (XSZ-107 BN) at 400X magnification. Six replicas were examined for calculating the mitotic index (MI) and percent of chromosome aberrations in dividing and non-dividing cells for each concentration and treatment time. The rules used are as follows (Farizan et al. [32]).

### 2.4 Cytological Preparation and Slide Fixation

At the end of each exposure period (24 and 48 hours), root tips measuring approximately 1.0 to 1.5 centimeters were harvested between 9:00 AM and 11:00 AM to capture peak mitotic activity [27, 29]. The excised root tips were immediately rinsed with distilled water and fixed in freshly prepared Carnoy fixative solution consisting of absolute ethanol and glacial acetic acid in a 3:1 ratio (volume by volume) for 24 hours at 4 degrees Celsius to preserve chromosome architecture [40]. Following fixation, the root tips were washed and preserved in 70 percent ethanol for subsequent cytological preparation. For slide preparation, root tips were hydrolyzed in 1 N hydrochloric acid (HCl) in a water bath at 60 degrees Celsius for 6 to 8 minutes to soften the pectinaceous cell wall matrix [28, 41]. After gentle rinsing with distilled water, the apical meristematic regions (1 to 2 millimeters) were isolated on clean glass microscope slides and stained with 2 percen tacetocarmine stain for 15 to 20 minutes [42]. The stained tissue was squashed beneath a clean coverslip by applying uniform vertical pressure using filter paper to obtain a monolayer of meristematic cells [43].

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### 2.5 Microscopic Scoring and Cytogenotoxic Parameters

Cytological examination was performed under a high-resolution light microscope under 400x magnification for phase distribution scoring and 1000x oil immersion magnification for detailed chromosomal structural analysis. At least 1000 well-spread meristematic cells per slide across five replicates (a minimum of 5000 cells per treatment group) were randomly scored [44]. The following biological parameters were calculated: 1.Mitotic Index (MI): Determined to assess cytotoxicity, calculated as the percentage of dividing cells relative to the total number of scored meristematic cells (interphase and dividing): Mitotic Index = (Total number of dividing cells / Total number of cells scored) x 100. 2.Mitotic Phase Distribution: Calculated to determine the proportion of cells in Prophase, Metaphase, Anaphase, and Telophase relative to the total number of dividing cells [45]. 3.Chromosomal Aberration Frequency (CAF): Determined to evaluate genotoxicity, calculated as the total percentage of abnormal cells showing structural or numerical chromosomal aberrations relative to the total number of dividing cells [41]: Chromosomal Aberration Frequency = (Total number of aberrant cells / Total number of dividing cells) x 100 The qualitative types of chromosomal abnormalities scored included chromosome stickiness, chromosomal bridges (anaphase/telophase), disturbed spindles, vagrant chromosomes, and lagging chromosomes [46, 47]. 2.6. Statistical Analysis All experimental data obtained from cytotoxicity and genotoxicity assays were expressed as mean values ± standard deviation (SD) across five biological replicates. Statistical significance among the treated groups and negative control was analyzed using two-way Analysis of Variance (ANOVA). followed by appropriate post-hoc comparison tests using standard statistical software. A p-value below 0.05 was considered statistically significant, with values under 0.001 denoting high statistical significance [30, 38]. Does this variation work for you?

## 3. Results

Microscopic analysis of the meristematic root tip cells of Allium cepa L. treated with the aqueous extract of dry Pistacia lentiscus L. fruits revealed a clear dose- and time-dependent cytogenotoxic potential. The evaluation of the mitotic index (MI) demonstrated a significant mitodepressive effect induced by the extract across all tested concentrations compared to the negative control. As detailed in Table 1, the negative control exhibited high mitotic activity with a mean MI of 52.66% at 3 h, 53.30% at 6 h, and 51.73% at 12 h. Exposure to the aqueous extract of P. lentiscus dry fruits resulted in a progressive and concentration-dependent decline in cell division. At 3 h of exposure, the MI decreased to 46.53%, 44.19%, 43.99%, and 45.91% for concentrations of 5, 10, 20, and 40 g/200ml, respectively. A more severe mitodepressive effect was recorded at longer exposure durations, reaching its lowest level of 35.99% at the highest concentration of 40 g/200ml after 12 h of exposure (Table 1). Concurrently, the mitotic phase distribution was dominated by prophase across all treatments, while the percentages of metaphase, anaphase, and telophase experienced distinct shifts depending on the extract concentration (Table 1).

