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RESEARCH ARTICLE

Cytotoxicity testing of the fruit extracts of *Rauvolfia tetraphylla* L. using the *Allium cepa* chromosome aberration assay

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Abstract

In the present study, *Allium cepa* root tip was used to evaluate the cytotoxicity of *Rauvolfia tetraphylla* L. fruit extract. For this the root tips were treated with fruit extracts at different concentrations. The mitotic indices of control and treatments were calculated and the chromosomal aberrations were also studied. The study revealed that the fruit extracts of *Rauvolfia tetraphylla* at different concentration has significant effect on mitotic index and can induce chromosomal aberrations.

Introduction:-

The vast reserve of global biodiversity coupled with wide-ranging customs and practices stemming from a heterogenous cultural heritage have led to the utilization of plant based products in folk and traditional medicines all over the world, for millennia. The increasing awareness on the side effects caused by synthetic drugs has resulted in a general preference for herbal medicine, with over 80 % of the world population using medicinal plants for primary medical treatments (Cordell, 1995). This long term use of plants by man in traditional medicines would naturally imply a low toxicity for plant-based medicinal products. However, it is also known that green plants in general are a primary source of antimutagens as well as natural toxic agents (Plewa et al. 1993), and many plants often cause cytotoxic and genotoxic effects *in vitro* and *in vivo* assays (Higashimoto et al. 1993, Schimmer et al. 1994). Therefore investigations on mitotic aberrations and their genetic consequences form an integral part of most of the mutation studies. It also provides a considerable clue to assess sensitivity of plants for different mutagens.

Cytological analysis with respect to either mitotic or meiotic behavior is one of the most dependable indices to estimate the potency of mutagens. Screening for antimitotic activity employing *Allium cepa* root tip meristematic cells has proved to be a reliable and cost-effective system for the rapid detection of cytotoxic and mutagenic properties of products of plant origin (Abhang et al. 1991; Latha et al. 1998 ; Kura's et al. 2006). Allium root tip meristem is characterized by a homogenous mass of rapidly dividing cells with only sixteen large chromosomes, making it an ideal system for bioassays (Havey et al. 2002). The mitotic variations so induced in root tip cells of *Allium cepa* may be useful in further cytological studies (Tariq Ahmad Bhat et al. 2007). Allaying the doubts regarding the extrapolation of results from plant tissues to animals and finally to humans, William and Omoh (1996) opined that molecules or chemical entities which affect plant chromosomes may also affect animals. This was further substantiated by Fiskesjo (1997) who reported that Allium test shows good correlation with mammalian test systems.

Ever since the *Allium* test was first introduced by Leven (1938), root tip systems of various plants like *Allium cepa* (Fiskesjo, 1985), *Vicia faba* (Kihlman, 1975), *Allium sativum* (Vera Lopez et al, 1995), *Tradescantia* (Ma and Grant,

1982) etc. have been widely used for determining the biological effects of chemicals. Consequently, cytotoxic and genotoxic effects have been reported from several plant extracts including the aqueous extract of *Lavandula stoechas* (Askin celik and Aslanturk, 2007), ethanolic extract of *Citrus limon* and *Citrus sinensis* (Ozmen and Askin celik, 2007), *Capparis spinosa* (Aslanturk et al 2009), *Vernonia amigdalina* (Adigbite and Sanyaolu, 2009), seed decoctions of *Cassia tora* (Pallavi Solanki et al 2008), leaf extracts of *Inula viscosa* (Tulay Askin Celik et al. 2010), fruit extract of *Cerbera odollum* (Kavitha et al. 2009). Reports on the cytotoxic effects of extracts from medicinal plants include those from *Azadirachta indica*, *Morinda lucida*, *Cymbopogon citrates*, *Mangifera indica*, *Carica papaya* etc. (Akinoboro et al. 2007).

Rauvolfia tetraphylla is a small woody shrub belonging to the Apocynaceae family, with whorled leaves, greenish-white or creamy-white flowers and drupaceous fruits which turn deep red or purple when mature. Commonly known as the Wild Snake Root, Devil–Pepper or Be Still tree, it is cultivated widely as both an ornamental and as a source of pharmaceuticals. The species was originally introduced from the West Indies, and is now naturalized throughout the tropics including Australia, Indochina and India (Sasidharan, 2004). The plant is of much ethnobotanical significance and is used widely by South Indian tribes for various ailments ranging from fever to mental illness, and as a remedy for snake bites, scorpion stings, insect bites etc. (Iqbal et al. 2013). A wide range of alkaloids such as reserpine, serpentine, reserpiline, ajamalicine and sarpagine have been reported from this plant (Anonymous, 1969), and the plant has been proved to possess antipsychotic, antimicrobial, anti-inflammatory, antioxidant, anticancer, and antihypertensive properties (Alagesaboopathi, 2009; Iqbal et al. 2013).

