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Cytotoxic assessment of aqueous extracts of *Heliotropium keralense* Sivar. & Manilal on *Allium cepa* root tip cells

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Abstract. *Heliotropium keralense* Sivar. & Manilal is an endangered medicinal plant native to the Indian state of Kerala. Cytotoxic effects of aqueous extracts of leaves, stems, and roots of *H. keralense* were evaluated using *Allium cepa* L. root tip method. *Allium cepa* bulbs were exposed to extracts of different parts of the plant for 24 hours. Compared to the negative control, a significant decrease in the length, root number and mitotic index of *Allium cepa* was observed with 5 to 25% aqueous extracts of *H. keralense.* Chromosomal abnormalities such as single, double, and multiple lesions in interphase, single and double lesions in prophase, diagonal metaphase, diagonal anaphase, bridged anaphase, strap-shaped nuclei, giant cells and chromosome loops are identified in positive control and treatments. The highest percentage of chromosomal aberration was observed in the (95.18±2.07%) positive control and 25% (71.76±7.46%) leaf extract. The analysis showed that the aqueous plant parts of *H. keralense* had anti-mitotic and cytotoxic effects. This study shows that *Heliotropium keralense* contains strong cytotoxic substances that can cause chromosomal aberrations.

Keywords: *Heliotropium keralense, Allium cepa*, Cytotoxicity, Chromosomal aberrations, Anti-mitotic.

INTRODUCTION

Phytomedicines play a crucial role in the treatment of human and animal diseases. They are safer than the synthetic drugs. These plant derived metabolites can be isolated, identified, tested and used against new diseases. Plant extracts are extensively used in medicine and the food industry; therefore, evaluating the cytotoxicity of plants against other cell lines and organisms appears crucial to determine non-toxic concentrations at which they can be safely used (Gazala et al., 2018). Many people use these plants for food and therapeutic purposes without having sufficient knowledge about the safe use of these medicinal plants and its products. Herbal products and herbal medicines must be properly evaluated and researched for the phytochemicals and their potential risk of adverse side effects due to overdose and toxicity.

It becomes necessary to investigate the toxicity of phytochemicals because of the adverse effects associated with their use in conventional medicine.

Heliotropium is a large genus of the Boraginaceae family with a wide distribution in tropical and temperate areas. *Heliotropium keralense* Sivar. & Manilal is endemic to Kerala, India. The plant is used as remedy against worms, skin diseases, scorpion and snake poisoning, asthma, cough, anemia, insanity, and epilepsy (Sivarajan, 1994). Flavonoids and terpenoids are abound throughout the plant. The plant contains several pharmacologically active compounds with therapeutic effects of antibacterial, antiviral, anti-inflammatory, and anticancer activities (Nayar, 1996). *Heliotropium keralense* Sivar. & Manilal (Thelkatta) is a tribal medicinal plant used by Mullukuruma tribes in Wayanad district of Kerala. A paste made from the leaves of the plant is applied on the bitten area in the treatment of sting bite (Silja et al., 2007). Due to the lack of knowledge about the genotoxic and cytotoxic potential of the plant, it is important to evaluate the effect of the plant extract on the cell and genetic material. The present study deals with the anti-mitotic and cytotoxic effects of aqueous extracts of different parts of *H. keralense*. The cytotoxicity was tested on *Allium cepa* root tip cells. The simplest and most ideal method for examining the impact of mitosis in plant cells was to examine the root tip of *Allium cepa*. It is a fast and inexpensive system for measuring the cytotoxic effects of pollutants, chemical substances, and plant extracts (Barman et al., 2021; Das et al., 2021).

MATERIALS AND METHODS

Plant collection and preparation of extract

Heliotropim keralense was collected from a paddy field in Pathanamthitta District, Kerala, India (latitude 9°15'26.4"N; longitude 76°49'35.6"E). Plant parts are separated and cleaned. Shade-dried plant parts are powdered. 5g, 10g, 15g, 20g, and 25g of plant parts (leaf, stem, and root) were weighed and boiled in 100ml of distilled water for 10 min. The extracts were filtered using Whatsman No.1 filter paper and used for the treatment.

