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Cytotoxic Effect of *Crotalaria laburnifolia* L. Leaf Extract on *Allium Cepa* Root Tip Cells

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Received on 20-7-2017
Accepted on 15-10-2017

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. *Crotalaria laburnifolia* L. is an agroforestry crop which is a green manure crop with good leaf production. Pyrolizidine alkaloids such as anacrotine and madurensine have been extracted from the seeds, the former being antispasmodic and possibly hepatotoxic. The leaves of *Crotalaria laburnifolia* were collected from different locations of Thiruvananthapuram city. 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 percentage of 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, though normal prophase was observed in all treatments. The most frequently observed abnormalities were nuclear lesions, stickiness, binucleate cells, and nuclear budding.

Keywords: *Crotalaria laburnifolia*, *Allium cepa*, cytotoxicity, chromosomal abnormalities.

Introduction

Several plant based drugs are in extensive clinical use against cancer. Antimitotic agents constitute a major class of cytotoxic drugs, and among them are plant-derived compounds such as paclitaxel, vincristine, and combretastatin (Iwasaki, 1993). Agents capable of inhibiting cell proliferation are currently used for the treatment of cancer (Trichopoulos and Willett, 1996). However, due to the great number of still non-treatable kinds of cancer and their tendency to produce resistance during anti-cancer treatment, we are faced with a current need to find new compounds and new lead structures for cancer chemotherapeutical purposes (Latha and Panikkar, 1998). An assessment of cytotoxic and anti-mutagenic activity of plant extracts is necessary to understand their antiproliferative activity. The effects of toxicants can be observed at the level of chromosomes (clastogenesis) through alterations in chromosome structure (chromosomal aberrations) and number (aneuploidy, polyploidy). The *Allium* test is a short-term test with many advantages: low cost, easy to handle, good chromosome condition for the study of chromosome damage or disturbance of cell division including the evaluation of risks of aneuploidy. It has been widely used for detection of cytostatic, cytotoxic and mutagenic properties of different compounds, including anticancer drugs of plant origin (Anjana and Thoppil, 2013).

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Crotalaria laburnifolia L. is an agroforestry crop which is a green manure crop with good leaf production. The plant has medicinal properties as well. Medicines prepared from the seed have a blood purifying effect and are used to treat sore throats and skin diseases. Pyrolizidine alkaloids such as anacrotine and madurensine have been extracted from the seeds, the former being antispasmodic and possibly hepatotoxic (Roder et al., 1992). The present study was done to evaluate the cytotoxic effects of aqueous extracts of the leaves of *Crotalaria laburnifolia* through *Allium cepa* test.

Materials and Methods

Plant material

Crotalaria laburnifolia L. plants were collected from different parts of Thiruvananthapuram city. The identification and verification of the plants were done by Dr. Shweta Subramaniam, Department of Botany, University of Delhi. Voucher specimen was deposited at All Saints' College Herbarium.

Preparation of Aqueous extract

The leaves of *C. laburnifolia* 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.

Allium cepa assay

The antimitotic activity of the test plant extract was screened using *Allium cepa* root tip meristematic cells. The bulbs were germinated over water before being transferred to each of the test plant extracts. When the roots were 5mm long, 5 bulbs were placed on petriplates containing aqueous extracts of *Crotalaria laburnifolia* such that the roots were immersed in the extracts. The duration of treatment was 2h. The sprouted roots were also treated with well water, which served as water control. The experimental setup had three 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 5min. The squashing was done over 4% acetocarmine stain. The slides were then scanned under Leica DM1000 trinocular research microscope and photomicrographs were taken.

The number of cells, dividing and non-dividing, were recorded. 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.

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

Percentage of abnormalities was calculated using the following formula:

$$\text{Percentage of abnormalities} = \frac{\text{Total number of abnormal cells}}{\text{Total number of cells observed}} \times 100$$

Results and Discussion

Allium cepa Assay

The effect of aqueous leaf extracts of *Crotalaria laburnifolia* on mitotic index is given in Table 1. The treatments also induced a wide spectrum of mitotic abnormalities in the root tips compared to the control. Most prominant abnormalities observed is presented in Fig 2. Normal mitotic stages in control onion root are presented in Fig 1.

