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RESEARCH ARTICLE

Cytogenetical Effects of *Terminalia bellerica*, Roxb. on Root Meristem of *Vicia faba.*

Monika Asthana, Avnish Kumar & Satyadev Sharma
Dept. of Biotechnology, School of Life Science, Khandari Campus,
Dr. B.R. Ambedkar University, Agra (U.P.)
Email: mailtomonikasaxena@gmail.com

ABSTRACT

The cytogenetical effects of the extract of *Terminalia bellerica* belongs to the family Combretaceae of order Rosales, have been studied on root meristem of *Vicia faba* to access the clastogenic potentials. The active principle is beleric myrobalans consists of gallo-tannic acid and glycoside bellericanin present in the extract, is known to act as anti-helminthic, anti-inflammatory, useful in bronchitis and asthama etc. The percentage of chromosomal aberration recorded in present investigation is directly proportional to the concentration and duration of the treatment. The clastogenic abnormalities observed are stickiness, lagging chromosomes, chromosomal bridge, fragments, unequal distribution etc. The results of the present findings indicate that injudicious use of drug lead to several genetic deformities in bio-organism which are said to be safe and potentially with no side effects.

INTRODUCTION

Ayurveda is the earliest medical science having positive concept of health and is based upon certain basic principles of physical, chemical and biological sciences. These drugs are said to be potentially safe casting no side effects over synthetic drugs like antibiotics, analgesics, antihelminitics etc. *Terminalia bellerica* vern Hindi –Bahera, English- Beleric myrobalans have folklore used as medicinal plant for common diseases of eye, nose, etc., in India. The fruit consist of gallo-tannic acid and glycoside bellericanin, which is bitter, pungent acrid, digestible, laxative, anti-helminthic, anti-bronchitis, sore throat, anti-inflammatory, anti-asthamic. The fruit in combination with other drugs is prescribed for snake bites.

MATERIAL AND METHODS

Preparation of mother tincture

The mother tinctures of *Terminalia bellerica* have been prepared by grinding 100 gm. dried fruits with sand particles in mortar and pestle adding 100 ml of 30% alcohol prepared in distilled water. This grinded mixture was boiled in flask fitted with reflux condenser on water bath for 4 – 6 hrs. The solution was cooled and filtered and stored in dark coloured labelled bottles as mother tincture. Different concentrations of these tinctures 5%, 10%, 20%, 30%, 40%, 50% used in this study were prepared from mother tincture or stock solution.

Root-meristem treatment: Somatic effects have been studied on root meristem. Healthy seeds of *Vicia faba* germinated on moist filter paper in petridishes. Germinated seeds having root tip of about 0.5- 1cm length were transferred into varying concentrations (5% to 50%) of mother tincture for 4, 8, 12 and 24 hrs duration. The root meristems were fixed in acetic alcohol at the time of maximum mitotic index and their clastogenic effects on somatic chromosomes have been studied.

Mitotic divison: Important stages of mitotic division were photographed from temporary and permanent slides at a magnification of 750 x and 1500 x using high power (50x) and oil immersion (100 x) objectives and 15 x eyepiece Olympus pm-6 35 mm. microscope camera.

RESULTS AND DISCUSSIONS

The cytological observation revealed that both the mother tincture of *Terminalia bellerica* had a strong mitostatic effect on *Vicia faba* root as evident by the mitotic index which decreases with the

increases in duration from 4 to 12 hrs and concentration from 5% to 50% in the test plant (table 1, 2). The reduction in mitotic index might be due to turbagenic or physiological changes induced by the drug in nuclear chromation and chromosomal anomalies resulting in deficiency of nucleic acid and associated proteins or due to inhibition in respiration and energy generating A. T. P. synthesis.

