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Cytogenetic effects of *Tribulus terrestris* L. on meristematic cells of *Allium cepa* L. and *Vicia faba* L.

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Abstract. *Tribulus terrestris* is a plant of the Zygophyllaceae family frequently used worldwide to treat various diseases due to the therapeutic effects of its pharmacological components. This study examines the cytotoxic and genotoxic effects of *T. terrestris* using two plant models, *Allium cepa* and *Vicia faba*. Extracts of 0.00625, 0.0125, 0.025, 0.05 and 0.1mg/mL were tested on meristematic cells of *A. cepa* and *V. faba* roots. This assessment includes the study of root growth, structure and coloration, as well as the determination of the mitotic index (MI) and chromosomal aberrations (CAs) as accurate indicators of toxicity. Our results showed a significant decrease in the mean length of roots treated with 0.025, 0.05 and 0.1 mg/ml for *A. cepa* and 0.1 mg/ml for *V. faba*. Cytotoxicity and genotoxicity results showed a significant decrease in MI from 0.025 mg/ml in *A. cepa* and from 0.05 mg/ml in *V. faba*, and this decrease in MI is linked to the increase in concentration and treatment time with *T. terrestris*. Furthermore, a significant increase in CAs was observed in A. cepa and *V. faba* from the 0.025 mg/ml concentration. The significant reduction in MI and CAs abundance suggests the genotoxicity of *T. terrestris*. Therefore, *T. terrestris* is a medicinal plant that should be used with caution, appropriately and based on essential therapeutic needs.

Keywords: *Tribulus terrestris*, *Allium cepa*, *Vicia faba*, cytotoxicity, genotoxicity.

INTRODUCTION

Recently, there has been a rapid increase in the use of dietary supplements derived from bioactive compounds of plant origin (Izzo *et al*. 2016). Herbal medicines are often used because many people believe that all that is natural is not toxic or harmful to health. This is a mistaken belief, as many therapeutic plants have high toxicity and harmful effects on human health (Proença da Cunha *et al*. 2012). Many studies examine the biological effects of extracts from different plants for their potential therapeutic use. However, there is little data available on the cyto-genotoxic effects of most plants.

Therefore, there is a need for research on these plants to assess their potential cytotoxic and genotoxic effects (Chukwujekwu and Van Staden 2014). Previous studies using different bioassays have revealed significant cytotoxic and genotoxic effects in different plants. Abudayyak *et al*. (2015) reported that *T. terrestris* L. had estrogenic and genotoxic activities in rat kidney cell lines exposed to this plant. Another study by Kumar *et al*. (2013) on the effect of *Tinospora cordifolia* in *A. cepa* meristematic cells showed a significant cytotoxic and genotoxic at high concentrations. In addition, Results obtained from the genotoxic study of Ayubi *et al*. (2021) showed that the hydro-alcoholic extract of *Z. multiflora* had no genotoxic effect. Bocayuva Tavares *et al*. (2021) reported that Seed proteins extract of *S. saponaria* causes cytotoxic and genotoxic effects in the human liver cancer cell line. In another study to evaluate the genotoxic effects of the plant *Angelica keiskei,* Maronpot (2015) demonstrated that it's not genotoxic in Chinese hamster ovary cells.

Researchers have long been interested in the genotoxic and cytotoxic effects of natural substances such as plant extracts. Higher plants, including *A. cepa* and *V. faba*, are used frequently to assess the genotoxicity of these environmental pollutants (Leme and Marin-Morales 2009). Furthermore, *A. cepa* and *V. faba* tests were ideal for evaluating chromosome damage and mitotic cycle disruptions due to their excellent chromosome characteristics, including large and fewer in number with a stable karyotype (Fiskesjo 1985). Moreover, this test method has demonstrated high sensitivity, which depends on a quick response in root development dynamics and simple detection of the endpoints associated with genotoxicity (Firbas and Amon 2014). Genotoxic endpoints include changes in MI values compared to controls (Akgündüz et al. 2020), morphological and number chromosome modifications expressed as CAs (Bonciu et al. 2018), and the frequency of Micronuclei as a simple quantitative characteristic (Bonciu et al. 2018; Younis et al. 2019).

T. terrestris is an annual plant from the Zygophyllaceae family. It has mainly cultivated in the Mediterranean and subtropical areas (Zhu *et al*. 2017). The *T. terrestris* extract is one of the natural therapeutic products that is used most frequently. This extract has shown several pharmacological activities, most of which are linked to diverse flavonoid and terpenoid components. These activities include antioxidants, antimicrobial, antibacterial, antitoxic, antiapoptotic molecules, platelet aggregation inhibitors, and anti-inflammation (Almasi *et al*. 2017). Also, these activities treat cardiovascular diseases, tumors, diabetes, respiratory diseases, and reproductive dysfunction (Qureshi *et al*. 2014). The widespread distribution of *T. terrestris*, its high content of active compounds (especially sterol saponins, as well as flavonoids, terpenoids, tannins, phenol, alkaloids and carboxylic acids), and the prevalence with which it is used in traditional medicine, all highlight the importance of analysing the phytopharmacological characteristics of the plant (Stefănescu *et al*. 2020), and due to the potential toxic effect of its active compounds, an assessment of a potential cytotoxic and genotoxic effect is essential for its use is safe and effective (Celik 2012). Although the therapeutic effects of this plant have been studied by several researchers, but the evaluation of the potential cytogenotoxic effects of *T. terrestris* on meristematic cells has not yet been studied.