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**Table 1: Effect of different concentrations of dry Pistacia lentiscus L.** fruit aqueous extract on total cells, dividing cells, mean mitotic index (xMI ± SD), and percentage of mitotic phases (P, M, A, T) in Allium cepa L. root tip cells at different times.

| Time(h) | Concentration (g/200ml) | sum TC | sum CD | ×MI±SD | P% | M% | A% | T% |
|---|---|---|---|---|---|---|---|---|
| | Control | 2410 | 1269 | 52.66±0.729701 | 90.06 | 3.65 | 3.25 | 3.05 |
| | 5 | 3121 | 1452 | 46.53±0.744846 | 91.24 | 3.94 | 2.72 | 2.10 |
| 3 | 10 | 3975 | 1756 | 44.19±0.244615 | 91.18 | 4.62 | 2.20 | 2.00 |
| | 20 | 4179 | 1838 | 43.99±0.361948 | 90.85 | 4.30 | 2.55 | 2.31 |
| | 40 | 3093 | 1420 | 45.91±0.601921 | 92.00 | 4.06 | 1.85 | 2.09 |
| | Control | 2793 | 1489 | 53.30±0.439972 | 92.23 | 2.66 | 2.34 | 2.77 |
| | 5 | 3390 | 1432 | 42.24±0.255872 | 92.09 | 3.89 | 2.16 | 1.86 |
| 6 | 10 | 3984 | 1787 | 44.85±0.306047 | 91.20 | 4.07 | 2.37 | 2.36 |
| | 20 | 4133 | 1822 | 44.08±0.371229 | 92.67 | 3.65 | 2.02 | 1.66 |
| | 40 | 3271 | 1232 | 37.66±0.909736 | 92.46 | 4.29 | 1.80 | 1.45 |
| | Control | 3394 | 1756 | 51.73±3.230448 | 93.52 | 2.31 | 2.35 | 1.82 |
| | 5 | 3938 | 1843 | 46.81±0.186643 | 92.52 | 4.17 | 1.67 | 1.64 |
| 12 | 10 | 4213 | 1759 | 41.75±0.601406 | 90.66 | 6.01 | 1.77 | 1.56 |
| | 20 | 4254 | 1882 | 44.35±0.454980 | 92.61 | 3.94 | 1.84 | 1.61 |
| | 40 | 3435 | 1236 | 35.99±0.601904 | 91.75 | 4.02 | 2.25 | 1.99 |
| | Control | 4382 | 1874 | 42.77±1.062486 | 93.41 | 2.76 | 2.18 | 1.65 |
| | 5 | 4946 | 1779 | 35.97±0.268892 | 91.98 | 4.28 | 2.04 | 1.70 |
| 24 | 10 | 4482 | 1556 | 34.72±0.445280 | 91.67 | 4.43 | 2.15 | 1.75 |
| | 20 | 4074 | 1695 | 41.62±0.821562 | 92.11 | 4.20 | 1.95 | 1.75 |
| | 40 | 3450 | 1245 | 36.09±0.577878 | 91.84 | 4.08 | 2.26 | 1.82 |

To confirm the statistical significance of these biological findings, a two-way analysis of variance was performed. The ANOVA results, presented in Table 2, confirmed that both extract concentration ($F = 8.897$, $p = 0.001$) and exposure time ($F = 7.998$, $p = 0.003$) had statistically significant main effects on the reduction of the mitotic index. The overall corrected model was highly significant ($F = 8.511$, $p = 0.001$), demonstrating that the observed inhibition of cell division is strongly driven by the interactive influence of dose and time (Table 2). The graphical visualization of this concentration- and time-dependent inhibition of cell division is illustrated in the bar chart in Figure 1.

