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Research Article

ANALYSIS OF CYTOTOXIC POTENTIAL OF THE AQUEOUS LEAF EXTRACTS OF *POGOSTEMON AURICULARIUS* (L.) HASSK. USING *ALLIUM CEPA* ROOT TIP ASSAY

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ABSTRACT

Pogostemon auricularius is an aromatic herb belonging to the family Lamiaceae. **Objectives:** In the present study we have utilized the *Allium cepa* root tip meristem model to evaluate the cytotoxic and antimitotic potential of *P. auricularius*. **Methods:** The roots of *Allium cepa* were exposed to different concentrations of the aqueous leaf extracts (0.01%, 0.05%, 0.1% and 0.5%), for four different time durations, using distilled water as the control. **Results:** Chromosome anomalies including formation of sticky chromosomes, chromosome bridges and several other metaphasic and anaphasic disorders were induced by all the extract concentrations. **Conclusions:** Mitotic index was found to be decreasing, which was concentration dependent. All the extracts induced lowering of the mitotic index when compared to the distilled water control.

Keywords: *Pogostemon auricularius*, cytotoxicity, *Allium cepa* assay, aberrations, mitotic index.

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 anti-cancer agents.

Herbs have always been the principal form of medicine in India. In recent years the use of complimentary medicines has increased. Moreover, about 50% of all modern clinical drugs are derived from natural products. Some plants contain anti-tumour compounds and such plant derived compounds can be used for the development of chemo-preventive agents against cancer. Plant substances continue to be a valuable source of drugs for the world population and several plant based drugs are in extensive clinical use. Agents capable of inhibiting cell proliferation, including apoptosis modulating signal transduction are currently used for the treatment of cancer [(1)].

An assessment of cytotoxic and antimutagenic activity is necessary to understand their antiproliferative activity. Recent reports have suggested the use of several plant derived compounds as antitumour agents.

Allium test is a sensitive test that has often been used for the determination of cytotoxic and/or genotoxic effects of various substances [(2, 3)]. *Allium* assay has been shown to have correlation with tests in other living systems and serve as an indicator of toxicity of the tested material [(4)]. *Allium cepa* root tip meristems have been widely used for the evaluation of cytotoxicity, anti-mitotic activity, and genotoxicity, by employing the growing roots of *A. cepa*. Root meristematic

cells of *A. cepa* have been used extensively in screening of drugs to evaluate their antimitotic activity.

The use of plant tissues, primarily root tip for studying the induction of chromosomal aberration is one of the oldest, simplest, most reliable and inexpensive method [5]. The assessment of anti mitotic activity using *A. cepa* root meristematic cells has been used extensively in the screening of drugs with antimitotic activity. The division in these cells is similar to normal human cells and cancer cell division. Hence, these meristematic cells can be used for screening of drugs with potential human anticancer activity [6].

Several authors have reported the cytotoxic activities of different medicinal plants [7-9]. The cytotoxic potential of *Pogostemon quadrifolius* was evaluated by Ancy and Thoppil [10].

P. auricularius is an aromatic herb and an oil rich taxon of the family Lamiaceae. There are no previous reports regarding the cytotoxic activities of this plant. The present study was therefore aimed at investigating the cytotoxic effects of the aqueous leaf extracts of the above said species using *A. cepa* root tip assay.

MATERIALS AND METHODS

Collection of plant materials

An aromatic species of *Pogostemon* viz., *P. auricularius*, was used for the present investigation. The plants were collected from Wayanad district of Kerala. The identification and verification of the plants were done by Dr A. K. Pradeep, Assistant Professor, Department of Botany, University of Calicut. Voucher specimen was deposited at Calicut University Herbarium (CALI 123731).

Preparation of leaf extracts

The fresh leaves were collected and washed thoroughly with tap water and air dried at room temperature. It was then powdered and extracted with hot water by boiling for 30 minutes to get the aqueous extract. This was then filtered to remove particulate matter. Distilled water was used as medium for dilution.

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 before being transferred to each of the test plant extracts. When the roots were about 5 mm long, the bulbs were placed on beakers containing the leaf extracts of four different concentrations (0.5%, 0.1%, 0.05% and 0.01%), such that the roots were immersed in the extracts. The duration of treatments for each extract was 2h, 1h and 30 minutes. The sprouted roots were also treated with distilled water, which served as control. The experimental set up had five replicates.