Thus, the present work aimed to evaluate the cytotoxic and genotoxic effects of *T. terrestris* methanolic extract on *A. cepa* and *V. faba* roots by assessing the root growth, structure and color as well as the MI and CAs.

MATERIALS AND METHODS

Plant methanolic extract preparation

600 g of *T. terrestris* aerial parts (Trunk, branches and leaves) were obtained from a local medicinal plant market (Setif-Algeria). The plant material was identified by Dr. Sakhraoui Nora (a botanist) and then dried in the dark and powdered with a domestic mixer. 500 g of *T. terrestris* powder was dissolved in 4L of 96% hydro-methanolic solution (80%) for 24 hours to obtain a methanolic extract. The solution obtained was then double filtered using a Whatman No.1 paper filter. The filtrate obtained was then evaporated in a rotavapor (RE-100 pros) at 45°C to obtain a final dry residue. During processing, the dry residue was dissolved in distilled water to prepare the different concentrations of 0.00625, 0.0125, 0.025, 0.05 and 0.1 mg/mL for the different treatments.

Plants assay and application concentrations

Healthy onion bulbs (*A. cepa*, 2n = 16) and *V. faba* seeds were obtained from a local market. Both species were kept in tap water until their roots reached 1.5-2 cm. Then they were divided into six groups, one serving as a control, while the other five were given different concentrations (0.00625, 0.0125, 0.025, 0.05 and 0.1 mg/mL) of *T. terrestris* at 24°C. Each concentration was tested on a minimum of three bulbs, and seven seeds with solutions changed daily. Using a ruler, root length was measured

at 24 h, 48 h, 72 h, 96 h, and 120 h. Other indicators of toxicity, including root structure and color changes, are also assessed.

Genotoxicity assessment

To investigate the potential effect of genotoxicity, *A. cepa* bulbs and *V. faba* seeds with a root length of 1.5-2 cm were exposed to *T. terrestris* at different concentrations (0.00625, 0.0125, 0.025, 0.05 and 0.1mg/mL) for 12 h and 24 h. After treatment, the bulbs and seeds were washed thoroughly and then placed in an ethanol/acetic acid solution (3v/1v) for 24 h before storage in 70% ethanol at 4°C. After a brief wash with distilled water, the root tips were hydrolysed in 1 N HCl solution for 5 min at 60°C and stained with Schiff's reagent for 20 min. The slides were prepared following the method of Sharma and Sharma (2014). After the first pre-treatment, the root tips were carefully washed in distilled water several times. Then they were subjected to hydrolysis in a 1 N HCl solution for five minutes at a temperature between 60°C and 70°C. Then the apical 2 mm were crushed in a 45% acetic acid solution. The apical meristems were analysed at 40x magnification after being crushed in a drop of 45% acetic acid. The mitotic index and chromosome aberration index were calculated according to the methods of Fiskesjo (1985). using at least 1000 cells per slide and five slides for each concentration.

The following formulae (Akwu *et al*. 2019) were used to determine the proportion of chromosomal aberrations (CAs), the mitotic index (MI), and the chromosomal aberration frequency (AF) in the cells:

$$\text{Mitotic index (MI) (\%)} = \frac{Total number of dividing cells}{Total number of cells observed} \text{x } 100$$

$$\text{Chromosome Aberrations (CAs) (\%)} = \frac{Total number of abnomal cells}{Total number of cells observed} \text{x } 100$$

$$\text{Aberrations type frequency (AF) (\%)} = \frac{Number of a type of aberrant cell}{Total number of aberrant cells} \text{ x } 100$$

Statistical data analysis

Graph pad prism 9.2.0 (Graph Pad Software, LLC, CA, USA) was used for statistical analysis of root length, MI (%), and ACs (%). Data Results were compared statistically using one-way ANOVA and Dunnett's multiple comparison test. All values were expressed as mean ± SD and were determined statistically significant when $P<0.05$.

RESULTS

The changes in root length, form and color of roots are illustrated in Table 1 (*A. cep*a) and Table 2 (*V. faba*) following different treatment with *T. terrestris* compared to the control. It was observed that the inhibition effect of *T. terrestris* increased with the increase in concentration and duration of treatment. Therefore, control showed highest root elongation, with mean lengths of 10.77 ± 0.06 cm and 10.48 ± 0.05 cm after 120 h in *A. cepa* and *V. faba*, respectively.

In other hand, throughout the five-day treatment, the mean root lengths of *A. cepa* were unaffected by the 0.00625 mg/mL and 0.0125 mg/mL concentrations and 0.00625, 0.0125, 0.025, and 0.05 mg/mL concentrations in *V. faba*.