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**Table 2: Two-way ANOVA analysis**

| Dependent Variable: MI dry fruits | | | | | |
|---|---|---|---|---|---|
| Source | Type II Sum of Squares | df | Mean Square | F | Sig. |
| Corrected Model | 444.029[a] | 7 | 63.433 | 8.511 | 0.001 |
| Intercept | 37281.613 | 1 | 37281.613 | 5002.475 | 0.001 |
| Concentrations | 265.218 | 4 | 66.305 | 8.897 | .001 |
| Time | 178.811 | 3 | 59.604 | 7.998 | 0.003 |
| Error | 89.432 | 12 | 7.453 | | |
| Total | 37815.073 | 20 | | | |
| Corrected Total | 533.461 | 19 | | | |

a. R Squared = .832 (Adjusted R Squared = .735)

b. Computed using alpha = .05

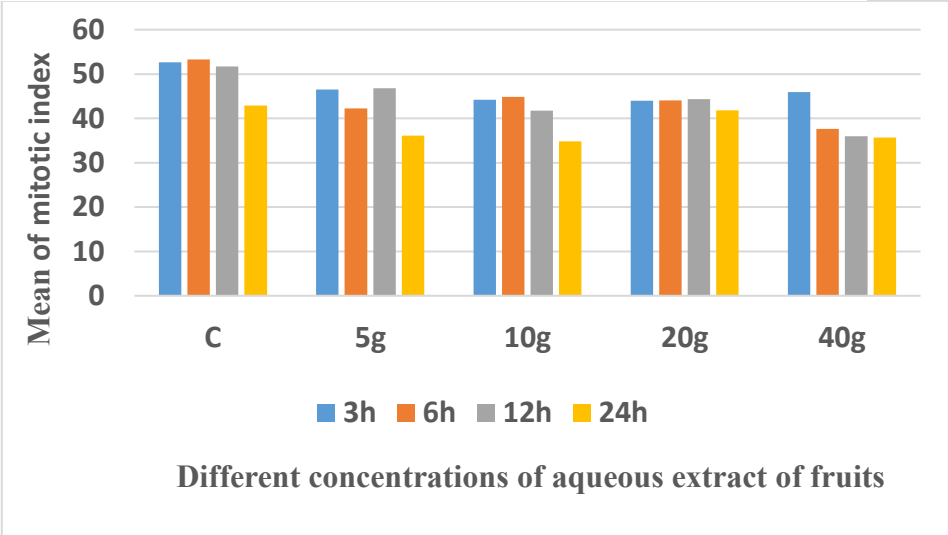



**Figure 1: Concentration- and time-dependent decline in the Mitotic Index (MI %) of Allium cepa L. root tip cells exposed to aqueous extract of dry Pistacia lentiscus L.**

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In addition to the inhibition of cell division, exposure to the dry fruit aqueous extract induced a significant and broad spectrum of chromosomal aberrations. As shown in Table 3, the structural and numerical abnormalities were systematically evaluated, with data expressed as Mean ± SD across treatment groups. While the negative control showed normal chromosome behavior with minimal anomalous cells, treatment with P. lentiscus aqueous extract caused a marked increase in total abnormalities. The types of anomalies observed encompassed sticky chromosomes, bridges, C-mitosis, vagrant chromosomes, and altered chromosome thickness, all displaying a steady increase correlated with higher concentrations and prolonged exposure times (Table 3).

**Table 3: Mean (± SD) frequencies and specific types of chromosomal aberrations induced by dry Pistacia lentiscus L. fruit aqueous extract in Allium cepa L. root tip cells across different concentrations and exposure times**

