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In Vivo Cytogenetic Effects of *Ephedra alata* L. Stems Extracts in Mitosis of Meristematic Cells in Onion Roots

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Abstract: *Ephedra alata.* is a plant that widely available around the world and long used in folk medicine as a natural medication, was employed in the current work to prepare extracts rich in alkaloids and to test their cytotoxic potential. Alkaloids-rich and crude extracts of *E.alata* were compared to pure ephedrine medication for mitosis on *Allium cepa.* test system. Alkaloids and crude aqueous extracts of *A. cepa* root tips were examined for a total of five hours at five different concentrations compared to ephedrine standard. Mitotic index, phase index, and chromosomal aberration as part of the study. IC50 values of 35 mg/ml were found for each extract, indicating a sub-lethal influence on cell viability. (Toxic and sublethal effects are thought to be the result of this abnormalities, chromosome stickiness abnormalities may be caused by some faulty nucleic acid metabolic pathways and an aneugenic agents caused by the extracts with a high percentage for 75 mg/ml treatment. It was concluded the variations in the chemical constituent of the extracts represented by events caused by various chromosomal and nuclear aberrations give us promising Look of the plant as anti-tumor drug.

Keywords: *Ephedra.alata*, mitosis, Allium cepa, aberration, sticky prophase

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Introduction

Many secondary metabolites such as alkaloids, terpenoids, and phenylpropanoids are being considered for drug development. Since the beginning of human civilization, people have used medicinal herbs. However, due to a shortage of improved access to healthcare, inexpensive conventional medications, and other considerations, the uncontrolled use of herbal remedies has grown widespread in underdeveloped nations (1). Due to the effects of *E. alata* stems, it is now often used in medicine to treat sinusitis and the common cold. *E. alata* has long been used in folk medicine in Palestine to cure conditions including the common cold, hay fever, and asthma. In recent years, it has also been widely utilized to treat cancer (2). From 2 % of the population to 3.4 % of six optically active alkaloids are localized in the stems of the aerial sections of several Ephedra species (3). The presence of ephedrine-type alkaloids is associated to the diversity of pharmacological effects shown by Ephedra species. Ephedrine is the primary alkaloid found in *E. sinica* Stapf, while (+)-pseudoephedrine is the primary alkaloid in *E. intermedia* and *E. lomatolepis*.(4). A study has reported that many alkaloids isolated from Ephedra plants, has antiproliferative and analgesic effects (5). The purpose of this research is to ascertain if two types of *ephedra alata* stem extracts have any influence on the *in vivo* mitosis of onion root meristematic cells. The he international programmed on chemical safety (IPCS) and (WHO) has validated the *Allium cepa L* assay as an effective and standard test for chemicals

screening, in situ monitoring of the genotoxicity of environmental substances, and to assess the genotoxic potential of medicinal plants. It is an *in vivo* assay and one of the established plant bioassays (6,7,8,9,10) By employing chromosomes to screen for genotoxicity, the cepa L assay may identify structural and numerical changes to chromosomes (11).

Materials and methods

Plant material preparation

The plant was collected from Al-Muthanna Governorate during the month and of October, the botanical identification was performed by directorate of seed testing and certification, the plant stems was washed and dried for 15 days at room temperature and ground in an electric mill and weighed.

Extraction Method

a. Crude preparation

Three hundred (300 g) of the plant was ground and dissolved in 500 ml of ethanol: water 5:1 It was dried at room temperature to obtain a residual concentrated extract of 14 g, bringing the yield to 4.6% (12).

b. Alkaloids extraction from *E. alata* stems

Fresh plant about 250 gm. was minced and underwent comprehensive extraction with n-hexane for defatting. After removing fat from the plant, the plant was extracted with 1000 ml of 5% hydrochloric acid: distilled water for 24 hours and filtered twice (13).