However, *A. cepa* roots exposed to the 0.025, 0.05, and 0.1 mg/mL concentrations range showed a significant decrease in root growth with values of 4.71, 3.92, and 3.16 cm, respectively. Moreover, a significant decrease ($P<0.05$) in root length was seen in *V. faba* roots treated with the 0.1 mg/mL concentration (3.33 cm) compared to the control. Simultaneously, the inhibitory effect of *T. terrestris* increased positively with concentration and time of exposure, whose value increased from 26.84% (0.006125 mg/mL) to 62.77% (0.1 mg/mL) after 120 h of exposure. Furthermore, it was remarked that *A. cepa* roots were more sensitive to *T. terrestris* than *V. faba* roots (62.77% and 55.92% inhibition, respectively).

Concerning the morphology of the roots, structural and color modifications were observed, particularly in *V. faba* roots treated from the 0.05 mg/mL concentration, which appeared slimy to slimy dark brown compared to the control. However, after 120 h of treatment, roots treated to the 0.05 and 0.1 mg/mL concentrations showed necrosis.

Figure 1 shows the effect of different *T. terrestris* concentrations on *A. cepa* and *V. faba* mitotic index. Meristematic cells of these two plants that are treated with different concentrations of *T. terrestris* showed a significant decrease in MI compared to the control. Our result showed that the control has the highest MI in both *A. cepa* and *V. faba* (59.26 ± 0.88% and 59.90 ± 0.40%, respectively) (12 h), and (60.74 ± 0.45% and 60.14 ± 0.48%, respectively) (24 h). In addition, Cell division was unaffected by the concentrations of 0.00625, 0.0125, and 0.025 mg/mL in *A. cepa* and 0.00625 and 0.0125 mg/mL in *V. faba*. In contrast, the values were as high as the control (12 h and 24 h) in *A. cepa* (60.80 ± 1.12%, and 60.24 ± 1.00%, respectively) and *V. faba* (59.88 ± 0.32% and 58.14 ± 0.35%, respectively). However, cytotoxic

Table 1. The change in mean root length (cm) of *A. cepa* after application of different concentrations (%) of *T. terrestris* at different exposure times .

Treatments (mg/ml)	Nr	Mean root length (cm) and inhibition (%) affected by *T. terrestris* treatment in *A. cepa.*											
		00 h	24 h		48 h		72 h		96 h		120 h		Mean of longth ± SD	Form and color
		Mean ± SD	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %		
Control	15	1.78 ± 0.08	4.18 ± 0.03	-	5.65 ± 0.02	-	7.32 ± 0.05	-	9.57 ± 0.09	-	10.77 ± 0.06	-	7.49 ± 2.71	Straight, white
0.00625	15	1.87 ± 0.07	4.01 ± 0.04	4.07	5.02 ± 0.03	11.16	5.84 ± 0.04	20.22	7.03 ± 0.07	26.55	6.91 ± 0.03	26.84	5,95± 1,54	Straight, white
0.0125	15	1.87 ± 0.07	3.83 ± 0.07	8.38	4.55 ± 0.06	19.47	5.09 ± 0.04	30.47	6.66 ± 0.07	30.41	5.09 ± 0.03	31.30	5.50 ± 1.48	Straight, white
0.025	15	1.71 ± 0.07	3.56 ± 0.04	14.84	4.20 ± 0.11	25.67	4.93 ± 0.06	32.66	5.35 ± 0.04	44.10	4.71 ± 0.03	48.57	4.71 ± 0.82*	Straight, white
0.05	15	1.86 ± 0.05	2.98 ± 0.03	28.71	3.3 ± 0.04	41.60	3.97 ± 0.03	45.77	4.45 ± 0.09	53.51	4.30 ± 0.04	54.14	3.92 ± 0.80**	Slimy, white
0.1	15	1.69 ± 0.02	2.24 ± 0.06	46.42	2.95 ± 0.05	47.79	3.01 ± 0.08	58.88	3.59 ± 0.05	62.49	4.19 ± 0.04	62.77	3.16 ± .67***	Slimy, white

Data are shown as mean ± SD, Nr: Number of roots, In (%): the inhibition percentage, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus control group using one-way ANOVA; Dunnett's test.

Table 2. The change in mean root length (cm) of *V. faba* after application of different concentrations (%) of *T. terrestris* at different exposure times.

Treatments (mg/ml)	Nr	Mean root length (cm) and inhibition (%) affected by *T. terrestris* treatment in *V. faba*											
		00 h	24 h		48 h		72 h		96 h		120 h		Mean of longth ± SD	Form and color
		Mean ± SD	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %		
Control	7	1.78 ± 0.08	3.38 ± 0.04	-	4.78 ± 0.06	-	6.24 ± 0.04	-	8.55 ± 0.06	-	10.48 ± 0.04	-	6.68 ± 2.85	Straight, white
0.00625	7	1.79 ± 0.07	3.20 ± 0.04	5.53	4.27 ± 0.05	10.67	5.01 ± 0.04	19.72	6.77 ± 0.07	20.82	8.21 ± 0.06	21.67	5.49 ± 2.00	Straight, white
0.0125	7	1.77 ± 0.02	3.01 ± 0.06	10.95	4.17 ± 0.02	12.77	4.77 ± 0.03	23.56	6.02 ± 0.05	29.60	7.32 ± 0.06	30.16	5.05 ± 1.66	Straight, white
0.025	7	1.74 ± 0.06	2.89 ± 0.07	14.50	3.68 ± 0.04	23.02	4.04 ± 0.05	35.26	5.22 ± 0.08	38.95	6.01 ± 0.03	44.56	4.36 ± 1.24	Slimy, white
0.05	7	1.80 ± 0.03	2.32 ± 0.06	31.37	2.98 ± 0.03	37.66	3.67 ± 0.06	41.19	4.52 ± 0.05	47.14	4.99 ± 0.02	52.39	3.69 ± 1.09	Slimy, dark brown
0.1	7	1.81 ± 0.06	2.22 ± 0.07	34.32	2.58 ± 0.10	46.03	3.24 ± 0.06	48.08	3.99 ± 0.09	50.34	4.62 ± 0.05	55.92	3.33 ± 0.98 *	Slimy, dark brown