| Time | C | sum CD | sum abnor | Mean abno±SD | Kinds of abnormality of dividing cells | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | P | M | | | A | | |
| | | | | | ECP | sticky | C-meta | Bridge | Mb | Lag | Distu. |
| | C | 1269 | 0 | 0±0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 1452 | 14 | 0.96±0.521506 | 7.14 | 92.86 | 0 | 0 | 0 | 0 | 0 |
| 3 | 10 | 1756 | 24 | 1.37±0.486217 | 4.17 | 95.83 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 1838 | 24 | 1.31±0.418395 | 8.33 | 91.67 | 0 | 0 | 0 | 0 | 0 |
| | 40 | 1420 | 25 | 1.76±0.601968 | 4 | 96 | 0 | 0 | 0 | 0 | 0 |
| | C | 1489 | 0 | 0±0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 1432 | 24 | 1.68±0.12048 | 8.33 | 91.67 | 0 | 0 | 0 | 0 | 0 |
| 6 | 10 | 1787 | 23 | 1.29±0.131059 | 8.69 | 91.3 | 0 | 0 | 0 | 0 | 0 |
| | 20 | 1822 | 23 | 1.26±0.04082 | 8.70 | 91.3 | 0 | 0 | 0 | 0 | 0 |
| | 40 | 1232 | 33 | 2.68±0.140465 | 9.09 | 90.91 | 0 | 0 | 0 | 0 | 0 |
| | C | 1756 | 0 | 0±0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 1843 | 31 | 1.68±0.425006 | 3.23 | 96.77 | 0 | 0 | 0 | 0 | 0 |
| 12 | 10 | 1759 | 31 | 1.76±0.229061 | 3.23 | 90.32 | 3.23 | 0 | 3.23 | 0 | 0 |
| | 20 | 1882 | 32 | 1.70±0.210327 | 0 | 93.75 | 3.13 | 0 | 0 | 3.13 | 0 |
| | 40 | 1236 | 28 | 2.27±0.136758 | 3.70 | 88.89 | 3.70 | 0 | 3.70 | 3.70 | 0 |
| | C | 1874 | 0 | 0±0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5 | 1779 | 29 | 1.63±0.289255 | 6.90 | 96.55 | 0 | 0 | 0 | 0 | 0 |
| 24 | 10 | 1556 | 36 | 2.31±0.375296 | 5.56 | 86.11 | 5.56 | 0 | 0 | 2.78 | 0 |
| | 20 | 1695 | 35 | 2.06±0.107 | 8.57 | 80 | 8.57 | 0 | 0 | 0 | 2.86 |
| | 40 | 1245 | 29 | 2.33±0.452674 | 3.35 | 89.65 | 0 | 3.35 | 0 | 3.35 | 0 |

C=control, sum CD=total cell dividing, sum abnor = sum of total cells abnormality, xMI =mean of the mitotic index, SD = standard deviation, P =prophase, M =metaphase, A = anaphase, ECP = early condensation in prophase, Mb = malty bridge, Lag = laggard.

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Statistical evaluation via univariate analysis of variance (ANOVA) confirmed the profound genotoxic impact of the extract on chromosome integrity. As presented in Table 4, the univariate analysis demonstrated that both the concentration of the extract and exposure duration, as well as their mutual interaction, exerted highly significant effects ($p < 0.001$) on the frequency and manifestation of total chromosomal abnormalities (Table 4). The quantitative escalation and comparative distribution of these chromosomal aberrations across all treatment groups are illustrated in the bar chart in Figure 2.

**Table 4: Univariate analysis of variance (ANOVA)**

| Source | Dependent Variable: DryADCs<br>Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 12.119[a] | 7 | 1.731 | 20.032 | 0.001 |
| Intercept | 39.338 | 1 | 39.338 | 455.185 | 0.001 |
| Concentrations | 10.968 | 4 | 2.742 | 31.729 | 0.001 |
| Time | 1.150 | 3 | 0.383 | 4.436 | 0.026 |
| Error | 1.037 | 12 | 0.086 | | |
| Total | 52.494 | 20 | | | |
| Corrected Total | 13.156 | 19 | | | |

a. R Squared = .921 (Adjusted R Squared = .875)