Effect of plant extracts on root tip cells of Allium cepa

The *Allium cepa* test was used to investigate the cytotoxic activity of the aqueous extracts from the leaf, stem, and root of *H. keralense*. Commercially available *A. cepa* bulbs of the same size (4-5g) were used, carefully de-scaled and placed on top of test tubes filled with distilled water for germination for 48h. The germinated bulbs were then transferred to various concentrations (5, 10, 15, 20 & 25 g/100ml) of aqueous extract of *H. keralense* plant parts for 24h. *Allium cepa* germinated in distilled water was used as negative control. Onions germinated in distilled water followed by treatment with hydrogen peroxide (7%) for 1h were considered as positive control. After treatment, the roots were counted and the length of the roots in each bulb was measured. The roots were fixed in Carnoy's fluid (ethanol: acetic acid, 3:1). After fixation, the roots were hydrolyzed in 1M/L Hydrochloric acid for 1 min at 60°C. The roots were placed on microscopic slides, crushed using 2% acetocarmine and observed under a microscope. Mitotic index was expressed as number of dividing cells/total number of cells counted (Ozmen and Summer, 2004). Chromosomal aberrations were determined by randomly selecting five zones per slide. Mitotic index was determined using the equation

Mitotic Index = (Number of dividing cells) ÷ (Total number of cells) × 100

The chromosome aberration frequency was expressed as a percentage. This was calculated by using the equation

Chromosome aberration frequency = (Number of cells with chromosome aberration) ÷ (Total number of cells) × 100

Statistical analysis

Ten random samples were taken to analyze the root growth of *Allium cepa* grown in different concentrations of aqueous extracts. Mitotic index was counted under the oil emersion (100x) microscopy. Mitotic index and chromosomal aberrations were determined by randomly picking five zones per slide. Photo documentation was taken using microscope Olympus CX 41 attached with camera Cmos Cam (3.0m pixels). Data on root number, root length, mitotic index and chromosomal aberration percentage in *Allium cepa* were subjected to statistical analysis. One way ANOVA was performed to determine the significance of tests using SPSS free trial Software.

RESULTS

Effect of various treatments on root growth of Allium cepa

The effects of different concentrations (5, 10, 15, 20, and 25%) of aqueous extracts of *H. keralense* plant parts (leaf, stem, and root) on root number and root length were significant (P<0.001). The mean numbers of root in the negative and positive controls (Table 1) were 56.4±12.54 and 7.4±1.81 respectively. The average number of roots of *Allium cepa* bulbs is higher in 5% extracts (leaf, stem, and root) of *H. keralense* is 39.8±2.77, 53.8±9.03 and 35.8±2.38. The mean root number in 25% of extracts of leaf, stem, and root are 8±2.44, 11.2±2.16, and 13.4±2.96 per individual bulb of *Allium cepa*. The number of roots decreases with the increasing concentration of plant extracts.

The mean root length was found to be4.32±0.80 cm in negative controls and 0.06±0.56cm in the positive control (Table 1). A continuous decrease in root growth was observed from lower concentration of the treatments to its higher concentrations. Root growth in 5% aqueous extracts of leaf, stem, and root of *H. keralense* is 5.66±0.86, 4.52±0.32, and 3.6±0.43cm, respectively. *Allium cepa* root growth is reduced in 25% of leaf, stem, and root extracts (0.6±0.38, 0.28±0.08, and 0.86±0.43cm, respectively). *Allium cepa* root lengths decreased with increasing the concentration of extracts.

Average root length and root number of treatments are (5, 10, 15, 20, and 25%) lower than the negative control. Root number and root length of *Allium cepa* were reduced from a lower concentration of extracts to a higher concentration of extracts of different plant parts. The highest concentrations of plant extract showed maximum inhibitory effects on root growth. The mean values of treatments are significantly (P<0.001) lower than the negative control. Treatments of *H. keralense* in *A. cepa* root apical meristem cells showed a concentration-dependent inhibitory effect on root growth.