Data are shown as mean ± SD, Nr: Number of roots, In (%): The inhibition percentage, * $p < 0.05$ versus control, used one-way ANOVA; Dunnett test.

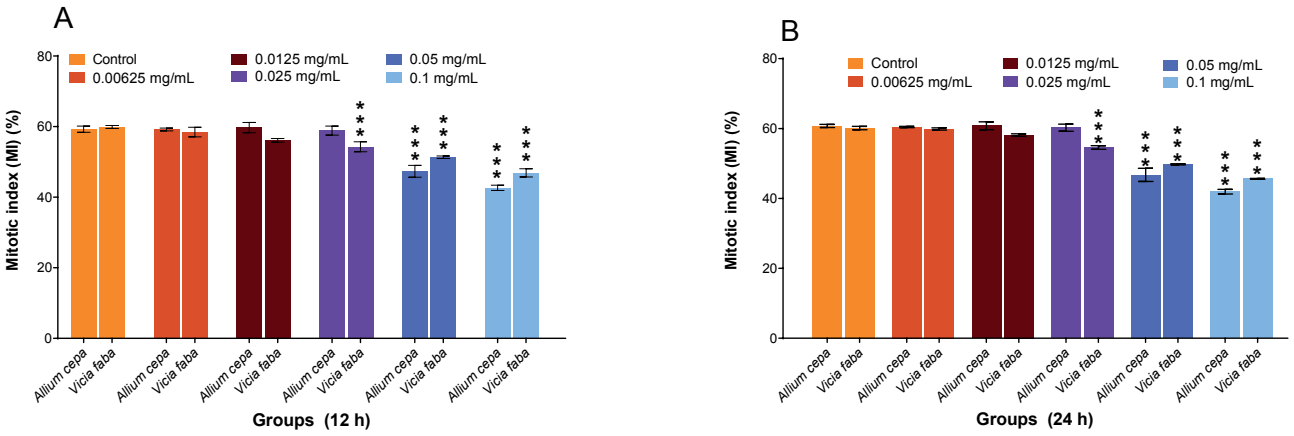

Figure 1. The effect of different *T. terrestris* concentrations (mg/mL) and exposure times on the MI (%) in *A. cepa* cells and *V. faba* cells after 12 h (A) and 24 h (B). Three bulbs and seven seeds were treated in each concentration, including the control. Five roots for each concentration were used. At least 1000 cells on each slide and five slides for each concentration were examined.

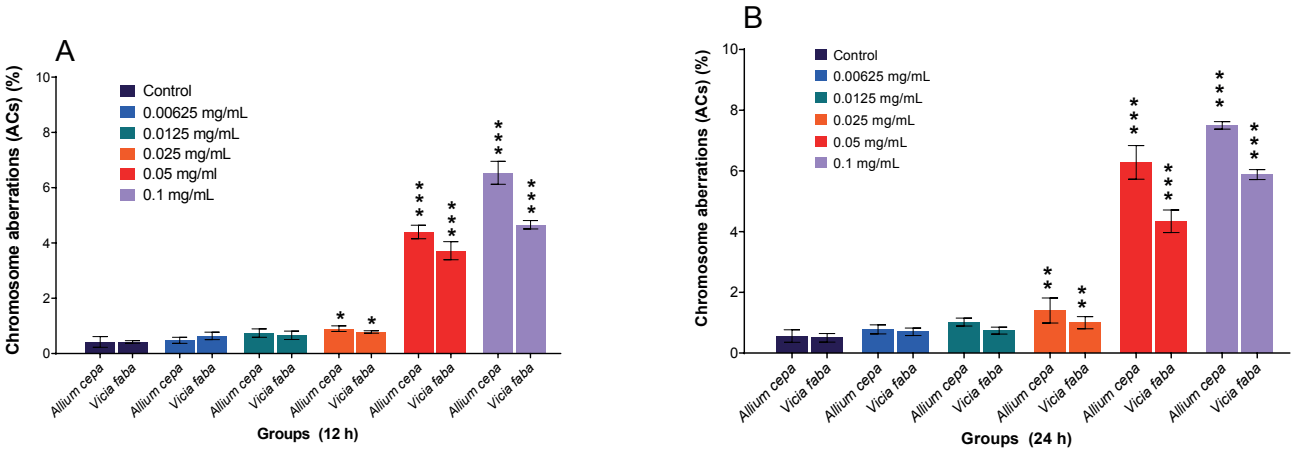

Figure 2. Percentage of CAs in *A. cepa* and *V. faba* cells induced by different concentrations of *T. terrestris* (mg/mL) after 12 h (A) and 24 h (B). Three bulbs and seven seeds were treated in each concentration, including the control. Five roots for each concentration were used. At least 1000 cells on each slide and five slides for each concentration were examined.

effects were observed from the 0.025 mg/ml concentration in *V. faba* and 0.05 mg/mL in *A. cepa* accompanied by a significant decrease in MI ($P<0.001$) (12 h and 24 h).