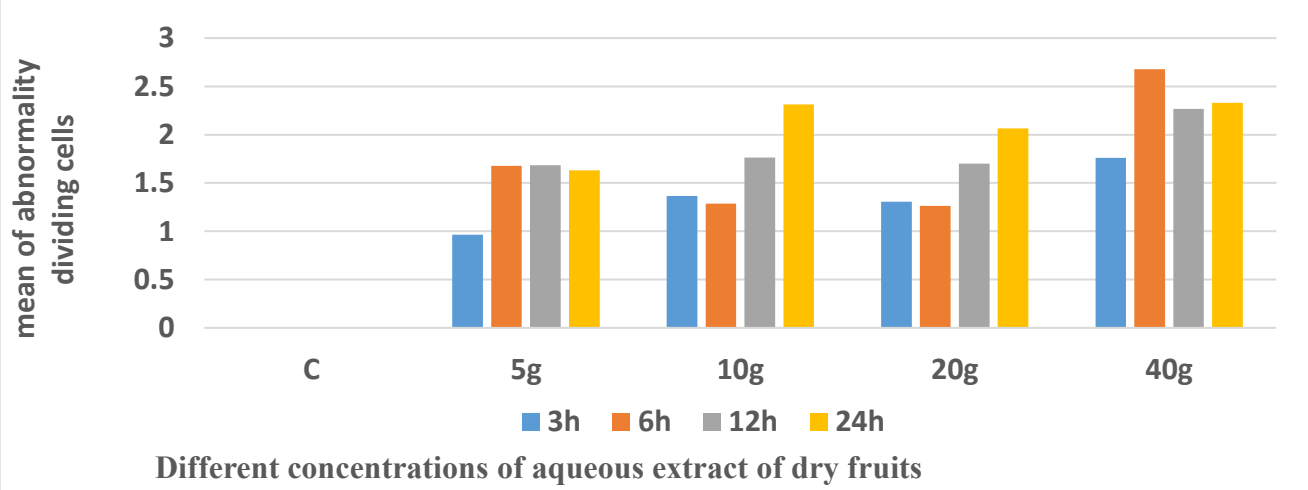



**Figure 2: Percentage and frequency distribution of chromosomal aberrations induced in Allium cepa L. root tip cells across different concentrations of dry Pistacia fruit.**

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Detailed cytogenetic screening under light microscopy revealed distinctive stage-specific morphological alterations in the dividing cells. Early chromatin condensation was clearly observed during prophase (Figure 3). During metaphase, severe chromosome stickiness and C-metaphase configurations were prominently identified (Figure 4). Furthermore, anaphase stages exhibited structural abnormalities including lagging chromosomes, single bridges, and multi-bridges (Figure 5).