The purpose of this study is to assay the cytotoxicity of *Rauvolfia teraphylla* fruits using *Allium cepa* root model which has not been attempted so far and hence the significance of the present investigation.

Materials and Methods:-

Fresh ripe fruits of *Rauvolfia tetraphylla* were collected from Thalassery of Kannur District, Kerala. The plant was taxonomically identified by referring to standard flora (Gamble, 1967; Sasidharan, 2004). Extracts of fruits were prepared using a homogenizer and diluted to various concentrations as needed and stored in labeled bottles. The locally available Onion bulbs (*Allium cepa*, 2n=16) of equal size were chosen as the test material. The bulbs were grown in pots containing sand and sufficient water was given. When fresh roots came out and developed up to 2-3 cm long, the bulbs were taken out, washed and kept in bottles containing different concentrations of *Rauvolfia tetraphylla* fruit extract (10%, 20%, 30%, 40%, 50%, and 100%) and distilled water (control).

Care was taken to ensure that all the roots were properly immersed in the test solution. After one hour of treatment with the test solution, the root tips were thoroughly washed in distilled water and fixed in acetic alcohol for 24hours.

Cytological preparations were made according to haematoxylin squash technique devised by Marimuthu and Subramanyam (1960). The fixed root samples of every treatment were washed in distilled water and hydrolysed in 1N HCl at 60% for 1 minute. The root tips were then washed in distilled water and were stained with Haematoxylin for 4 hours. The stained roots were squashed with a drop of 45% acetic acid and examined under the microscope. The mitotic index was calculated by the following formula

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

At least 1000 cells from about 10 root tips were scored to determine the frequency of mitotic index. Changes in chromosome morphology and number and spindle orientation were observed to gather information about chromosomal aberrations.

Results:-

The results obtained from the present study revealed that Rauvolfia fruit extract induces a wide range of mitotic disturbances in the root tips of *Allium cepa* as compared to control (Table-1). The mitotic index decreased significantly with increasing concentration of fruit extract as compared with the control. The divisional frequency were 17.36, 17.30, 13.91, 14.21, 12.60 and 11.49 respectively in the roots treated with 10%, 20%, 30%, 40%, 50% and 100% concentrations of the test solution (Table.1). This is statistically significant when compared to control ($P<0.05$). Chromosomal aberration was maximum at 100% test solution when compared to the others (Table.2). The plant fruit extract induced a wide range of mitotic disturbances in the Allium root tips when compared to control. The percentage of total abnormalities was increased significantly in all concentrations when compared to control.

The increase was dose dependent. The abnormalities included precocious movements of chromosomes, multinucleolated conditions, chromosomal stickiness, bridges, laggards etc. Among the aberrations observed, chromosomal bridge formation was found to be the most frequent change effected in all concentrations other than the control.

Discussion:-

The present *in vitro* study showed that the fruit extract of *Rauvolfia tetraphylla* induced cytotoxic and genotoxic effects on *Allium cepa* root tip cells. The treatment of root tips in fruit extracts of different concentrations showed a gradual decrease in mitotic index, with increasing concentration of the fruit extract. This antimitotic effect may be due to the arrest of cell division due to changes at in the chromosomal morphology or spindle orientation. A thorough screening of the chromosomal abnormalities showed that the total number of abnormal cells increased with increasing concentrations of the fruit extract in a dose dependent manner.

Multinucleolate condition was observed in all concentrations of the fruit extract, but was not seen in the control. Earlier Lakshmi Nandakumar et al (1984) observed multinucleolate condition in onion root tip cells treated with isovitexin isolated from the leaves of *Rhynchosia minima* and opined that they might have resulted from nucleolar fission. Nucleolus being the centre of protein synthesis has a strategic role in cell division. Consequently the fission of such key sources of mitotic proteins may reflect 'preprophase poisoning' leading to mitotic arrest.

Lagging chromosomes resultant from chromosomal fragmentation and failure of chromosomes to move to either of the poles (Kavitha, 2008), were also observed. However maximum laggard formation was observed in 20% concentration of the fruit extract, with the frequency decreasing at higher concentrations of the extract. The capacity of inducing chromosomal fragments or laggards is a property of several chemical agents as suggested by Sharma & Sharma (1990), and has been ascribed to stickiness of chromosomes at metaphase hindering normal separation and movement at anaphase. This stress in anaphasic movement may result in fragmentation of chromosomes. Laggards may also reflect the failure of chromosomes to attach to the spindle fibres (Khanna and Sharma 2013). The present observation of increase in chromosomal fragmentation is suggestive of the claustogenic effect of Rauvolfia fruit extract.