Effect of various treatments on mitotic index of Allium cepa root cells

Significantly high mitotic index was observed in negative control (Table 2). A significantly (P<0.001) low mitotic index was observed in the aqueous extracts of leaves, stems, and roots of *H. keralense* compared to the negative control (Table 2). Aqueous extract of the stem, leaf, and root of *H. keralense* actively inhibits cell division in *Allium cepa* roots. In different concentrations of extracts, the number of dividing cells decreases with increasing concentration of the extracts. Among the different treatments, the lowest mitotic index was observed in the leaf, stem, and root (25%) extracts (13.54±5.27, 18.37±3.84, and 14.53±4.49). In leaf extracts, the mitotic index of *Allium cepa* cells is reduced from 5% (62.62±8.56) to 25% (13.54±5.27) of the extracts. In stem extracts, the mitotic index was 51.88±10.38 in 5% and 18.37±3.84 in 25% extracts. In cells treated with root extract, 52.6±5.65 mitotic index in 5% and 14.53±4.49 mitotic index in 25% extracts. Mitotic index decreases with the increasing concentration of plant extracts (Table 2).

Cytological effect of various treatments on Allium cepa root tip cells.

Compared to the negative control (Fig. 1c,d,e), chromosomal aberrations were found to be very high in the treatments. The positive control shows the maximum percentage of aberration. All cells treated with the plant

Table 1. Effect of aqueous extracts of *Heliotropium keralense* on *Allium cepa* root growth.

Treatment	Leaf extract		Stem extract		Root extract	
	Root number	Root length (cm)	Root number	Root length (cm)	Root number	Root length (cm)
Negative control	56.4±12.54^{a}	4.32±0.80^{a}	56.4±12.54^{a}	4.32±0.80^{a}	56.4±12.54^{a}	4.32±0.80^{a}
Positive control	7.4±1.81^{d}	0.06±0.56^{d}	7.4±1.81^{c}	0.06±0.56^{c}	7.4±1.81^{c}	0.06±0.56^{c}
EX 5%	39.8±2.77^{b}	5.66±0.86^{a}	53.8±9.03^{a}	4.52±0.32^{a}	35.8±2.38^{b}	3.6±0.43^{a}
EX 10%	34.6±1.81^{b}	3.86±0.39^{b}	34±4.06^{a,b}	3.14±0.19^{a}	33±4.84^{b}	3.08±0.65^{a}
EX 15%	19.2±2.38^{c}	3.02±0.70^{b}	20.8±3.27^{b}	1.84±0.43^{b}	22.8±2.58^{b,c}	2±0.72^{b}
EX 20%	16.4±4.61^{c}	1.52±0.34^{c}	13.2±3.27^{c}	1.26±0.11^{b}	21.2±4.43^{b,c}	1.42±0.35^{b}
EX 25%	8±2.44^{d}	0.6±0.38^{d}	11.2±2.16^{c}	0.28±0.08^{c}	13.4±2.96^{c}	0.86±0.43^{c}
Main effect F df (n-1) = 6	99.14***	59.09***	62.59***	191.43***	32.43***	22.06***

EX: means the different concentrations of plant extract. *** Significant at P<0.001 level. Means within column followed by the same letters are not significantly (P<0.05) different as determined by DNMRT.

Table 2. Mitotic index of *Allium cepa* cells treated with *Heliotropium keralense* extract.

Treatment	Leaf extract	Stem extract	Root extract
Negative control	62.62±8.56[a]	51.88±10.38[a]	52.6±5.65[a]
Positive control	33.11± 3.55[b]	33.37±6.69[b]	39.36±9.49[b]
EX 5%	28.9±11.79[b,c]	32.62±2.02[b]	34.61±1.03[b]
EX 10%	23.05±8.26[b,c]	25.8±3.58[c]	23.99±6.8[c]
EX 15%	13.54±5.27[c]	18.37±3.84[d]	14.53±4.49[c]
EX 20%	68.58±5.8[a]	68.58±5.8[a]	68.58±5.8[a]
EX 25%	12.12±2.06[c]	12.12±2.06[d]	12.12±2.06[c]
Main effect F df (n-1) = 6	26.645***	21.041***	27.978***

EX: means the different concentrations of plant extract. ***Significant at P<0.001 level. Means within column followed by the same letters are not significantly (P<0.05) different as determined by DNMRT.