Figures 2, 3, 4, and 5 show *T. terrestris*-induced aberration percentages and different chromosomal abnormalities. It was showed that the increase in CAs (%) depends on *T. terrestris* concentration and treatment duration.

Compared to the control, no significant effect on CAs (%) was noted after treatment with the concentrations 0.00625 and 0.05 mg/mL (12 h and 24 h) in *A. cepa* and *V. faba*. While a significant increase ($P<0.001$) in ACs (%) was observed in cells treated with the concentrations 0.025, 0.05, and 0.1 mg/mL (12 h and 24 h) compared to the control.

The highest CAs (%) in *A. cepa* and *V. faba* were 7.5 ± 0.129% and 5.88 ± 0.16%, respectively, after 24 h treatment with *T. terrestris* at the 0.1 mg/mL concentration, and the lowest was 0.48 ± 0.10% and 0.64 ± 0.13%, respectively at the 0.00625 mg/mL concentration (12 h).

The most frequent types of CAs were multipolar in *A. cepa* (34.89 ± 2.30%) (12 h) and (33.97 ± 4.36%) (24 h) and in *V. faba* (34.08 ± 2.23%) (12 h) and (30.96 ± 4.13%) (24 h), followed by break in *A. cepa* (19.89% ± 1.75%) (12 h) and (26.07 ± 3.79%) (24 h) and in *V. faba* (24.20 ± 0.79%) (12h) and (24.19 ± 1.35%) (24 h), stickiness in *A. cepa* (17.49 ± 1.25%) (12 h) and (20.24 ± 3.58%) (24 h) and in *V. faba* (20.05 ± 3.79%) (12 h) and (19.18 ± 1.77%) (24 h), vagrant in *A. cepa* (18.94 ± 2.27%)