Table-1: Type and distribution of somatic chromosomal abnormalities in (%) induced by different concentration and duration of *Terminalia bellerica* (Root-tip treatment) in *Vicia faba*

Duration	Conc. Laggards in %	Total cell Multi observed	Mitotic Polyploidy Index	condensed Total % of and sticky	Multi polar	Fragments	chromatin bridges	
	control	785	12.29	1.08	-	-	-	-
	5	795	12.16	7.38	-	1.12	0.67	-
	10	794	12.07	8.49	-	1.01	0.91	-
4 hrs.	20	786	12.00	8.93	-	0.98	1.42	0.68
	-	-	12.01					
	30	780	11.80	9.55	-	0.88	1.51	-
	40	788	11.63	10.02	-	0.65	1.53	-
	Mean	**789.16**	**11.85**	**9.16**	**0.20**	**0.81**	**1.28**	**0.11**
	-	**-**	**11.56**					
	Control	812	13.46	1.41	-	-	-	-
	5	820	12.10	8.03	-	1.52	1.08	-
	10	817	12.00	9.11	-	1.50	1.23	-
8 hrs.	20	800	11.68	9.43	-	1.33	1.41	-
	30	798	11.31	9.98	-	1.05	1.62	0.45
	-	-	13.10					
	40	815	11.00	10.51	0.45	1.00	1.75	0.50
	-	-	14.21					
	50	821	10.67	10.96	0.90	0.93	1.80	-
	Mean	**811.83**	**11.46**	**9.67**	**0.22**	**1.22**	**1.48**	**0.15**
	-	**-**	**12.74**					
	Control	830	13.21	1.30	-	-	-	-
	5	818	12.05	8.91	-	2.01	1.16	-
	10	792	11.90	9.52	-	1.81	1.38	-
12 hrs.	20	790	11.50	10.23	-	1.63	1.62	-
	30	821	11.05	11.02	0.52	1.42	1.80	-
	40	789	10.82	11.52	0.93	1.20	2.01	-
	50	829	10.50	11.90	1.05	1.06	2.50	1.03
	1.38	-	18.57					
	Mean	**806.5**	**11.30**	**10.51**	**0.41**	**1.52**	**1.68**	**0.17**
	0.38	**-**	**14.67**					
	Control	800	12.50	1.00	-	--	-	-
	5	779	11.67	8.15	-	1.10	0.25	-
	10	785	11.25	9.00	-	1.00	0.58	-
24 hrs.	20	790	10.80	9.58	-	0.95	0.65	-
	30	801	10.21	9.91	-	0.80	0.87	-
	40	793	9.93	10.50	-	0.53	0.98	-
	50	798	9.50	10.81	2.05	-	1.08	-
	Mean	**791**	**10.56**	**9.65**	**0.34**	**0.73**	**0.73**	**-**
	0.84	**-**	**12.29**					

Terminalia bellerica used in this study resemble other medicinal plants regarding the ability to inhibit cell-division, for instance water extract of *Pulicaria crispa*, *Anastatica hierochuntica* L. [1], active constituents of *Peganum harmala* [2], extract of castor seed [3].

The stickiness of chromosomes (fig 1) was the most prominent aberration induced by the drugs in the test plant. The stickiness of chromosomes might have resulted due to loosening of nucleic acid from the protein architecture of the chromosomes or due to depolymerization of DNA [4] and partial dissolution of nucleoprotein. At higher concentration the stickiness of cells were very common. This effect was previously recorded by Haroun and Shehri [5], for *Calotrois procera* extract.