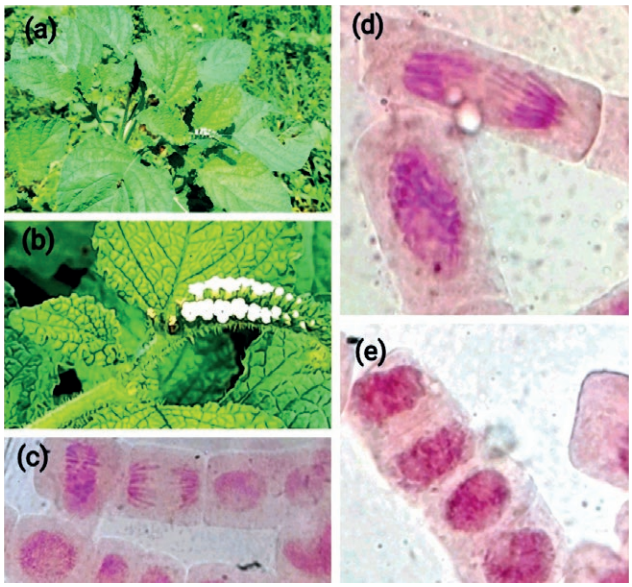

Figure 1. (a) *Heliotropium keralense*. (b) *H. keralense* infloresecence. (c) Normal mitotic phases (interphase,metaphase, and anaphase)in *Allium cepa* root tip .(d) Prophase and anaphase. (e) Telophase.

extract show aberrations. The percentage of aberrations increased with increasing concentration of extracts of individual parts of the *H. Keralense* plant. The leaf extract of *H. keralense* showed a higher percentage of aberration compared to other plant parts. The percentage of aberration increases with the increase with the concentration of aqueous extract of leaves, stem, and root of *H. keralense*.

The mean percentage of aberrations for all treatments is significantly (P<0.05) low compared to positive control. Among the treatments, lowest percentage of aberration was observed in 5% leaf extract was 21.88±3.78. The percentage of aberration of 25% of leaf, stem, and root extracts (71.76±7.46, 67.17±7.22, and 53.43±1.97%) was significantly (P<0.05) lower than that of positive control (95.18±2.07%). Lesions were the common abnormality seen in the interphase and prophase. From the analysis, it was found that plant extracts produce more aberrations in interphase and prophase.

In 25% leaf extract, 34.6±2.6% aberrations are observed in interphase, 17.4±1.14% aberrations in prophase, 11.8±2.49% in metaphase, 9.7±2.58% in anaphase (Table 3). The percentage of aberrations of the 25% leaf extract (71.76±7.46) was significantly (P<0.05) lower than that of the positive control (95.18±2.07%).

In the stem, a significantly low chromosomal aberration was recorded in the 5% stem extract (21.85±2.10) compared to higher concentrations (Table 4). Among

Table 3. Cellular abnormalities observed in *Allium cepa* exposed to the leaf extract of *Heliotropium keralense*

Conc.	Total no. of cells	Percentage of aberrant cells	Percentage of abnormality			
			Interphase	Prophase	Metaphase	Anaphase
5%	118.8±29.82[a]	21.88±3.78[b]	11.2±2.28[c]	8.2±1.3[c]	4.6±1.81[b,c]	2.2±0.83[b,c]
10%	112.6±24.37[a]	28.35±2.66[b]	13.4±2.07[c]	10.6±3.64[b,c]	4.4±1.14[b,c]	3.6±1.14[b,c]
15%	104.8±12.21[a]	33.73±8.30[b]	15.4±2.4[c]	10.8±3.03[b,c]	5±2.34[b]	4.4±0.83[b]
20%	91.6±3.5[b]	56.13±10.67[b]	24.4±2.3[b]	14.6±3.5[b]	6.8±1.3[b]	5.8±0.83[b]
25%	102.4±6.65[a]	71.76±7.46[a]	34.6±2.6[b]	17.4±1.14[b]	11.8±2.49[a]	9.7±2.58[a]
Negative Control	90.2±7.32[b]	9.9±1.65[c]	5±2[d]	2±0.7[d]	1±0[c]	1±0[c]
Positive Control	116.2±6.22[a]	95.18±2.07[a]	53.8±10.94[a]	23.8±2.77[a]	19.4±4.39[a]	13.7±2.86[a]
Main effect F df(n-1)=6	1.587[NS]	53.962***	85.419***	8.804**	13.35***	21.069**

[NS] non significant, **P<0.005,***P<0.001. Means within column followed by the same letters are not significantly (P<0.05) different as determined by DNMRT.

