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Effects of the flower extract of Ixora coccinea Linn. On the meristematic cells of Allium Cepa

P.G. Latha*, C.T. Chandralekha*, G. Vilasini* and K.R. Panikkar
Division of Ethnopharmacology, Tropical Botanic Garden and research Institute, palode, Trivandrum – 695 562, Kerala.

Department of Botany, Mahatma Gandhi College, Trivandrum – 695 004*

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ABSTRACT: *The aqueous extract of flowers of I. coccinea was evaluated for its cytotoxic and mutagenic effects on the meristematic cells of onion root tip. The percentage of abnormalities was found to increase with the increase in the concentration of the extract from 20 to 60 mg/ml. With 80 mg/ml completed arrest or total inhibition of cell division was observed. The major abnormalities were unprinted chromosomes at metaphase. Stickiness and clumping of chromosomes were induced by higher concentrations of the extract. Sticky anaphase bridges and formation of micronuclei were induced at the highest concentration (80mg/ml) tried. The significance of these abnormalities is discussed in detail. The results point to the potential use of the aqueous extract of flowers of I. coccinea in the chemotherapy of cancer.*

INTRODUCTION

Ixora coccinea (Rubiaceae) is a shrub occurring along the western coast of India, and the flowers are used as a flavoring agent. In traditional Indian medicine, the flowers find use in the treatment of dysentery, leucorrhoea, dysmenorrhoea, haemoptysis and catarrhal bronchitis (1). Though such medicinal properties are appreciable, the spectrum of drastic side effects, caused at the cellular level, due to its us at higher doses can never be ruled out. A yellow colouring matter related to quercetin, an astringent principle, a wax and a neutral crystalline substance have been isolated from the flowers of I. coccinea (2) subramonian and Nair (3) reported that the flowers contain the flavonoids, cyaniding-3-rutinoside and leucocyanidin glycoside. This paper deals with the cellular damage induced by the aqueous flower extract of *I. coccinea* on root meristem of *Allium cepa.*

MATERIALS AND METHODS

Commercially available bulbs of onion (*Allium cepa*) were dried in the sun. The scales were removed and e bulbs were allowed to sprout in wt sand. When the root tips were about 1cm long, the were treated with different concentrations (20,40 and 80 mg/ml) of the aqueous extracts of the powdered flowers of I.coccinea for 12h. sprouted bulbs in distilled water served as the control, after treatment, the root tips of each set were washed thoroughly under tap water, harvested and fixed in acetic – alcohol (1:3) for 24th squash preparations of the root tips were made by the acetocarmine technique.

5 slides of each treatment were made and observed under the Leitz orthoplan microscope. Data were collected from 10 different fields at random, as to the number

of dividing cells and abnormlities if any. Temporary preparations were used for collecting the data and for taking photomicrographs.

RESULTS

The process of mitosis was normal in control roots (Fig 1) The treated roots exhibited a wide spectrum of abnormlities such as unoriented metaphases, where the chromosomes were scattered in the cell in a disorganized manner (Fig 2) and sickiness and clumping of chromosomes at metaphase (Fig 3-5). The occurrence of high frequency of stick bridges at anaphase (Figs 6,7) may be attributed to chromosome stickiness andconsequent failure of free anaphasic separation. The formation of micronuclei (Fig 8) and nuclei of different sizes (Fig 9) were also observed.

In general, percentage of total abnormalities increased linearly with the increase in the concentration of the extracts, from 20-60 mg/ml, with total arrest if mitosis at 80mg/ml. The data on the number of dividing cells were analysed. Percentage of abnormalities scored, the concentration of the extract used and duration of treatment employed are recorded in Table 1.

DISCUSSION

A careful analysis of the data revealed that chromosome stickiness at metaphase and anaphase stages is the major abnormality met with, irrespective of the concentration used. It varied from light to severe stickiness. Darlington (4) pointed out that disintegration of DNA to a deploymerised and fluid condition and altered surface property of chromosomes may be responsible for chromosome stickiness. Stripping of the protein coat of DNA in chromatin may be another reason for chromosome stickiness (5) similar results were obtained by Narasimba Das working with asafetida (6) and shehab et al (7) working with *Achillea fragrantissima*. Unoriented metaphases might be due to the disturbance of the spindle apparatus (8). Condensation and stickiness of chromosomes due to treatment with *Ipomoea carnea* extract resulted in the formation of ring chromosomes in *Allium cepa* roots (9). Such ring chromosomes however were not observed in the present study. Tewari et al (9) have reported that triterpenoids present in *Ipomoea carnea* leaves may be responsible for these mitotic inhibitory effects. In this context, it is of interest to note that our preliminary phytochemical investigations (10) have indicated the presence of triterpenoids in *I. coccinea* flowers and may cause the observed antimitotic effects. Stick bridges at anaphase resulted from difficult in anaphasic separation due to adhesion or clumping of chromosomes (11) or due to crosslinking of the DNA of the chromosomes (12). According to George and geethamma (13), exposure of the chromosomes to the extract for a long duration, brought about changes in the viscosity of the ectoplasm and imbalance in the nucleocytoplasmic equilibrium, resulting in chromosome condensation and cells with micronuclei and nuclei of various shapes.