Table 1 Effect of aqueous extracts of *Crotalaria laburnifolia* L. on Mitotic Index of *Allium cepa* root tips

Treatment	No. of cells observed	Prophase	Metaphase	Anaphase	Telophase	MI
Control	734	84	62	42	56	33.25
C. laburnifolia (5%)	716	37	22	16	12	12.15
C. laburnifolia (10%)	725	39	24	12	9	11.59

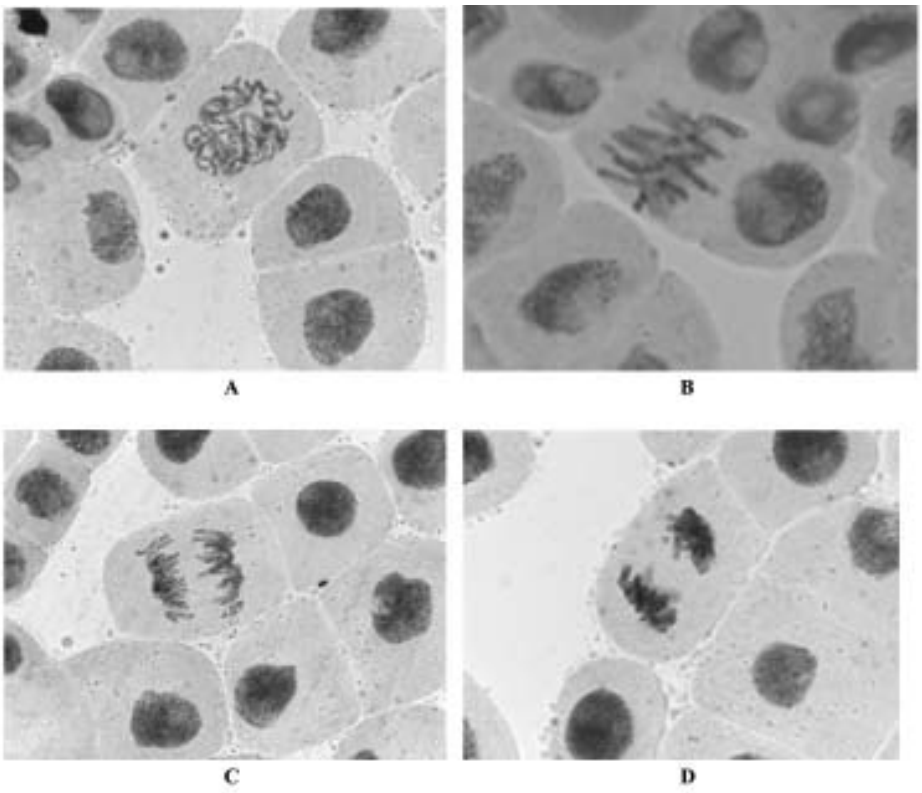

Fig. 1 – Normal mitotic stages in Control *Allium cepa* root tip. **A** – Prophase; **B** – Metaphase; **C** – Anaphase and **D** – Telophase

