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Cytotoxic effect of *Hemigraphis alternata (*Burm. F.) T. Anderson leaf extract on *Allium cepa* root tip

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ABSTRACT

Cytotoxicity is a major subject in pharmaceutical studies relevant to the area of cancer research. Low cytotoxicity to healthy cells and high cytotoxicity to cancerous cells is the ultimate goal of many chemotherapy drugs. *Hemigraphis alternata* (Burm. F.) T. Anderson of Acanthaceae family is an exotic plant adapted to India, a versatile tropical low-creeping perennial herb .The leaves of *Hemigraphis alternata* were collected .50% and 25% aqueous extracts of the plants were prepared from fresh leaves. Onion root tips treated with each concentration were used for cytology study. Mitotic index (M.I.) and various chromosomal abnormalities were studied. Present study showed lower Mitotic index frequency in roots treated with extract as compared to control. Chromosome aberrations were observed in all stages of mitosis. The most frequently observed abnormalities were nuclear lesions, chromosome stickiness, multipolar anaphase, chromosome laggards, stellate chromosome etc..

Keywords: Cytotoxicity, Mitotic index, Chromosomal aberrations, *Hemigraphis alternata*.

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INTRODUCTION

Medicinal plants continue to play an important role in the healthcare system of a significant portion of world population. There are several medicinal plants which are being widely used in the traditional systems of medicine for the prevention and treatment of diseases like cancer. Several plant derived compounds have been found to play significant role in the development of clinically useful anticancer agents. Moreover, about 50% of all modern clinical drugs are derived from natural products. Some plants contain antitumor compounds and such plant derived compounds can be used for the development of chemo-preventive agents against cancer.

Hemigraphis alternata (Burm. F.) T. Anderson of Acanthaceae family is an exotic plant adapted to India, a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm. It prostrates and spreads with rooting stems when grown on ground, and on hanging baskets it cascades over beautifully. In Kerala, the plant is popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds.

Allium test is a sensitive test that has often been used for the determination of cytotoxic and/or genotoxic effects of various substances [1,2].

MATERIALS AND METHODS

a. Collection of plant materials :

Hemigraphis alternata (Burm. F.) T. Anderson of Acanthaceae family was used for the present investigation. The plant materials were collected from home garden. Morphological studies were carried out using fresh plant specimens. The morphological identities were determined with the help of intended keys in 'The flora of Presidency of Madras '[3].

b. Preparation of Aqueous extract:

The leaves of H. alternata were segregated and pulverized mechanically; the extract was squeezed out manually and stored in refrigerator separately. 50% and 25% aqueous extracts of the plants were prepared by taking 10ml and 5ml aqueous extract respectively and making up to 20ml using distilled water.

c. Allium cepa assay:

The antimitotic activity of the test plant extract was screened using Allium cepa root tip meristematic cells which have been used extensively in the screening of drugs with antimitotic activity. The bulbs were germinated over water 48 hours before being transferred to each of the test plant extracts. When the roots were about 1cm long, the bulbs were placed on petridish containing the leaf extracts of two

different concentrations (50% and 25%), such that the roots were immersed in the extracts. The duration of treatments for each extract was 1h. The sprouted roots were also treated with distilled water, which served as control. The experimental set up had three replicates.

The root tips were harvested after the treatment duration and fixed in Carnoy's fluid (1 part of glacial acetic acid: 3 parts of absolute alcohol). The root tips were hydrolysed in 1N HCL for 5 minutes. The squashing was done over 2% aceto - carmine stain. The slides were then scanned under 100X objective of compound microscope and photomicrographs were taken. The numbers of cells, dividing and non- dividing, were recorded. Various chromosome aberrations was noted. Mitotic index was calculated by expressing the number of dividing cells as a percentage of total cells counted for each of the treatments and the control.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

OBSERVATION AND RESULT

The results showed that the aqueous extract of the *Hemigraphis alternata* had cytotoxic activity. Clastogenic abnormalities including nuclear lesions, chromosome bridges, and non clastogenic aberrations like ball metaphase, binucleate cell formation, stellate anaphase, chromosome laggards were observed. **Table 1** shows the data on the number of onion cells in various phases of cell division after treatment of *A. cepa* root tip on H. alternata leaf extract and **Table 2** shows the mitotic index and phase index observed in A. cepa root tip cells treated with the aqueous leaf extracts of the test plant material.

The mitotic indices of all the extract treated roots were significantly lower than that of the control. Also, the mitotic index values were observed to be decreasing with increasing concentrations of the extracts. The studies showed that maximum cells were under normal cell division in control treatment and the number reduced with increase in the concentration of leaf extract. In higher concentrations cells enter in the cell division but were unable to enter in to metaphase and anaphase. This may be caused due to damage to the spindle apparatus. The results of 2161 cells observed during this experiment were shown in above tables. The studies revealed adverse effects of H. alternata leaf extract on the mitotic index.

Table 1. Number of onion cells in various phases of cell division after treatment of H. alternata leaf extract.