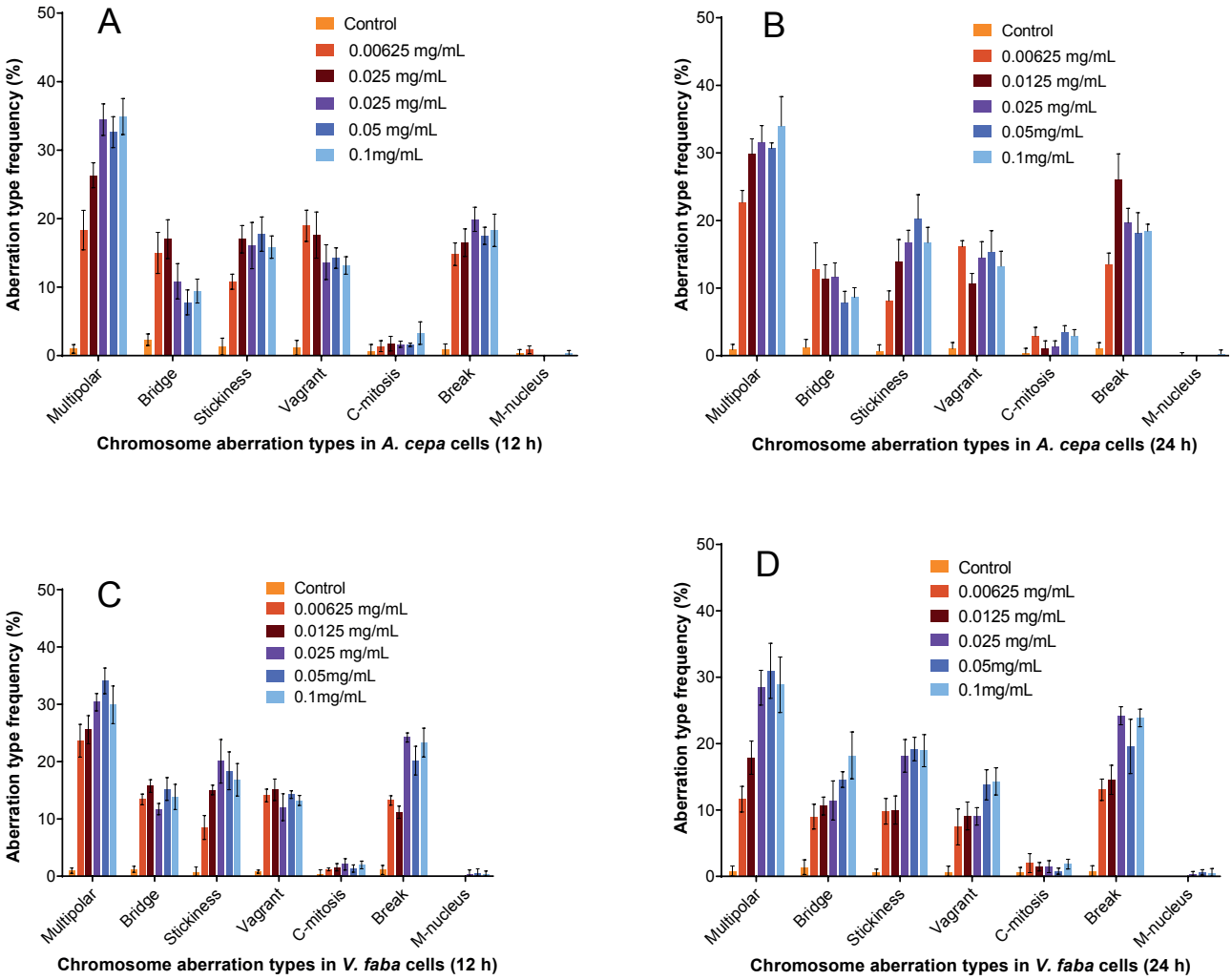

Figure 3. Frequency of different types of CAs induced by different concentrations of *T. terrestris* (mg/mL) in *A. cepa* cells after 12 h (A) and after 24 h (B) and in *V. faba* cells after 12 h (C) and after 24 h (D). Three bulbs and seven seeds were treated in each concentration, including the control. Five roots for each concentration were used. At least 1000 cells on each slide and five slides for each concentration were examined.

(12 h) and (15.38 ± 3.11%) (24 h) and in *V. faba* (15.07 ± 1.88%) (12 h) and (14.31 ± 2.05%) (24h), bridge in *A. cepa* (17.00 ± 2.82%) (12 h) and (12.80 ± 3.89%) (24 h) and in *V. faba* (15.73 ± 1.10%) (12 h) and (18.22 ± 3.52%) (24 h), C-mitosis in *A. cepa* (3.29 ± 1.63%) (12 h) and (3.52 ± 0.91%) (24 h) and in *V. faba* (2.06 ± 0.98%) (12 h) and (1.89 ± 1.34%) (24 h), and M-nucleus in *A. cepa* (0.35 ± 0.39%) (12 h) and (0.52 ± 0.75%) (24 h) and in *V. faba* (0.54 ± 0.74%) (12 h) and (0.59 ± 0.49%) (24 h) (Fig. 3A, 3B, 3C, 3D).

DISCUSSION

This study was conducted to assess the cytotoxic and genotoxic effects of *T. terrestris* methanolic extract by analysing the change in root growth, morphology, and color of *A. cepa* and *V. faba*. as well as the determination of MI and different types of CAs. Our results revealed that *T. terrestris* treatment induced cytotoxic and genotoxic effects manifested by inhibition of root growth, modification of root colour and structure, decrease in MI (%), and increase in CAs (%) with the appearance of various types of chromosomal aberrations. These changes are dependent on the different concentrations and duration of exposure to *T. terrestris*. Furthermore,