The acidic medium was extracted with chloroform (CHCl3; 4 × 4 L) by separation funnel to obtain a weak base. The aqueous phase was evaporated to give 300 mg of light brown residue as fraction number 2 which Dragendorff was performed on it.

Acidic medium was alkalized with NH3OH until pH= 10, white residue precipitated and isolated separately which weight 5g and Dragendorff was performed on it as fraction (e)which have the most alkaloidal content of extraction yield, Basified medium exhaustively extracted with chloroform (CHCl3; 4 × 4 L), the aqueous phase was evaporated to give 56 mg of dark brown residue, The obtained extract, which was Dragendorff positive which as fraction (1), all 3 fractions was fractioned with crude extract by thin layer chromatography on neutral Al2O3 (14).

Preparation of serial dilution

A set of serial dilution prepared from the highest extracts yield (normal whole crude of plant and alkaloids rich extract), The formula for calculating a dilution is $(C1)\ (V1) = (C2)\ (V2)$ where:

- C1 is the concentration of the starting solution.
- V1 is the volume of the starting solution.
- C2 is the concentration of the final solution.
- V2 is the volume of the final solution

Effects of *Ephedra* extracts on onion root tip cell division in vivo:

Onion bulbs were purchased from the local market. The roots were 2-3 cm long after 48 hours. At the height of the mitotic cycle, the root meristem was exposed to various radish aqueous extract concentrations. The cytotoxic and genotoxic potential of four concentrations 5, 15, 35, 55, and 75 mg/ml) was examined over the course of four hours using the Allium test. Root-tip preparations for mitotic examination were accomplished using enhanced method (15). At least five micro glass slides were made for each

parameter, and each experiment was performed three times.

Treatment of roots and preparation of slides

Onion root tips, both treated and untreated, had their tips dissected, and karyotypic research was conducted on the root tips. After 48 hours of growth at 25 to 30 oC in the dark, the extracted roots from the germinated bulbs were dissected, leaving 1-2 mm of the root tips. In order to analyze cell division, the root ends were stabilized in Carnoy fluid. One volume of glacial acetic acid was added to three volumes of 100% ethylene alcohol (16, 17) In vials at 55–60 oC, root tips were treated with a few drops of 1 N HCl, followed by ten minutes in the oven. Root pieces were cleaned with distilled water, put in a new vial with acetocarmine stain 2 percent, and then baked for 10 minutes. Compress the root tips after gently inserting slide covers after removing excess stain and adding one drop of fresh stain to a dot-sized piece of the root tip (18). At 40X and 100X magnifications, a compound light microscope was utilized to look at the meristematic zone of the root tip. iPhone cameras were used to take pictures of the chromosomes throughout various stages of mitosis.

Mitotic index

Based on a minimum of 1000 cells, the proportion of cells undergoing mitosis in each treatment was estimated. The following formulae were used to determine this index as well as the percentages of abnormally dividing cells:

% Mitotic Index (MI) = Total number of dividing cells /Total number of cells examined x 100 % phase Index = Total number of phase /Total number of dividing cells x 100 %

Aberration Index = Total number of aberrant cells /Total number of dividing cells in the same phase x 100 (19, 20).

The percentage of control for mitotic index and phase index were calculated as in the following equations: Percentage of control for Mitotic index = (Mitotic index in each treatments / Mitotic index in control) x 100 Percentage of control for phase index = (treatment phase index / control phase index) x 100 (21).

Statistical Analysis

The SPSS v25 program for the purpose of calculating comparisons between totals, concentrations and control, as well as calculating descriptive statistics for the study samples using Kruskal-Wallis Test (22).

Results and Discussions

Mitotic index

Mitotic index reduced significantly at all extract concentrations, recording (6, 5.10, 4.40, 2.70) for pure ephedrine extract and (8,4.30,3.50,2.70 %) for crude extract, (6.10,4.60, 5, 2.90%) for alkaloids extract 9% for control, (5, 15, 35, 55 mg/ml) respectively which have highly significant difference between concentration effect on mitotic index percent for both crude and alkaloids extracts extract ($p< 0.05$) correlated to control (Table1,2).