The root tips were harvested after the treatment duration and fixed in Carnoy's fluid (1 part of glacial acetic acid: 2 parts of absolute alcohol). The root tips were hydrolysed in 1N HCL for 5 minutes. The squashing was done over 2% aceto - orcein stain. The slides were then scanned under Leica DM 1000 trinocular research microscope and photomicrographs were taken.

The numbers of cells, dividing and non- dividing, were recorded. Incidence of chromosome aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment. 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.

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

Statistical Analysis

The data were subjected to statistical analysis using analysis of variance followed by appropriate post-hoc tests. The means, with 95% confidence limits and the standard errors for results of the root inhibition and chromosome aberrations of each concentration of the extracts were calculated. Data were expressed as Mean ± Standard Error of Mean (SEM). $P < 0.05$ was considered to be statistically significant. All statistical analyses were carried out using SPSS 17.0 statistical package.

RESULTS AND DISCUSSION

The results showed that the aqueous extract of the *P. auricularius* had excellent cytotoxic activity. Clastogenic abnormalities including nuclear lesions, chromosome bridges, and non clastogenic aberrations like ball metaphase, binucleate cell formation, stellate anaphase, chromosome laggards, equatorial separation, shift in microtubular

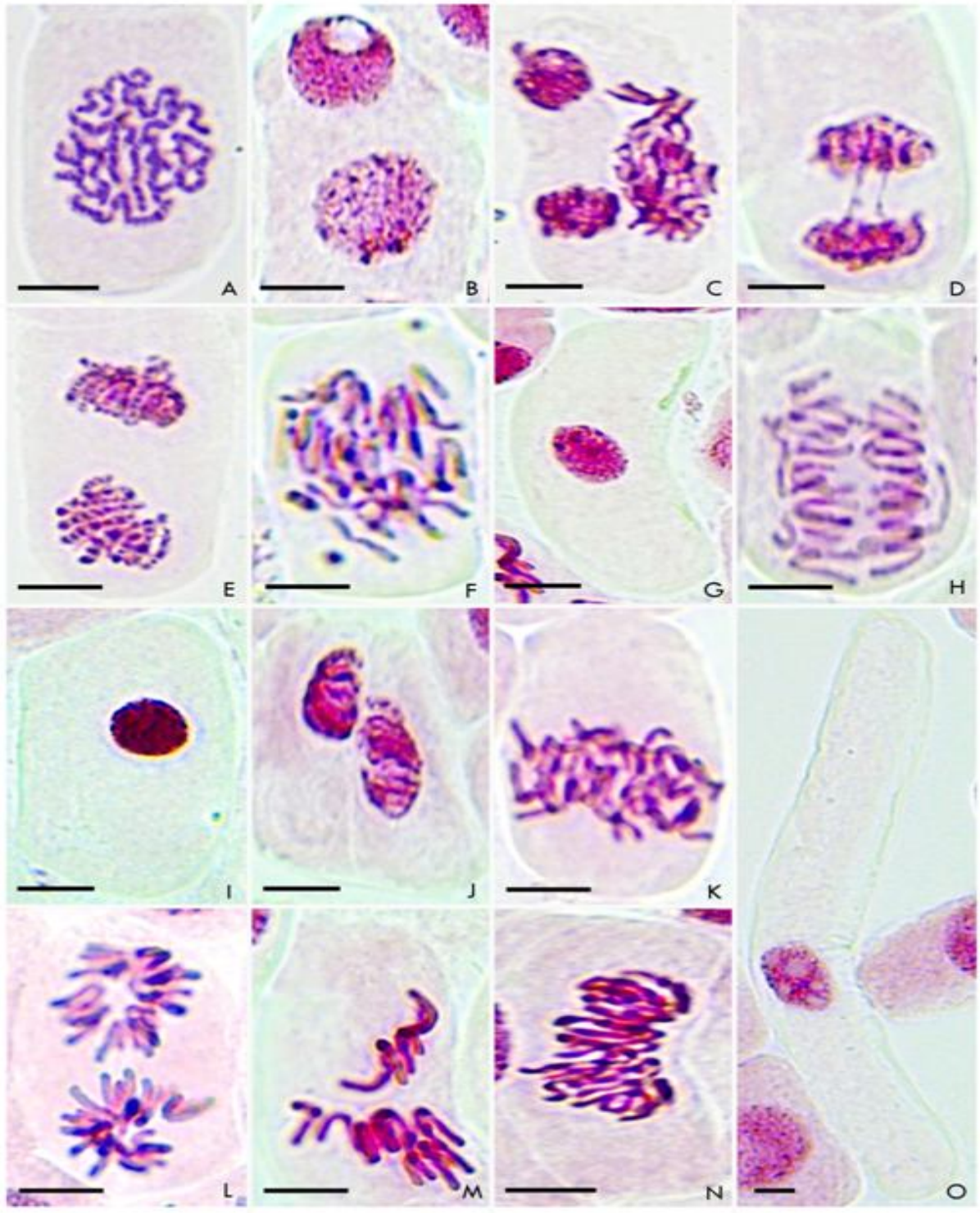

Fig. 1 Chromosomal aberrations caused by aqueous leaf extracts of *P. auricularius* A)Ball Metaphase B) Binucleate cell with one showing lesion C) Binucleate cell showing sticky Anaphase and Metaphase D)Chromosome bridges at Anaphase E) Chromosome gaps at Anaphase F) Chromosome laggards at Anaphase G) Cytoplasmic heteropyknosis H) Equatorial separation at Anaphase I) Heterochromasia J) Shift in MTOC at Cytokinesis K) Stathmo-Anaphase L) Stellate Anaphase M) Unequal grouping at Metaphase N) Unilateral Anaphase O) Multiple nuclear lesions in a giant cell

Table 1: Effect of varying concentrations of *P. auricularius* leaf extracts on the mitotic index of *Allium cepa* root meristems and the percentage of chromosomal aberrations observed