Table 4. Cellular abnormalities observed in *Allium cepa* exposed to the stem extract of *Heliotropium keralense.*

Conc.	Total no. of cells counted	Percentage of aberrant cells	Percentage of abnormality			
			Interphase	Prophase	Metaphase	Anaphase
5%	121.2±13.8^{a}	21.85±2.10^{b}	11±1.58^{c}	7.6±2.88^{c}	6.4±1.51^{c}	1.8±0.83^{b}
10%	93.6±20.18^{b}	26.68±2.69^{b}	11.8±2.68^{c}	4.6±1.14^{c}	6.4±2.07^{c}	2.2±1.3^{b}
15%	99.2±20.51^{b}	30.72±6.11^{b}	13±3.8^{c}	5.8±1.78^{c}	8.2±1.78^{c}	3.8±2.04^{b}
20%	109.8±23.95^{a}	44.98±12.06^{b}	17.4±4.03^{c}	12.2±1.92^{b}	10.4±2.51^{b}	9.6±1.81^{a}
25%	94.8±15.35^{b}	67.17±7.22^{b}	25.2±3.76^{b}	14.4±3.5^{b}	12.8±2.58^{b}	11.6±6.34^{a}
Negative Control	90.2±23.74^{b}	9.9±1.65^{c}	5±2.23^{d}	2±0.7^{c}	1±0^{c}	1±0 ^{b}
Positive Control	116.2±33.15^{a}	95.18±2.07^{a}	53.8±12.51^{a}	23.8±6.9^{a}	19.4±6.8^{a}	13.8±2.86^{a}
Main effect F df(n-1)=6	1.863^{NS}	41.541***	15.819*	15.392**	8.342*	10.089***

^{NS} non significant, *P<0.01, **P<0.005, ***P<0.001, ***P<0.001. Means within column followed by the same letters are not significantly (p<0.05) different as determined by DNMRT.

Table 5. Cellular abnormalities observed in *Allium cepa* exposed to root extract of *Heliotropium keralense.*

Conc.	Total no. of cells	Percentage of aberrant cells	Percentage of abnormality			
			Interphase	Prophase	Metaphase	Anaphase
5%	118.2±18.64^{a}	19.31±3.89^{b}	9.6±2.07^{c}	6.4±2.3^{c}	5.8±2.16^{b,c}	1.6±0.54^{c}
10%	88.6±10.59^{b}	25.29±5.91^{b}	9.8±1.64^{c}	6.6±2.96^{c}	4.4±1.51^{b,c}	1.8±0.44^{c}
15%	114.6±17.91^{a}	30.02±8.30^{b}	15.6±6.58^{b,c}	8.8±2.86^{c}	7.4±2.88^{b}	2.8±0.83^{c}
20%	98.4±3.91^{a}	41.8±19.27^{b}	17.8±5.49^{b,c}	9.8±3.42^{c}	8.8±1.92^{b}	5±1.58^{b}
25%	104.8±11.25^{a}	53.43±1.97^{b}	27.6±6.46^{b}	12.4±2.96^{b}	9.8±2.38^{b}	6.2±2.68^{b}
Negative Control	90.2±2.86^{b}	9.9±1.65^{c}	5±2.82^{d}	2±0.89^{d}	1±0^{c}	1±0^{c}
Positive Control	116.2±18.15^{a}	95.18±2.07^{a}	53.8±14.46^{a}	24±7.68^{a}	19.4±7.63^{a}	13.8±4.96^{a}
Main effect F df (n-1)=6	3.921*	13.630***	11.106***	3.586***	4.84**	9.47**

*P<0.01, **P<0.005, ***P<0.001, ***P<0.001. Means within column followed by the same letters are not significantly (p<0.05) different as determined by DNMRT.

the treatments, highest aberrations were noticed in 25% extract. In 25% stem extract, treated cells showed 25.2±3.76% aberrations in interphase, 14.4±3.5% in prophase, 12.8±2.58% in metaphase, and 11.6±6.34% in the anaphase (Table 4).