The IC50 of each extract is 35 mg/ml which have sub-lethal effect on cell viability is 35 mg/ml for each (alkaloids, crude extracts and standard ephedrine (23)

Crude extracts exhibit higher antagonistic effects than refined extracts, according to the present study's findings, which are supported by other investigations (24). The delay in cell division and altered cell cycle activities

brought on by the various solvent plant extracts exposed to the roots may be the cause of the dose-dependent drop in mitotic indices. The outcome is consistent with earlier study results (25).

Therefore, reduction in the mitotic index is an important parameter that can be implemented to examine the antimitotic as well as cyto-genotoxic effects of biochemicals which may interfere with the phases of division, thus preventing the nucleus of the cell from entering the cell prophase and thus stopping mitosis during anaphase interphase or by increasing the period of the G2 and S phases or inhibiting the process of building protein and DNA (26).

Prophase

The recorded values percentage of the control was decreased to 57.40, 36.20, 46.60, 22.5 and 35.10%, using 5, 15, 35, 55and 75 mg/ml respectively for alkaloids extract, and 56.00, 34.64, 38.26, 24.09 and 11.80% for whole plant crude extract. This confirms the strong antiproliferative effect of *E. alata* shown in Table (1),(2).

The pure standard ephedrine shows no significant differences in prophase index during the five treatments show 46.10% and 60.70% for 5 and 15 mg/ml, (29.20, 21.40 and 22.50 %) for 35, 55, 75, mg/ml respectively.

Chromosome division cannot take place in this situation, which might lead to the emergence of lagging chromosomes and genetic abnormalities (polyploidy, aneuploidy, etc.) (27).

Metaphase

Metaphase index of control was decreased recording the percentage (20.60%, 55.10, 71, 91.72%) of 75, 55, 35, 15 mg/ml respectively, while it was increased using 5 mg/ml recording 114.40%, crude extract shows less decrease in metaphase index at all concentrations treatment than alkaloids extract recording (71, 73.10, 84.80, 98.62%) respectively, 5 mg/ml show more in this phase index recording 227% increase as shown in Table 1, ephedrine standard recording the percentage 62, 55.17, 84.80, 64.13 and 103% respectively.

This might be evidence that the chemicals investigated in total plant alkaloids inhibit microtubules and polymerize tubulin to produce spindle filaments, preventing chromosomes from aligning properly during metaphase (28).

Anaphase and telophase

The crude extract shows significant decrease in control phase index percentage records 67, 67, 81, 40, 40.70 % for 75, 55, 35, 15 and 5 mg/ml respectively, while ephedrine pure standard show slightly decreases at 55 mg/ml while show phase index increase in percentage (100, 103 and 133 %) for 5,15, 35, 75 mg/ml respectively, anaphase increased recording 114.40 and 122.20% for 15 and 5 mg/ml respectively for alkaloids extract treatment. this case Typically, these cells develop as a result of the suppression of cell plate development (29), as demonstrated in table (1).

A likely cause of the binucleated condition is arrested or delayed anaphase cells, such as those discovered in the current research, The results in Table 4.6 show that alkaloids extract was decrease telophase index significantly recording (28.57, 37.14, 65.70, 85.70, 74.20%) for (75, 55, 35, 15 and 5 mg/ml) respectively, crude extract show28% telophase decreases percentage of control at 15, 35 and 75 mg/ml and 42.85 for 55and 57% for 5 mg respectively, Standard ephedrine

shows significant decrease for all concentration as mentioned Table(1) and show 151.42% increasing at 5mg /ml (30).

Chromosomal aberrations

In this study various cytological and chromosomal abnormalities induced by both extracts. The mitotic abnormalities revealed by the treated samples show a positive correlation with the both extracts concentrations, The most frequent chromosomal aberrations (stickiness at prophase, Bi-nucleated cells at anaphase, Micronucleus, nuclear lesion, multipolar (table (2).