Concentration	Mitotic index (± SE)			% of aberrant cells (± SE)		
	½ hr	1 hr	2 hr	½ hr	1 hr	2hr
Control	24.25 ± 0.25^{a}	26.10 ± 0.34^{a}	27.56 ± 0.05^{a}	0^{a}	0^{a}	0^{a}
0.01 %	15.74 ± 1.02^{b}	15.06 ± 0.30^{b}	14.58 ± 0.41^{b}	39.16±1.32^{bc}	41.81±0.56^{b}	46.07±1.30^{b}
0.05 %	14.03 ± 0.52^{bc}	13.40± 0.38^{bc}	12.89 ± 0.60^{c}	36.03±0.75^{b}	43.64±1.82^{bc}	48.14±0.93^{bc}
0.1 %	12.74 ± 0.79^{c}	12.63 ± 1.01^{bc}	12.49 ± 0.33^{c}	39.50±1.86^{bc}	48.32±1.61^{c}	51.60±2.61^{c}
0.5 %	12.27± 0.21^{c}	12.06± 0.46^{c}	11.88 ± 0.47^{c}	42.88±2.88^{c}	47.98±3.07^{c}	62.85±1.91^{d}

Figures with different superscripts are statistically significant ($p<0.05$) (Duncan's multiple range test)

organizing centres, unilateral anaphase and stathmo anaphase were observed. Table 1 shows the data on the mitotic indices and the percentage of chromosome aberrations 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 number of aberrant cells was also observed to be increasing with the concentration of extract. Cell division was normal in the root tips kept as control. A one way ANOVA showed that there was a significant effect of treatment on mitotic activity. Post-hoc analysis using Duncan's multiple range test showed that the activity of all the different extracts were significant when compared with that of control.

The chromosomal aberrations induced in the treated onion root cells were definitely caused by the chemical ingredients in the aqueous leaf extracts of the tested plant species, since such aberrations were not observed in the control. The observation of cells with laggards, chromosome gaps, and giant cells in the treated onion cells is an indication that the extract, especially at high concentrations, is capable of causing changes in chromosome number and structure. 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 [11].

Earlier reports suggest that the presence of nuclear lesions and nuclear dissolution offer cytological evidence for the inhibitory action on DNA biosynthesis [12, 13]. 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 [14]. Chromosome bridges may be caused by stickiness of chromosomes which make their separation and free movements incomplete and thus they remain connected by bridges [15].