In short, the flower extract of *I.coccinea*, at various concentrations may be acting trough different means, ultimaely disturbing nucleic cid metabolism, thereby inhibiting protein synthesis and thus resulted an array of abnormalities at the nuclear and chromosome levels on *Allium cepa* rot meristem. In certain cases the cell division was completely arrested and there were indications of the death of actively growing cells, whereas nature and differentiated cells were not affected. Latha et al. cells were not

affected latha et al. (14) reported that 50% ehtanolic extract of *I. coccinea* flowers is cytotxic to Dalton's lymphoma ascites and Ehrlisch ascetic carcinoma cells in vitro even at very low concentrations. These findings are in accordance with those of khandelwal (15) and point to its potential in the chemotherapy of cancer. Similarly, aqueous extracts of *Heliotropium curassavicum L.*, though widely used in terapeutics as been found to induce chromosomal aberrations and anaphase delay in a CHO cell line, due to a process of in vitro metabolism (16).

Further studies are needed to identify the component present in the extract which is responsible for the cellular damage produced in root meristem.

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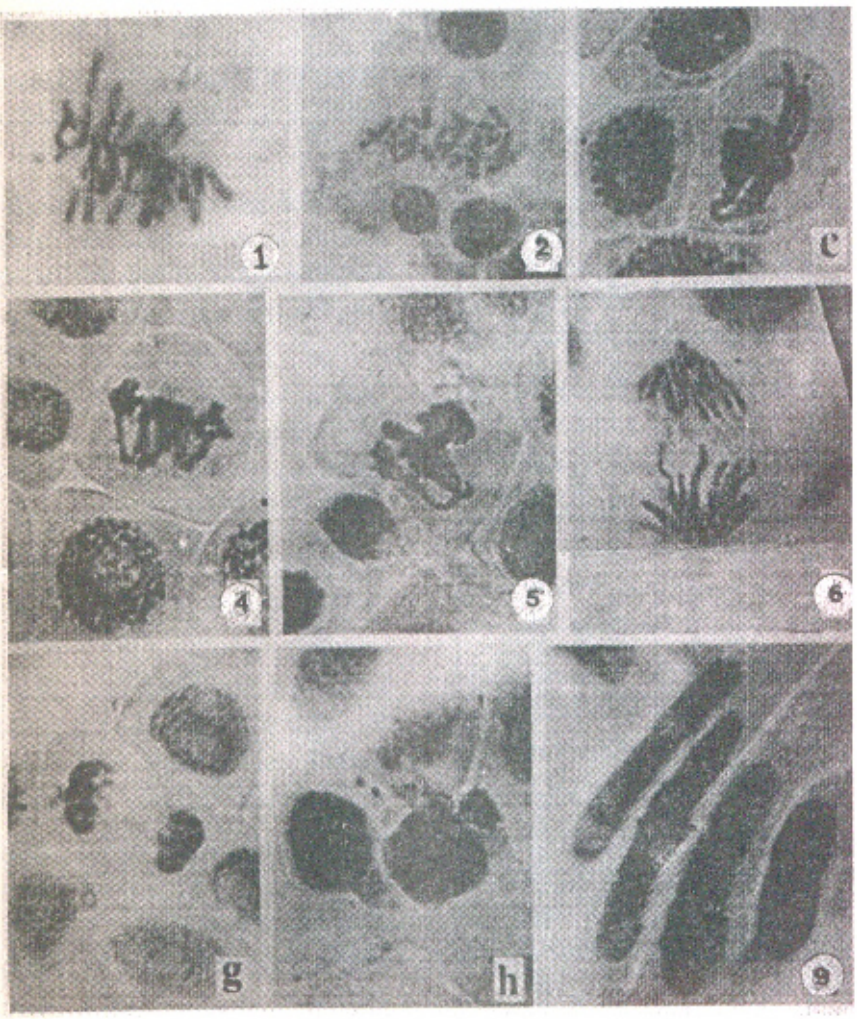

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Fig1: Normal metaphase in root tip cell of *Allium cepa* x 850

Figs. 2-9: Chromosomal abnormalities seen in root tips of allium cepa treated wit flower extract of *Ixora coccinea* x 850 2, Chromosome condensation. 3,4,5 clumping and stickiness of chromosomes. 6,7 chromosome bridges at anaphase. 8, micronucleus. 9. nuclei of different size and shape.

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Table – 1: Abnormalities at metaphase and anaphase in root tip cells of Allium cepa treated with aqueous flower extract of *I. coccinea*

Concentration of extract (mg/ml)	Duration of treatment	Total number of dividing cells	Total number of abnormal cells	Chromosome stickiness cell No. (%)		Unoriented metaphase cell No. (%)		Anaphasic sticky bridges cell No. (%)		Micronuclei formation cell No. (%)		Total abnormality (%)
0	12	1000	5	0	-	5	0.2%	0	-	0	-	0-2%
20	12	1710	485	165	9.65	26	1.52	38	2.22	18	1.05	14.44
40	12	1391	1648	317	22.78	100	7.18	167	12.00	71	5.10	47.06
60	12	1110	2049	365	32.88	290	26.13	136	12.25	68	6.13	77.39
80	12	249	2281	101	40.56	15	6.02	47	18.87	49	19.6	85.21

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