The percentage of anomalies was gradually increased over the mitotic phases while extracts concentration increased respectively. stickiness appeared during 4 hr. treatment in prophase, as shown in (table 2), reach (1.70%, 3.30%, 0%) also has reached in 35mg/ml concentration (1.70%, 0%, 4.10%), for 55mg/ml (2.40%, 4.60%, 6.50%) and (4%, 3%, 10%) for 75 mg/ml While treated with pure ephedrine standard, alkaloids table (3), figure (1), This aberrant phenomenon caused by defective metabolic pathways in nucleic acids and an aneugenic agents, this observation shown in previous studies (31, 32).

Bi-nucleated aberration percentage appeared 20% and 15% only at 75 mg/ml concentration, an abnormal spindle division during early anaphase or lack of cytokinesis following telophase results in binucleated cells. Treatment for both crude and alkaloids extracts, respectively, and we have no findings for this condition in ephedrine standard extract (33).

Alkaloids extract shows highest Multipolar percentage highest percentage at 75 mg/ml which show 33.0 %, ephedrine has less observation of this condition than previous extract which have 4%and 4.25% for both 55and 75 mg/ml (table 2, figure2), this condition was indicative of abnormal DNA condensation and chromosome coiling and inactivation of the spindles which observed in other previous researches (34,35,36), appeared at alkaloids (55, 75mg/ml) concentration as (2.30,3%) respectively, in crude extract treatment shows only at 55mg/ml as 4.30 % while pure ephedrine records 2% in 75mg/ml, before the DNA and chromosomal material has been replicated during the DNA synthesis cycle, these lesions form in interphase (G1 and G0).

The following mitosis will reveal chromosomal damage as a result of this (37, 38). Micronuclei typically result from fragments or lagging chromosomes that do not integrate into either of the daughter nuclei during telophase of the mitotic cells (39,40); the highest percentage of micronuclei is reached at a dose of 75 mg/ml for alkaloids, while 33 mg/ml for crude extract treatment and 40 mg/ml for pure ephedrine respectively.

Table (1): MI% and PI % of 4hr concentration treatment on Allium *cepa* L.

solvent	conc (%)	MI (%)	PI (%)			
			prophase	metaphase	anaphase	telophase
pure	5 mg/ml	66.60 %	46.10%	103.00%	133.00%	151.42%
	15 mg/ml	56.60 %	60.00%	64.13%	103.00%	28.57%
	35mg/ml	50%	29.20%	84.80%	100%	28.57%
	55mg/ml	30%	21.40%	55.17%	88.80%	45.71%
	75mg/ml	31.10 %	22.50%	62.00%	100.00%	9.40%
alkaloids	5 mg/ml	67.70 %	57.40%	114.40%	122.20%	74.20%
	15 mg/ml	51.10 %	36.22%	91.72%	114.40%	85.70%
	35mg/ml	55.50 %	46.60%	71.00%	84.40%	65.70%
	55mg/ml	55.50 %	22.50%	55.10%	77.70%	37.14%
	75mg/ml	38.80 %	35.11%	20.60%	77.00%	28.57%
crude	5 mg/ml	85.50 %	56.60%	227.00%	67%	57%
	15 mg/ml	47.70 %	34.60%	98.62%	67%	28.57%
	35mg/ml	50%	38.20%	84.80%	81.10%	28.57%
	55mg/ml	33.30 %	24.60%	73.10%	40.00%	42.85%
	75mg/ml	27.70 %	11.80%	71.00%	47.70%	28.00%

Table (2): *Allium cepa* mitotic index of 4hr concentration treatment.