	Total cells examined	Prophase	Metaphase	Anaphase	Telophase
Control	729	248	47	51	40
H.alternata (25%)	712	198	22	18	12
H.alternata (50%)	720	166	16	11	7

Table 2. Effect of H. alternata extract on % Mitotic index and % Phase Index in onion root.

	%Mitotic Index	%Prophase Index	%Metaphase Index	%Anaphase Index	%Telophase Index	Total cells examined
Control	52.9	34.0	6.44	6.99	5.48	729
H. alternata (25%)	35.1	27.8	3.08	2.52	1.68	712
H. alternata (50%)	27.7	23.0	2.22	1.52	0.97	720

Chromosomal aberrations observed:

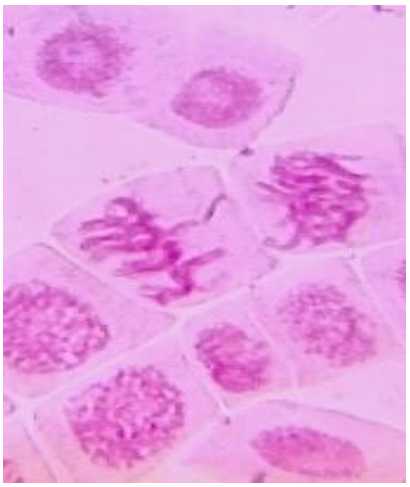

Fig :1 Metaphase stickiness

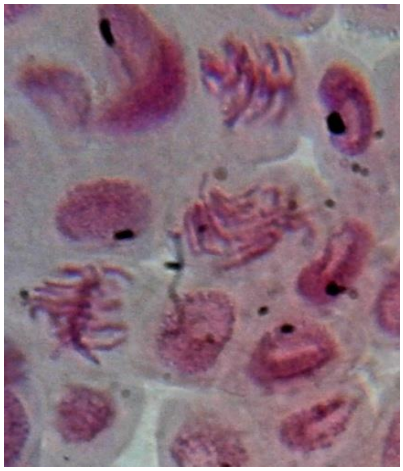

Fig :2 Multipolar anaphase

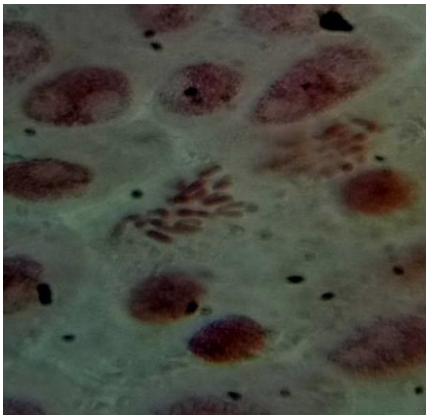

Fig : 3 Chromosome laggard

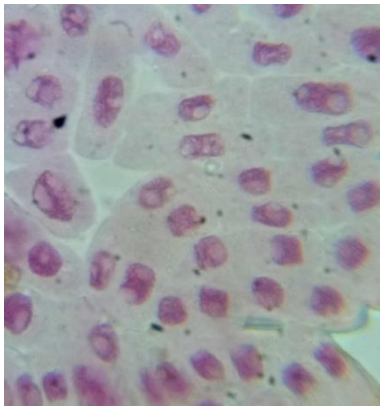

Fig :4 Nuclear lesion

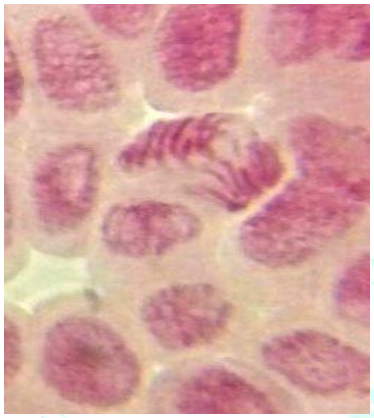

Fig :5 Disturbed Anaphase

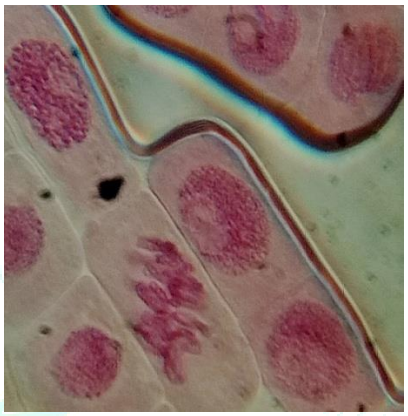

Fig:6 Diagonal Metaphase & puffed prophase

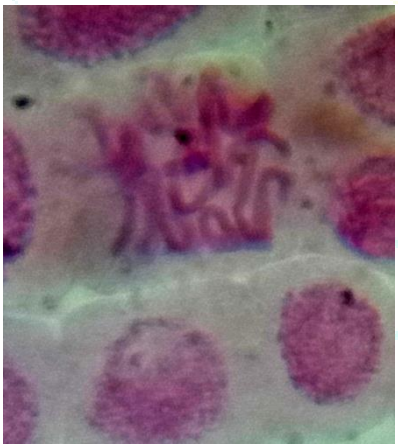

Fig :7 Stellate chromosome

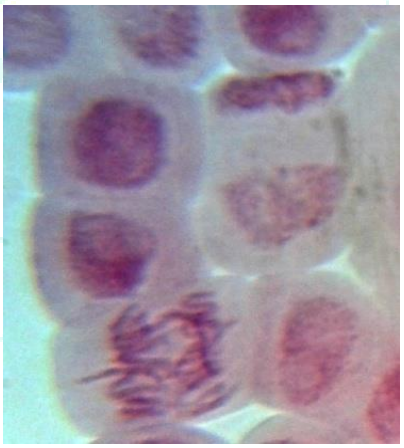

Fig: 8 Delayed anaphase & puffed telophase

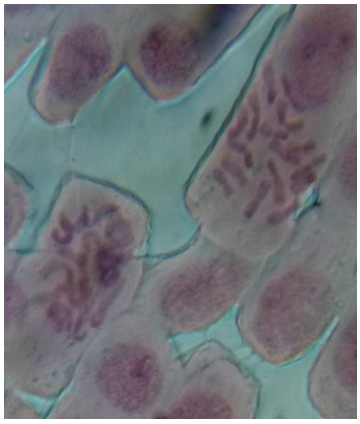

Fig: 9 Laggard chromosome & ball metaphase

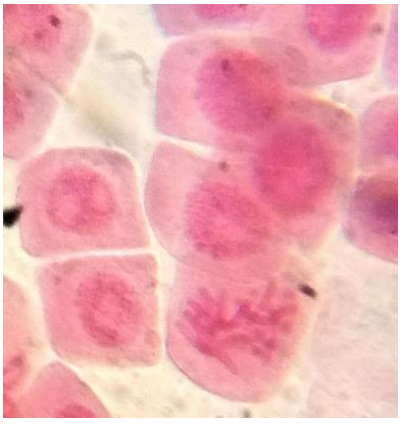

Fig : 10 Cell with 2 nuclear lesion & multipolar anaphase

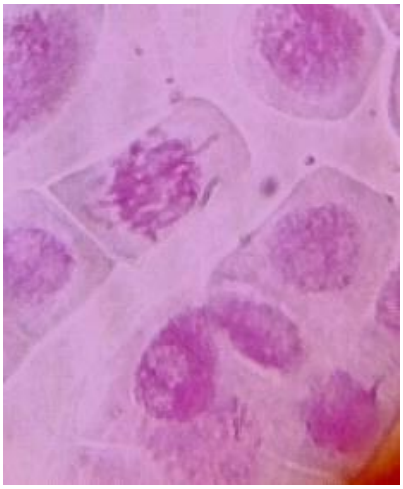

Fig :11 Equatorial metaphase with vagrant chromosome

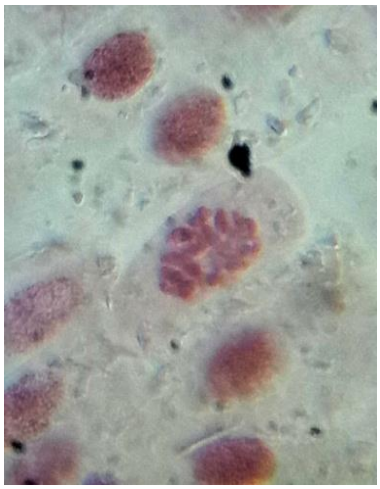

Fig :12 Ball metaphase

DISCUSSION

Mitotic index is an acceptable measure of cytotoxicity in all living organisms [2]. The cytotoxicity level can be determined by the decreased rate of mitotic index. The decreased mitotic index values in the treated onion roots may be an indication of the presence of cytotoxic substances in the aqueous leaf extracts, which causes inhibition of mitotic activities, while the aberrant cells in the treated onion root tip