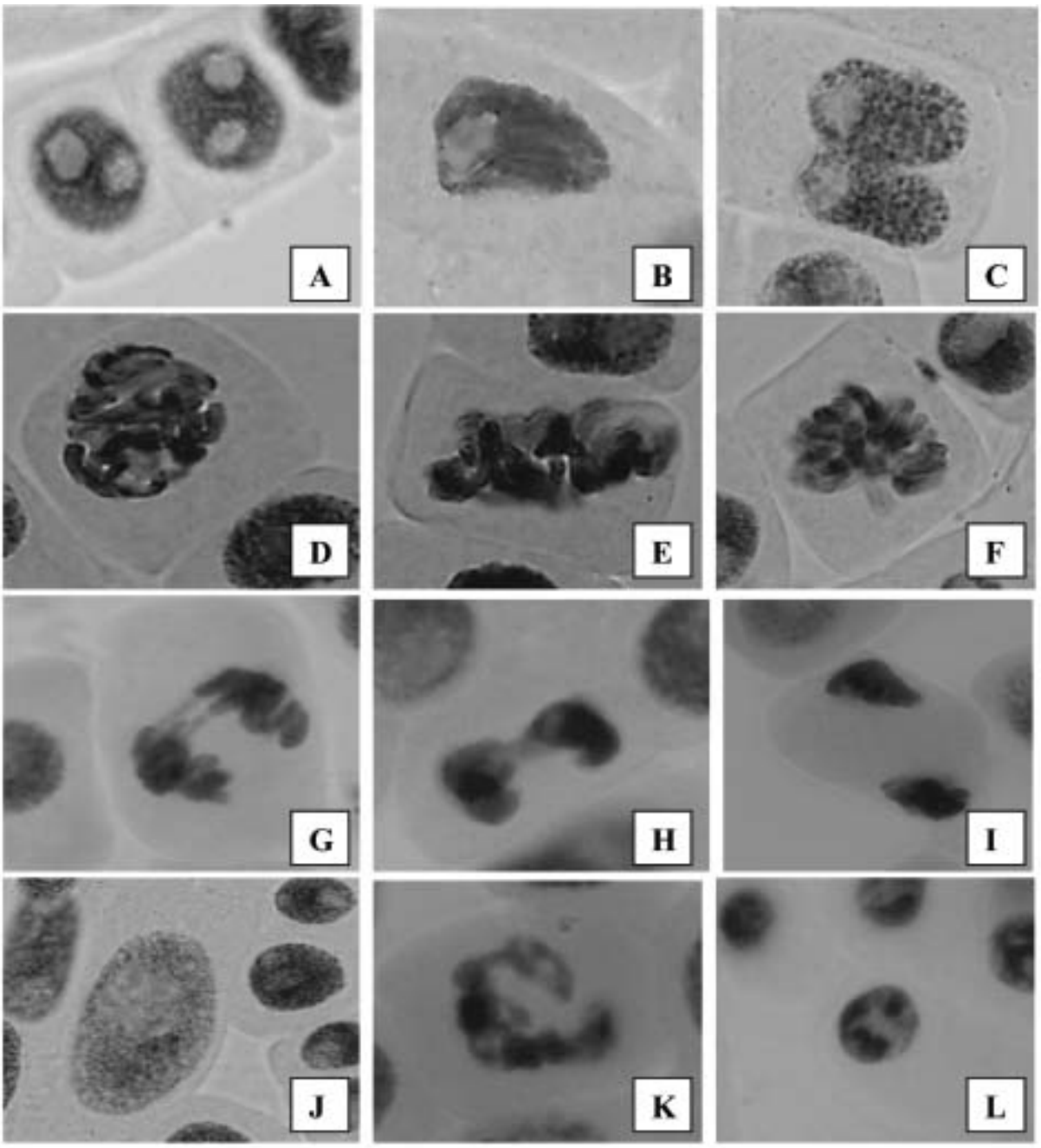

Fig 2 Cytological aberrations observed in *Allium cepa* root meristem treated with aqueous extract of *Crotalaria laburnifolia*. **A** – Nuclear lesion, **B** – Nuclear budding (initial stage), **C** – Nuclear budding (later stage), **D** – Clumbing in prophase, **E** – Clumbed diagonal metaphase, **F** – Star metaphase, **G** – Chromosome bridges & clumbing in anaphase, **H** –Multiple bridges & clumbing in telophase, **I** – Diagonal sticky telophase, **J** – Giant nucleus, **K** – Nuclear disintegration, **L** – Nuclear vacuolization

Mitotic index

Mitotic index is an acceptable measure of cytotoxicity in all living organisms (Smaka-Kinel et al., 1996). 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 (El-Shahaby et al., 2003). 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 (Sudhakar et al., 2001).

Chromosome aberrations

Genotoxins can induce mutations in chromosomes (clastogenesis) or in a small number of base pairs (mutagenesis). The effects of toxicants can be observed at the level of chromosomes (clastogenesis) through alterations in chromosome structure (chromosomal aberrations) and number (aneuploidy, polyploidy). Chromosome aberrations were observed in all stages of mitosis. Many clastogenic and non-clastogenic

abnormalities were detected during the different treatments. The major clastogenic abnormalities observed were nuclear lesions, chromosome stickiness and chromosome bridges whereas the non-clastogenic abnormalities observed were chromosome clumping, binucleate cell, polyploidy, early movement of chromosomes, non-synchronous movement of chromosomes, diagonal metaphase, anaphase and telophase. The most frequent abnormalities observed were nuclear lesions, stickiness, chromosome bridges, and nuclear budding.

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. Earlier reports suggest that the presence of nuclear lesions offer cytological evidence for the inhibitory action on DNA biosynthesis (Akaneme and Iyioke, 2008). Chromosome bridges may be caused by stickiness of chromosomes which make their separation and free movements incomplete and thus they remain connected by bridges (Kabarity et al., 1974). Nuclear buds were observed to originate from the nuclear envelope in situ at certain regions of the interphase nucleus of the treated cells and these might be a result of the excessive production of nucleic acids and proteins, induced by cytotoxicants (Akaneme & Iyioke 2008).

Conclusion

Thus the present study suggested that the aqueous extract of leaves of *Crotalaria laburnifolia* L. exhibited significant inhibitory and mitodepressive effects on the cell division of *Allium cepa*. 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.

Acknowledgement

The author thanks University Grants Commission (UGC), New Delhi, India for the financial support in the form of Minor Research Project.

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