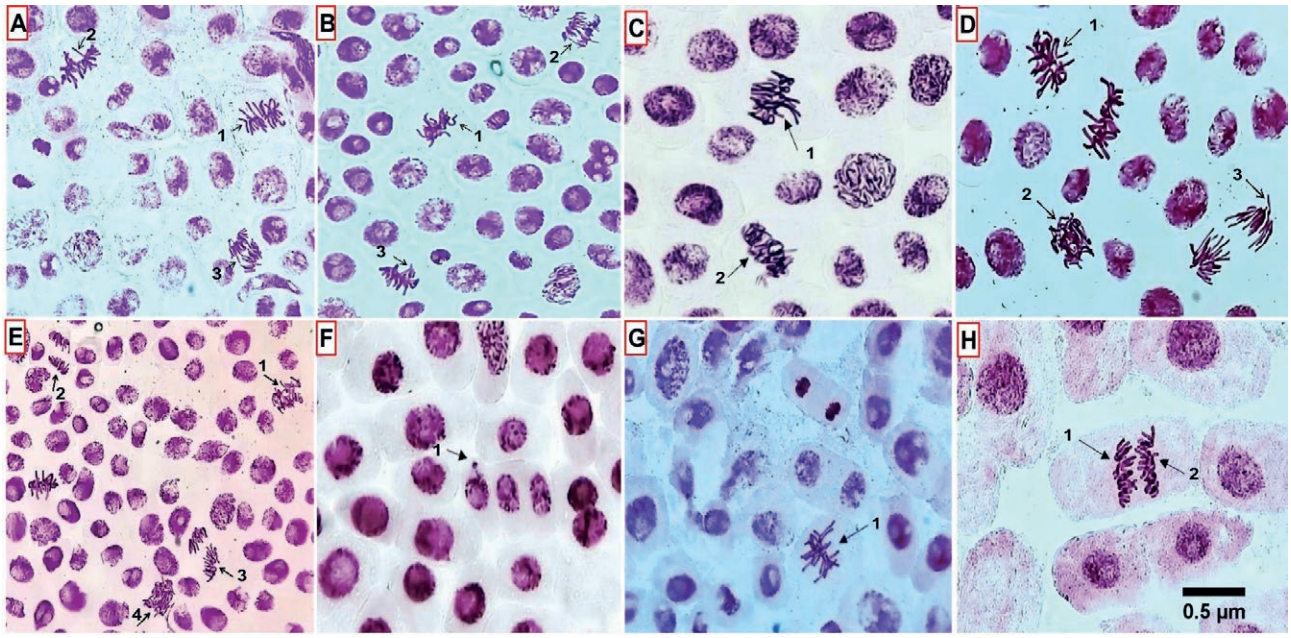

Figure 4. Photomicrographs showing different CAs induced by different concentrations of *T. terrestris* in *A. cepa* root tip cells: vagrant (A1) with break (A2), and bridge (A3), multipolar and bridge (B2), break (B1) and stickiness (B3), stickiness (C1, C2), c-mitosis (D1), stickiness (D2) and break (D3), stickiness (E1, E4) and multipolar (E2, E3), micro-nucleus (F1), c-mitosis (G1), stickiness (H1, H2). After 12 h and 24 h of treatment. scale bar = 0.5 μm.

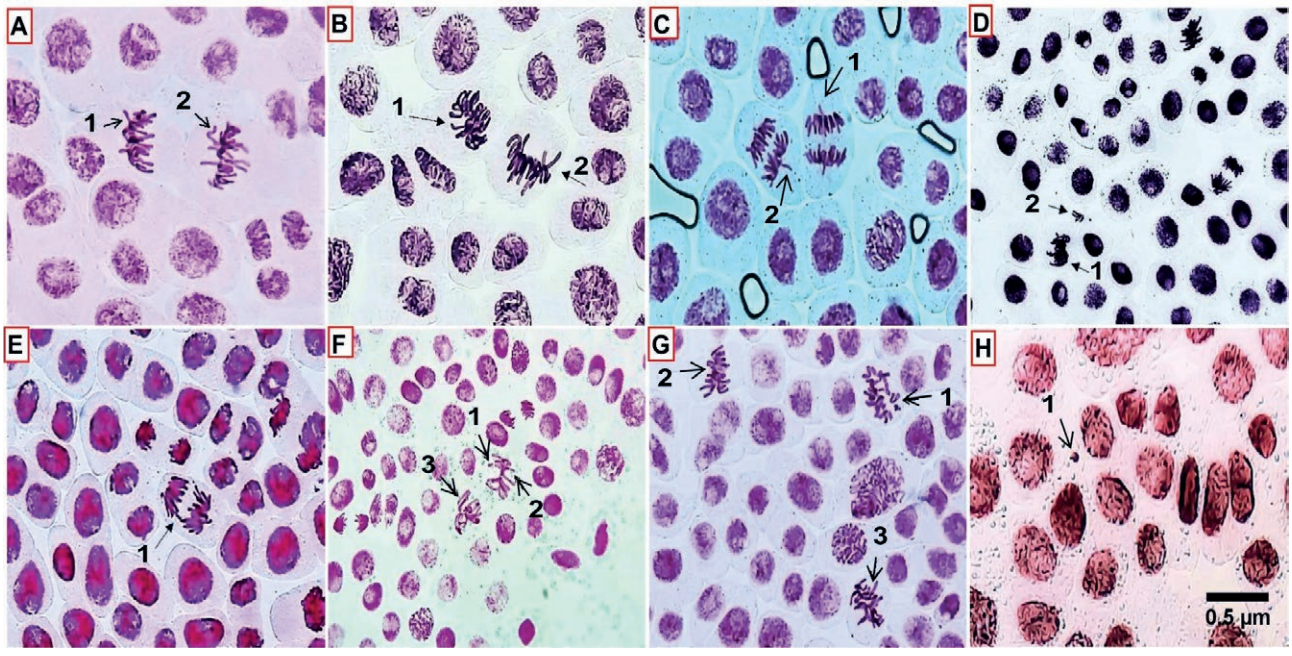

Figure 5. Photomicrographs showing different CAs induced by different concentrations of *T. terrestris* in *V. faba* root tip cells: stickiness (A1) and vagrant (A2), stickiness (B1, B2), multipolar (C1) and multipolar with break (C2), stickiness (D1) and vagrant (D2), multipolar with bridge (E1), break (F1, F2, F3), c-mitosis (G1) and stickiness (G2, G3), micro-nicleus (H1). After 12 h and 24 h of treatment. scale bar = 0.5 μm.

the results obtained on root growth and structure are in agreement with the results of Basu and Tripura (2021) on *Cascabela thevetia* who found a decrease in root growth, turgescence, and color change in *A. cepa* and *V. faba* treated with a high concentration of *Cascabela thevetia* extract. Similar results were approved by the studies of Issa *et al*. (2020) on the roots of *Avena fatua* and *Echinochloa crus-galli* exposed to a high concentration of *Vitex negundo*. According to Wierzbicka (1988), root growth is directly related to the enzymatic activity and cell elongation of the meristematic zone. This activity promotes cell elongation and membrane release during cell differentiation (Silveira *et al*. 2017). Thus, the slowing of root growth may be due to the inhibitory effect of *T. terrestris* on the enzymatic activity that promotes the elongation of the meristematic region.