Conc.	Solvent		
	Ephedrine	Crude	Alkaloids
5	0.06	0.08	0.061
15	0.051	0.043	0.046
35	0.045	0.045	0.050
55	0.27	0.030	0.029
75	0.28	0.025	0.035
Control	0.09		
p-value	0.02 ($p<0.05$)		
Kruskal-Wallis H	13.402		
df	5		
Asymp. Sig.	0.020		

Table (3): *Allium cepa* aberration's of 4hr concentration treatment on *Allium cepa* L.

Time (hrs)	Conc. (%)	Percentage of abnormalities (%)				
		Prophase (%)	Metaphase, Anaphase and Telophase (%)			
		Stickiness	Bi-Nucleated	Micro Nucleus	Nuclear lesion	Multipolar
pure ephedrine	5mg/ml	0.00%	__	__	__	__
	15 mg /ml	1.70%	__	__	__	__
	35mg/ml	1.70%	__	40.00%	__	__
	55mg/ml	2.40%	__	__	__	18.70%
	75mg/ml	4.00%	__	__	2.00%	6.00%
alkaloids	5mg/ml	__	__	__	__	__
	15 mg /ml	3.30%	__	11.00%	__	__
	35mg/ml	__	__	28.50%	__	4.30%
	55mg/ml	4.60%	__	25.00%	2.30%	4.70%
	75mg/ml	3.00%	20.00%	50.00%	3.00%	33.00%
crude	5mg/ml	__	__	__	__	__
	15 mg /ml	___	__	__	__	__
	35mg/ml	4.10%	__	__	__	__
	55mg/ml	6.50%	__	__	4.30%	__
	75mg/ml	10.00%	15.00%	33.00%	__	7.60%

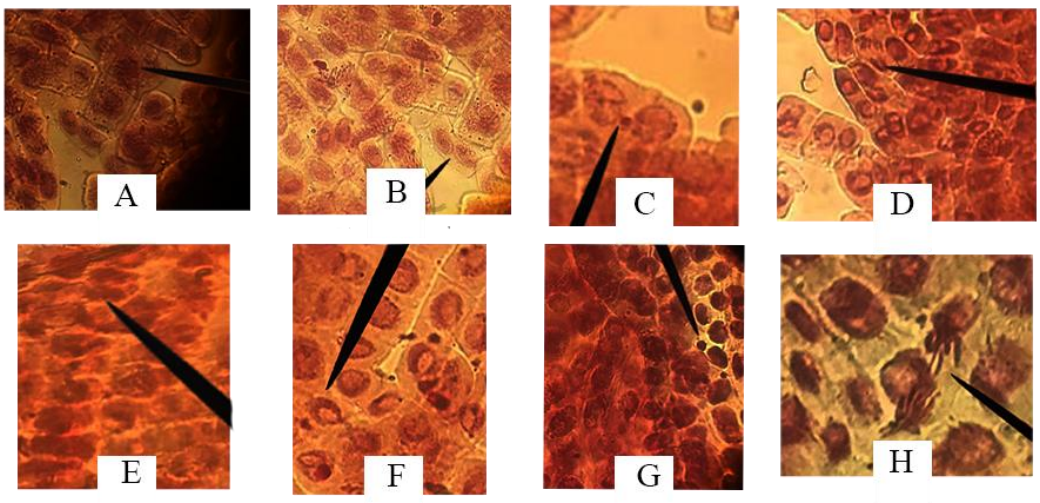

Figure (1): chromosomal aberrations types appeared by *Allium Cepa* L.: (A): Stickiness (B): Bi-Nucleated, (C): Micro Nucleus, (D): Multipolar, (E): Stickiness, (F): Nuclear lesion, (G): Micro Nucleus, (H): Multipolar.

Conclusion

The total plant and alkaloidal crude extract had a strong cytotoxic effect on Allium cepa cells, which was dosage dependent. The differences in the chromosomal aberration and MIs of the extracts were attributed to variations in the chemical constituent of the extracts represented by events caused by various chromosomal and nuclear aberrations, as well as the assessment of cell culture systems for this plant that seem to show us a promising Look of plant as anti-tumor drug.

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