In root, a significantly low chromosomal aberration was noticed in 5% (19.31±3.89%) extract compared to higher concentrations. In root tip cells treated with 25% root extract, 27.6±6.46% of aberrations in interphase, 12.4±2.96% aberration in prophase, 9.8±2.38% in metaphase, and 6.2±2.68% in the anaphase (Table 5). All parts of this plant show an undifferentiated range of aberration on the cells of the *Allium cepa* root tip. Lesions are seen in interphase and prophase. More chromosomal aberrations are seen in metaphase and anaphase.

Chromosomal lesions and chromatid bridges are high in 20 and 25% of the plant extracts and in the positive control. All treatments showed varying levels of chromosomal aberrations, but this was less compared to the positive control. Major abnormalities (Fig. 2) such as giant cells, strap-shaped nuclei, single, double, and multiple lesions in interphase, single and double lesions in prophase, metaphase clumping, diagonal metaphase, diagonal anaphase(Fig. 2g), bridged anaphase (Fig. 2e), lagging chromosome, metaphase clumping (Fig. 2f) and chromosome loops were observed in treatment and positive control. Sticky chromosomes and chromosome loops are high in higher concentrations (20 & 25%) of the leaf extract and on the positive control.

Most of the aberrations are noticed in metaphase and anaphase and are in the higher concentrations (20 & 25%) of the plant extracts. Prophase and interphase lesions also increase in higher concentrations of the plant extracts. These aberrations inhibit the cell division and also cause the cell death.

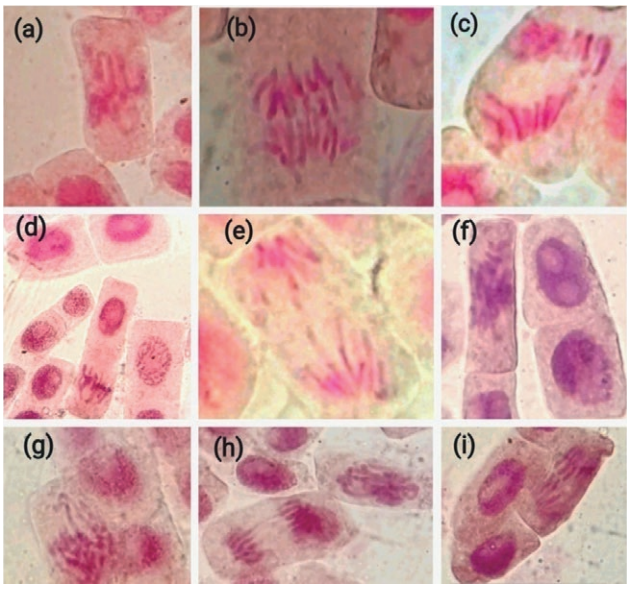

Figure 2. *Allium cepa* root tip cells exposed to the extracts of *Heliotropium keralense*(a)Metaphase clumping (b) Chromosome fragments.(c) Diagonal anaphase (d) Diagonal metaphase, giant cells (e) chromosome fragments (f)Diagonal metaphase and double lesions(g) Disoriented anaphase (h) Chromosome bridge and metaphase clumping(i) polar deviation.

DISCUSSION

In this study, the effect of *H. keralense* extracts was evaluated by root growth and cytology of root tip cells of*Allium cepa*. In *Allium cepa,* aqueous extracts of *H. keralense* reduces the root length and prevent root formation. .As the concentration of leaf, stem, and root extracts increased, the number and length of roots decreased. This growth gradation indicates an inhibitory effect of *H. keralense* on growth and cell division in *Allium cepa* roots and is similar to previous studies where in aqueous extracts of *Capparis spinosa* caused decreased mitotic index in *A.cepa* root tips(Sultan and Celik, 2009).The aqueous extract of *Campomanesia xanthocarpa* also showed the same effect in *A. cepa* root cells (Pastori et al., 2013).