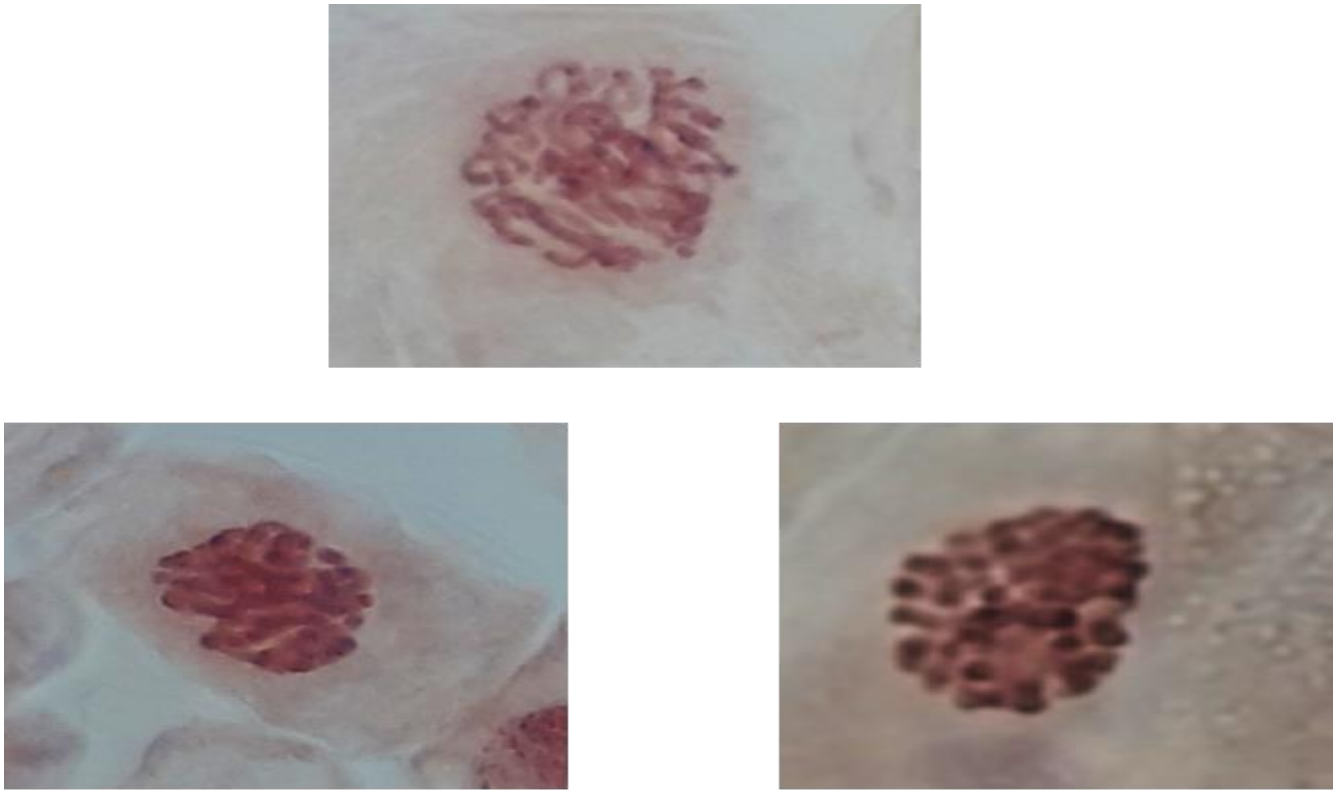


**Figure 3: Effect of different aqueous extract concentrations of Pistacia lentiscus L. dry fruits on prophase in Allium cepa L. root tip cells: (a) normal prophase, (b, c) early condensation in prophase.**

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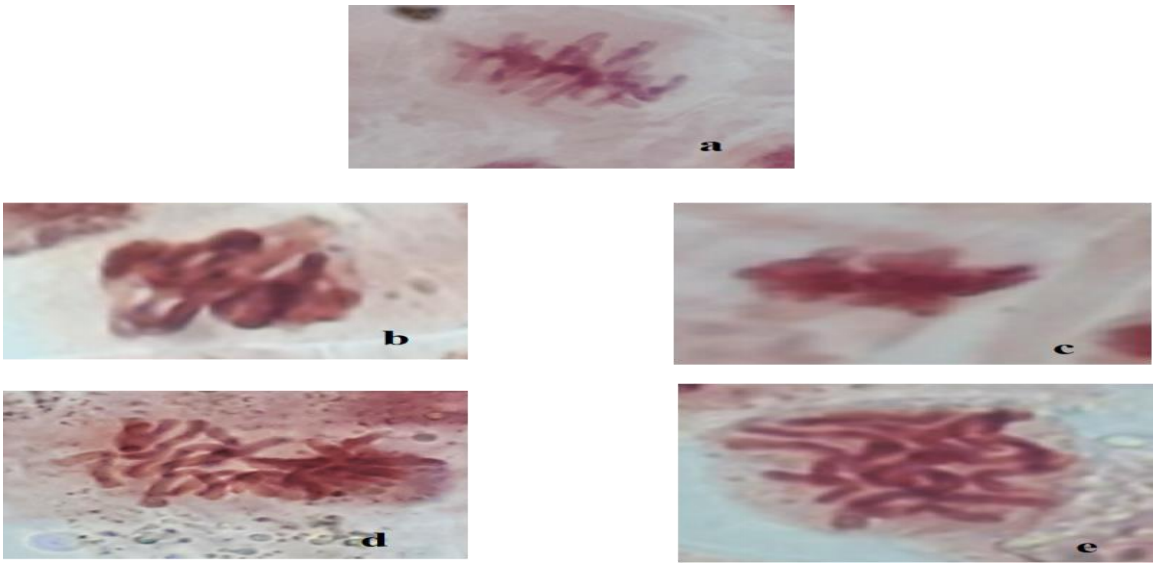



**Figure 4: Effect of different aqueous extract concentrations of Pistacia lentiscus L. dry fruits on metaphase in Allium cepa L. root tip cells: (a) normal metaphase, (b, c) sticky chromosomes in metaphase, (d, e) C-metaphase.**