Inhibition of cytokinesis following telophase is responsible for binucleated cell formation [16]. The occurrence of lagging chromosomes was attributed to the hindrance of pro-metaphase movement accompanied by adhesion of centromere to the nuclear membrane or to the surrounding surface of plasma membrane [17]. Chromosome bridges may arise due to stickiness or due to the formation of dicentric chromosomes by breakage and reunion [18]. Stathmo-anaphase may be due to the abnormal functioning of the spindle fibres [19].

CONCLUSIONS

Mitotic index is an acceptable measure of cytotoxicity in all living organisms [3]. The cytotoxicity level can be determined by the decreased rate of mitotic index. Mitotic index was found to be decreasing with increasing extract concentration and duration of treatment. Mitotic index observed in the

treated root tip meristems were significantly different from the control group. Significant difference in the percentage of chromosomal aberrations was also observed in the test samples, when compared to the distilled water control.

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 [20]. Reduction in the mitotic activity could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis [21].

Chromosome aberrations were observed in all stages of mitosis. The abnormalities of chromosomes could be due to the blockage of DNA synthesis or inhibition of spindle formation. The cells of *A. cepa* root tips after treatment with extracts of *P. auricularius* showed decreased mitotic index with increasing extract concentration. The results of the present investigation suggest the potential use of *P. auricularius* as a therapeutic agent. The mitodepressive effects induced by this plant extract suggest that it has some effect on the cell division of *A. cepa* which may be due to the conditions induced by the chemical components of the extracts. These results also suggest that, although the plant has beneficial effects as medicinal herbs, it can cause harm and damage on cells if not used in appropriate dose and period.

Further studies are required to isolate the compounds, responsible for the cytotoxic activity, from this plant and establish the antitumor activity of the isolated compounds *in vivo* and *in vitro* with different human cancer cell lines. More works are also essential to prove the specificity of this plant to be used as an anticancer agent.

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REFERENCES

1. Trichopoulos D, Willett WC. (1996). *Cancer Causes Cont*. 7: 3-4.
2. Grant WF. (1982). *Mutat. Res*. 99: 273-291.
3. Smaka-Kinel V, Stegnar P, Lovaka M, Toman J. (1996). *Mutat. Res*. 368: 171–179.
4. Fiskesjo G. (1985). *Hereditas* 102: 99-112.
5. Auti S, Pagare R, Ahire D, Sawale V. (2010). *J. Cell Tissue Res*. 10(3): 2331-2335.
6. Saboo S, Deore SL, Khadabadi SS, Deokate UA. (2007). *Nig. J. Nat. Prod. Med*. 11: 75-78.
7. Adegbite AE, Sanyaolu EB. (2009). *Sci. Res. Essays* 4 (11):1311-1314.
8. Bhattacharya S, Haldar PK. (2010). *Pharmacognosy Res*. 2(6): 355–358.
9. Akinboro A, Bakare AA. (2007). *J. Ethnopharmaco*l. 112(3): 470–475.
10. Ancy CA, Thoppil JE. (2009). *J. Cytol. Genet*. 10: 59-64.
11. Christopher HB, Kapoor MB. (1988). *Cytologia* 54**:** 203-209.
12. Akaneme FI, Iyioke IV. (2008). *Bio – Research*. 6(1): 293-297.
13. Mercykutty VC, Stephen J. (1980). *Cytologia* 45: 769-777.
14. Ford JH, Correl AT. (1992). *Genome* 35: 702-705.
15. Kabarity A, El-Bayoumi A, Habib A. (1974). *Biol. Plantarum*. 16: 275–282.
16. Majewska AE, Wolska E, Sliwinska M, Furmanowa N, Urbanska A, Pietrosiuk A, Zobel A, Kuras M. (2003). *Caryologia* 56: 337-351.
17. Nagpal A, Grover IS. (1994). *Nucleus* 37: 99-105.
18. Raj A, Rao R. (1972). *Cytologia* 37: 245-246.
19. Shehab AS. (1979). *Cytologia* 44: 607-613.
20. El-Shahaby OA, Abdel-Migid HM, Soliman MI, Mashaly IA. (2003). *Pak J. Biol. Sci*. 6(1): 23-28.
21. Sudhakar R, Ninge Gowda KN, Venu G. (2001). *Cytologia* 66(3): 235–239.