Reduced mitotic index of *Allium cepa* root cells treated with different concentrations of extracts was observed in the present study. This indicates the inhibition of cell growth and cell death. It was found that the mitotic index decreased with increasing concentrations of the plant extracts. This result indicates the inhibition and suppression of mitotic division in the root cells of *A. cepa* by the chemical compounds present in the aqueous extract of plant parts. The reduction of cell division and cell differentiation in *A. cepa* root cells indicates the cytotoxic effect of the components in *H. keralense* aqueous extract.

The current findings demonstrate that as the concentration of leaf, stem, and root extracts increases, so do interphase and prophase lesions. Giant cells are generated as a result of endoreplication or endomitosis, while binucleated cells appear as a result of interrupted cytokinesis (Das et al., 2022). Chromosomal bridges, polar deviation during different mitotic phases and metaphase clumping, were the most frequent abnormalities: all of these aberrations are regarded as being notably cytotoxic (Askin and Aslanturk 2010, Barman et al., 2020, Roy et al., 2021). As treatment concentration increases, a variety of cytological abnormalities occurred throughout both metaphase and anaphase. At a 25% leaf extract concentration, the frequency of mitotic abnormalities such as diagonal metaphase and bridged anaphase is greater. The anaphase and metaphase abnormalities were low in different concentration of leaf, stem and root extracts compared to onion root tips treated with positive control. Chromosome stickiness can result from excessive elongation of chromatin filaments, which causes their improper condensation and can alter the physiochemical properties of nucleic acids, there by arresting the normal process of cell division and promoting cell death (Joti et al., 2012, Renjana et al., 2013, Moustafa et al., 2016, El-Ghamery and Mousa, 2017, Barman and Ray, 2022).This study found that the aqueous extract of *Heliotropium keralense* was lethal to the cell division of *Allium cepa* root tips.

Comparing extracts of leaf, stem, and root to negative control, a reduction in mitotic index was noted. A decrease in the mitotic index indicates that the extracts inhibit the DNA synthesis or block the G2 phase in the cell cycle (Akinpelu et al., 2019), thereby preventing the cell from entering mitosis (Sudhakar et al., 2001). Polar deviation of chromosome can occur due to intra- spindle filament distribution the distribution and indicates the presence of compounds that can interrupt the spindle fiber formation (El-Ghamery and Mousa, 2017). Anaphase bridges and sticky chromosomes are indicative of abnormal DNA condensation and destabilization of mitotic spindles (aneugenic effects) in *A. cepa* root tip cells (Barman et al., 2020, 2021 and 2022).

Mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death or a delay in the kinetics of cell proliferation (Rojas et al.1993). It is an acceptable measure of cytotoxicity in all living organisms (Smaka-Kinel et al., 1996). A decreased rate of mitotic index was determined because the extracts contained cytotoxic compounds. This result explains that the

extracts suppress cell division and proliferation. Other reports suggested that the occurrence of various chromosomal aberrations after treatment with plant extracts related to their cytotoxicity (Barman et al., 2020, Roy et al., 2021). The plant extract of *H. keralense* may contain chemicals that are capable of producing cytotoxic effects. Previous reports in *H. keralense* show two hepatotoxic compounds; iso-lycopsamine and intermedine and the plant can be considered as a toxic species (Subban et al., 1990). Ivana Boskovic et al. (2021) confirmed that plant extracts from the Boraginaceae family have cytotoxic potential on cancer cells. In the present study, it is evident that the leaf, stem and roots of the *Heliotropium keralense* induce chromosome aberrations and have a strong cytotoxic effect on other organisms.

To our knowledge, this is the first report of a cytotoxicity study of *Heliotropium keralense*. The plant prompted cytotoxic effects in *A. cepa* likely due to the phytochemicals that can interact synergistically and antagonistically on distinct activities of the genetic material in the test system. The uncontrolled use of this plant can cause negative physiological results to crucial organs. Therefore, further study should be conducted to standardize the concentration of this plant material for medicinal purposes. These phytochemicals from *H. keralense* may be potent anticancer agents. Further studies are needed for phytochemical profiling of this plant. Isolation of potential active compounds from plants and testing them against cancer calls would be of great importance.

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