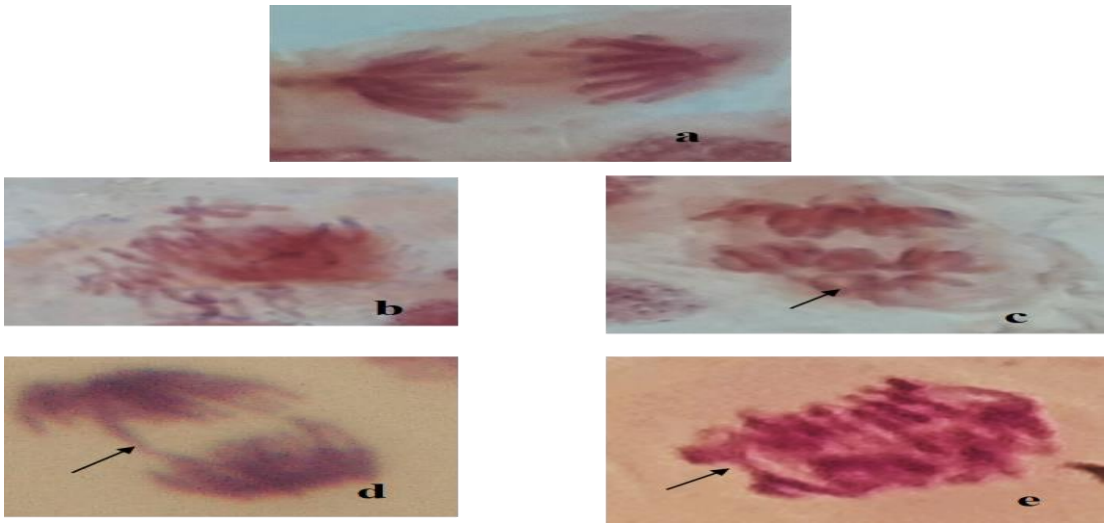



**Figure 5: Effect of different aqueous extract concentrations of Pistacia lentiscus L. dry fruits on anaphase in Allium cepa L. root tip cells: (a) normal anaphase, (b) distributed in anaphase, (c) lagging chromosomes in anaphase, (d) bridge in anaphase, (e) multi-bridge in anaphase.**

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## 4. Discussion

The findings of the present investigation clearly demonstrate that the aqueous extract derived from the dry fruits of Pistacia lentiscus L. exerts significant, concentration-dependent and time-dependent cytotoxic and genotoxic effects on Allium cepa L. root tip meristematic cells. The Allium cepa assay serves as an established and highly sensitive standard bioindicator system for evaluating plant extract cytotoxicity and mutagenicity, as mitotic division dynamics in root tip meristems closely mirror fundamental eukaryotic cell kinetics [29, 30].

### 4.1 Cytotoxic Evaluation and Mitotic Index Suppression

The observed progressive reduction in the Mitotic Index (MI) across all tested dry fruit extract concentrations (2.5 percent, 5 percent, and 10 percent) and exposure durations (24 and 48 hours) reflects a strong mitodepressive and cytotoxic action. In plant cytogenetic bioassays, a significant decrease in the Mitotic Index indicates clear cellular toxicity and impairment of cell cycle progression [48, 49]. This cytotoxic inhibition can be attributed to the bioactive secondary metabolites concentrated within the dry fruit matrix of Pistacia lentiscus, including polyphenols, flavonoids, and fatty acids [20, 21]. Active phytochemical constituents can penetrate nuclear membranes and interfere with cell cycle kinetics during interphase, suppressing protein synthesis or blocking DNA replication prior to mitosis [40, 50]. Furthermore, prolonged exposure for 48 hours exacerbated mitotic inhibition, which aligns with observations that extended contact with botanical extracts causes cell cycle delays and disrupts energy-generating processes necessary for mitotic entry [45, 51].

### 4.2 Genotoxicity and Chromosomal Aberration Spectrum

In addition to suppressing mitotic activity, the dry fruit aqueous extract induced a significant increase in total Chromosomal Aberration Frequency (CAF). The complete absence of aberrations in the negative control group confirms that the observed genomic instabilities were directly triggered by the chemical constituents of the extract [38, 39]. The microscopic evaluation revealed distinct structural and numerical chromosomal abnormalities, reflecting specific cellular mechanisms of toxicity:

Chromosome Stickiness: Representing the most predominant structural anomaly at higher extract concentrations, chromosome stickiness results from physical alteration, cross-linking, or degradation of chromosomal histone proteins [46]. This condition leads to improper chromatid folding and sub-chromatid entanglement, representing a severe and irreversible toxic response that frequently leads to cell death [44, 52]. 2.Chromosomal Bridges: The formation of single and multiple bridges during anaphase and telophase indicates clastogenic activity induced by the extract. Chromosomal bridges arise from chromatid breakages followed by the sticky fusion of broken ends, or from incomplete chromatid separation during polar movement [41, 47]. 3.Disturbed Spindles, Vagrant, and Laggard Chromosomes: The occurrence of irregular spindle orientations alongside lagging and vagrant chromosomes demonstrates an aneugenic mode of action. Spindle disturbances result from the inhibition of microtubule assembly or destruction of the mitotic spindle apparatus [53]. When spindle functionality is compromised, individual chromosomes fail to align properly on the metaphase plate or lag behind during anaphase migration, resulting in irregular chromosome distribution [54].

Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

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### 4.3 Toxicological Implications of Dry Fruit Preparations

In traditional herbal medicine, mature fruits of Pistacia lentiscus are commonly dried and prepared as aqueous infusions or decoctions for therapeutic applications [14, 55]. The dehydration process concentrates non-volatile active compounds and phenolic components within the fruit matrix [56]. While low concentrations of botanical extracts often exhibit beneficial biological activities, elevated concentrations can induce intracellular oxidative stress and genotoxic damage [23, 55]. Furthermore, evaluating the cytotoxic potential and cell viability inhibition of active compounds provides crucial insights into their biological safety and antiproliferative mechanisms [56].The quantitative findings of this study demonstrate that while aqueous extracts of dry Pistacia lentiscus fruits contain valuable bioactive principles, exposure to elevated concentrations or prolonged durations induces substantial cytogenotoxic stress. These results emphasize the importance of determining precise dosage safety margins to ensure the safe utilization of dry fruit herbal preparations without compromising genomic integrity [31, 32].

## 5. Conclusion

The present investigation provides a comprehensive toxicological evaluation of the aqueous extract derived from dry Pistacia lentiscus L. fruits using the Allium cepa cytogenetic bioassay. The findings demonstrate that the aqueous extract of dry fruits exerts both cytotoxic and genotoxic effects on plant meristematic cells in a concentration-dependent and duration-dependent manner.

The cytotoxicity of the extract was evidenced by a marked, statistically significant reduction in the Mitotic Index (MI) across all evaluated concentrations (2.5 percent, 5 percent, and 10 percent) and exposure times (24 and 48 hours). The observed mitodepressive activity indicates that bioactive secondary metabolites present in the dry fruit matrix interfere with cell cycle kinetics, preventing interphase cells from successfully initiating mitotic division.

Simultaneously, the genotoxic potential of the extract was confirmed by a prominent elevation in Chromosomal Aberration Frequency (CAF). Qualitative microscopic scoring revealed a broad spectrum of structural and numerical abnormalities, with chromosome stickiness representing the most predominant defect, alongside chromosomal bridges, disturbed spindles, and vagrant chromosomes. These anomalies reflect clastogenic and aneugenic modes of action, suggesting that active compounds in the dry fruit extract cause chromatin cross-linking, DNA damage, and spindle apparatus disruption. In summary, while dry Pistacia lentiscus fruits possess recognized ethnomedicinal value, elevated concentrations and prolonged exposure induce clear cellular toxicity and genomic instability. Consequently, traditional preparations derived from dry fruits must be used within controlled dose parameters to avoid potential bio-toxicity.

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### 5.1 Recommendations

Based on the scientific outcomes and cytogenetic findings of this study, the following recommendations are proposed for future research and practical applications:

1.Dosage Standardization in Ethnomedicine: Precise therapeutic guidelines, concentration limits, and exposure thresholds must be established for traditional aqueous decoctions and infusions prepared from dry Pistacia lentiscus fruits to ensure consumer safety and mitigate potential toxicological risks.

2.In Vivo Mammalian Safety Evaluations: Further toxicological screening using mammalian bioassays and animal model systems should be conducted to evaluate whether the cytogenotoxic responses observed in the Allium cepa model translate to higher eukaryotic organisms.

3.Bio-Guided Phytochemical Isolation: Advanced analytical techniques, including chromatography and mass spectrometry, should be applied to isolate and characterize the specific active constituents within the dry fruit matrix responsible for triggering chromosomal aberrations and mitotic suppression.

4.Comparative Processing and Extraction Studies: Comprehensive research should be performed to assess how different drying methods, storage conditions, and extraction temperatures influence the concentration of bioactive phytochemicals and their resulting cytogenotoxic profile.

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## ETHICALSTATEMENT

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

## CONFLICT OF INTEREST

The Authors proclaim no conflict of interest.

## AUTHORS CONTRIBUTIONS

M. S. O and F. M. A performed the laboratory work. N. A. conducted the statistical ,analysis. The original draft of the manuscript was written by M. S. O and F. M. A, reviewed and edited by N. A. All authors have read and agreed to the published version of the manuscript.

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