He stickiness of chromosome observed in here may be due to any upset in the spindle formation (Borah & Jayanta Talukar, 2002). However Onyenwe (1983) opined that stickiness may result from DNA depolymerization, partial dissolution of nucleoproteins, breakage and exchange of folded units of chromatids and the stripping off of the protein coat of DNA.

In all the concentrations single, double and multiple chromatin bridges were found. Chromosomal bridge formations have been attributed to chromosome breakage, stickiness and reunion of broken ends. The stickiness in turn ensured gluing together of chromosomal ends, leading to the formation of connecting bridges (Badr *et al*.1992). Such chromosomal bridges eventually result in inhibition of cytokinesis causing mitotic arrest. The results of the present investigations indicated that the *Rauvolfia* fruit extract can cause cytotoxicity.

Table 1. Mitotic index and chromosomal aberrations in the root tip cells of *Allium cepa* treated with *Ravolfia tetraphylla* fruit extract.

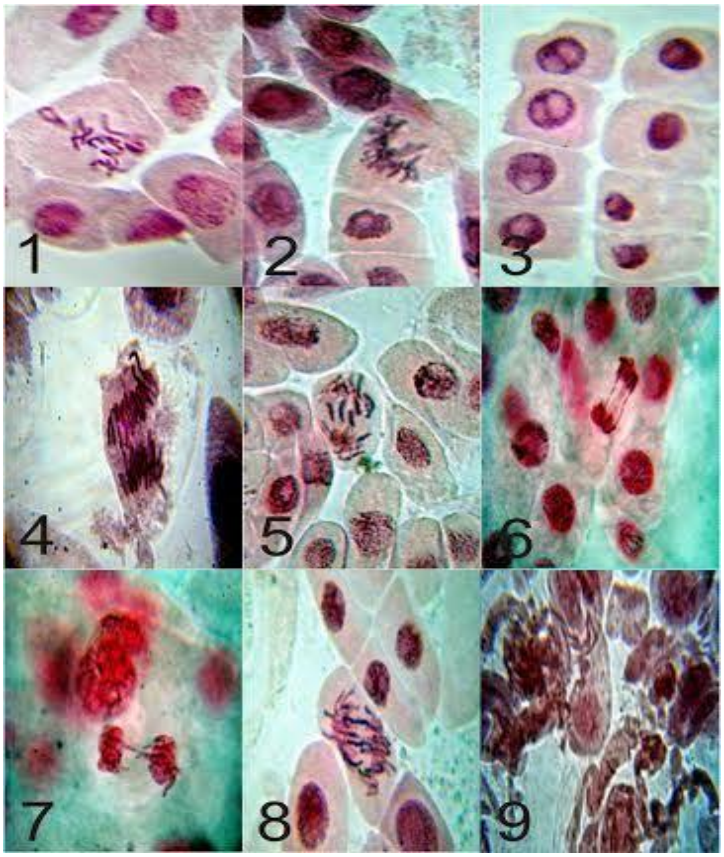
A	B	C	D	E	F	G
Control	1043	162	162	-	15.53 ± 0.89	-
10%	1048	182	172	10	17.36 ± 0.82	5.49 ± 0.32
20%	1092	189	179	10	17.30 ± 0.53	5.29 ± 0.32
30%	1092	152	141	11	13.91 ± 0.65	7.23 ± 0.22
40%	1161	165	149	16	14.21 ± 0.48	9.69 ± 0.36
50%	1190	150	132	18	12.60 ± 0.56	12 ± 0.7
100%	1218	140	115	25	11.49 ± 0.42	17.85 ± 0.85

A- Concentration; B- No. of cells analyzed; C- No. of mitotically dividing cells (Normal & Abnormal); D- No. of normal cells; E- No. of abnormal cells; F- Mitotic index (Mean & SD); G - Chromosomal aberrations (Mean & SD)

Table2: Types of aberrations observed in *Allium cepa* root tips treated with fruit extracts of *Rauvolfia tetraphylla*

Chromosomal aberrations	**Concentration of fruit extract**						**Control**
	10%	**20%**	**30%**	**40%**	**50%**	**100%**	
Precautious movements	1	0	1	1	3	2	-
Bridge formation	2	1	6	8	8	9	-
Laggards	2	5	2	2	2	3	-
Sticking of chromosomes	-	2	1	1	0	3	-
Multinucleolated condition	5	2	1	4	5	8	-
Total No. of aberrations	10	10	11	16	18	25	-

Plate-1



Figs. 1-9: Root tip cells of *Allium cepa* showing abnormalities induced by fruit extracts of *Rauvolfia tetraphylla*. 1. Precocious movement of chromosomes. 2. Oblique orientation of metaphase chromosomes. 3. Multinucleolate condition. 4. Anaphase with bridges and precocious movement. 5. Laggard formation. 6. Clumping with bridge and laggard formation. 7. Late anaphase showing bridge formation. 8. Multibrige formation. 9. Denatured cells.

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