meristems indicates genotoxic effects of the leaf extract[4]. The reduction of the mitotic index might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis [5]. Earlier reports suggest that the presence of nuclear lesions offer cytological evidence for the inhibitory action on DNA biosynthesis [6,7]. Ball metaphase results from the complete destruction of spindle fibres and a subsequent clumping of chromosomes into a tight ball. Separation of daughter chromosomes parallel to the equator rather than towards the poles is an acute aberrant condition that arises as a result of errors in mitotic spindle assembly and dynamics [8]. Chromosome bridges may be caused by stickiness of chromosomes which make their separation and free movements incomplete and thus they remain connected by bridges [9].

Inhibition of cytokinesis following telophase is responsible for binucleated cell formation [10]. The occurrence of lagging chromosomes was attributed to the hindrance of prometaphase movement accompanied by adhesion of centromere to the nuclear membrane or to the surrounding surface of plasma membrane [11].Chromosome bridges may arise due to stickiness or due to the formation of dicentric chromosomes by breakage and reunion [12].

CONCLUSION

Thus the present study suggested that the aqueous extract of leaves of *Hemigraphis alternata* exhibited significant inhibitory and mitodepressive effects on the cell division of Allium cepa. As H. alternata inhibits cell division, it may be used for checking uncontrolled cell division like as in tumor formations and cancer treatments. Further studies are required to isolate the compounds responsible for the cytotoxic activity from this plant. There is a need for a closer look at the genotoxicological effects of the tested extracts in animal test systems for human welfare.

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