The mitotic index (MI) is an indicator to determine the cytotoxicity induced by toxic substances (Leme and Marin-Morales 2009). The MI is also used to measure the portion of dividing and arrested cells during the cell cycle (Rojas *et al*. 1993). In this study, the decrease in MI after 12 h and 24 h of treatment with *T. terrestris* suggested the significant cytotoxic effect of this plant. Furthermore, our results indicate that *T. terrestris* inhibits cell division in *A. cepa* and *V. faba*, significantly reducing MI at high concentrations. The cytotoxic and genotoxic effects of plants are evaluated by several studies, including *Vitex negundo* (Issa *et al*. 2020), *Citrus aurantiifolia* (Fagodia *et al*. 2017) and *Plantago major* (Ždralović *et al*. 2019). However, research investigating the genotoxicity of *T. terrestris* on meristematic cells is scarce.

The current study showed a correlation between the increase in *T. terrestris* concentration and the reduction of MI. According to the results of Qari and El-Assouli (2019), the aqueous extract of *T. terrestris* fruit can inhibit the proliferation of human lymphocytes in culture. This decrease in IM could be caused by the arrest of mitotic phases or by decelerating the cytokinesis process (Kundu and Ray 2017). However, our results suggest that this inhibition is caused by the genotoxic effects of one or more components of the *T. terrestris* extract that can damage DNA strands in a specific way (Qari and El-Assouli 2019). In addition, Kundu and Ray (2017) found that *T. terrestris* fruit extract can inhibit cell division due to a DNA defect, suggesting that it could be used as an anticancer agent based on its ability to inhibit cell proliferation, its safety on the DNA molecule at lower doses, and its antioxidant component.

Different types of chromosomal aberrations (CAs) were observed at all concentrations applied. There is a significant increase in CAs (%) from the concentration of 0.025 mg/mL. It also produced aberrant chromosome segregation and caused the formation of various anomalies such as multipolar, chromosome bridge, stickiness, vagrant, c-mitosis and micro-nucleus. Similar results were obtained by *Anita Sharma et al*. (2019) in *A. cepa* root cells treated with *H. suaveolens* extract. Furthermore, Sabeen *et al*. (2020) reported that CAs are produced by proteolysis and by blocking DNA synthesis. Data analysis showed that multipolar anaphases were the most common CAs. Khallef *et al*. (2019) suggests that mitotic spindle instabilities may produce anaphase multipolarity. In addition, Sabeen *et al*. (2020) suggests that CAs, like chromosomal breaks and bridges, indicate clastogenic activity. However, stickiness, which causes cell death, may be caused by excess chromosomal condensation or inappropriate nucleoprotein biosynthesis (Sabeen *et al*. 2020). According to Mercykutty and Stephen (1980), stickiness may be caused by the depolymerization of DNA, the partial dissolution of nucleoproteins, the breakage and exchanges of the basic folded fiber units of chromatids, and the stripping of the protein covering of DNA in chromosomes.

This study typically found chromosome break formation and stickiness. Kuchy *et al*. (2015) suggests that chromosomal condensation or excessive nucleoprotein production can form stickiness. Another notable abnormality was the chromosomal vagrant. Due to spindle abnormality, vagrant chromosomes are induced, resulting in the dissociation of an unequal distribution of chromosomes in the daughter nuclei and the generation of daughter cells with abnormally small or sized nuclei during interphase (El-Ghamery *et al*. 2003). C-mitosis and micro-nucleus were rare in our results and their presence may be due to the spindle apparatus' incapacity to arrange and function appropriately (Rosculete *et al*. 2019).

T. terrestris has been traditionally used for medicinal purposes throughout history, addressing various health issues such as impotence, rheumatism, edema, hypertension, and kidney stones (Chhatre *et al*. 2014). Pharmacological studies conducted on *T. terrestris* have demonstrated its aphrodisiac, analgesic, antibiotic, antihyperglycemic, antihyperlipidemic, larvicidal, repellent, antioxidant, cytotoxic, immunomodulatory, hypolipidemic, anticancer, antibacterial, and antifungal properties (Chhatre et al., 2014). Furthermore, in order to ensure safe therapy, it is important to test the cytogenotoxicity of *T. terrestris*. Our current study on the cytogenetic impact of *T. terrestris* revealed cytotoxic and genotoxic effects depending on the concentrations applied and the duration of treatment. It observed that signs of toxicity appeared from a concentration of

0.05 mg/mL, resulting in a significant reduction in the mitotic index and a significant increase in chromosomal aberrations, as well as a change in the shape and colour of the roots of *A. cepa* and *V. faba*, indicating necrosis. Our results underline the interest in using *T. terrestris* as an effective medicinal plant for various diseases, but its use must respect appropriate therapeutic doses and not be anarchic.

CONCLUSION

This study is the first to investigate the cytotoxic and genotoxic effects of *T. terrestris* on meristematic cells of *A. cepa* and *V. faba*. Decreased root growth with decreased mitotic index and increased chromosomal abnormalities resulting from treatment with *T. terrestris* are signs of cytotoxicity and genotoxicity. Therefore, this plant should be used with caution in traditional medicine.

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