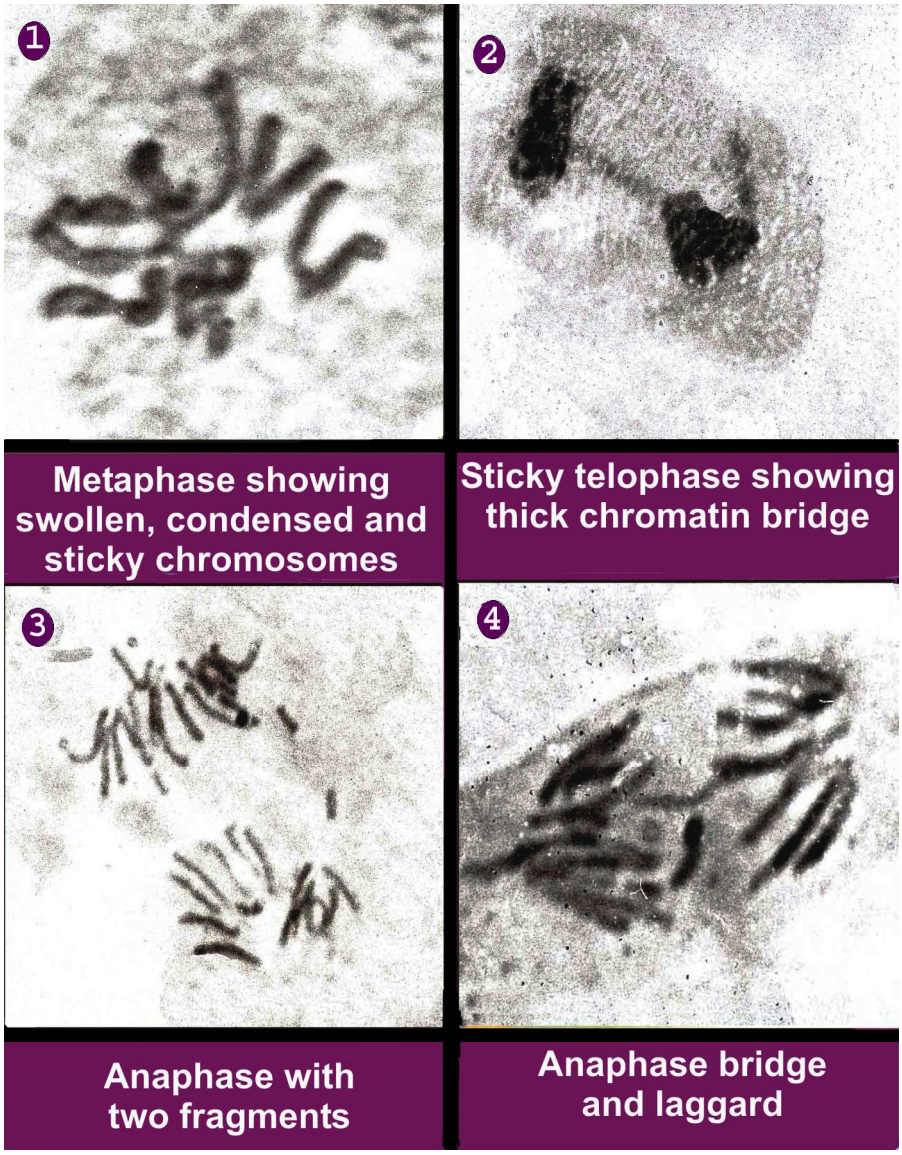

FIGURE: 1-4: Indicating chromosomal abnormalities induced by *Terminalia bellerica* (Root-tip treatment) in *Vicia faba*

Table 2: Mean percentage (M%) of somatic chromosomal abnormalities induced by *Terminalia bellerica* on root meristem of *Vicia faba*

Type of treatment and duration	Total cell observed	Mitotic Index	Condensed and sticky chromosome	Multipolar spindle	Fragments	Chromatin Bridges	Laggards	Multi nucleate cell	Total % of abnormalities
RT04	789.16	11.85	9.16	0.20	0.81	1.28	0.11	-	11.56

RT08	811.83	11.46	9.67	0.22	1.22	1.48	0.15	-	12.74
RT12	806.50	11.30	10.51	0.41	1.52	1.68	0.17	0.38	14.67
RT24	791.00	10.56	9.65	0.34	0.73	0.73	-	0.84	12.29

The chromatin bridge (fig - 2) was observed due to the sticky nature of chromosomes, inversions and high frequency. This effect was previously recorded by Ayse Nihal Gomurgen [6].
The fragments (fig 3) were noticed either due to terminal breaks in the chromosome or failure of chromosome thread to rejoin [7].
The lagging chromosome (fig 4) was possibly formed due to the inhibition of centromeric and spindle activity which inhibits chromosome movement and due to presence of acentric fragments or to the interaction of drug with protein of the spindle apparatus [8]. Lagging chromosomes were resulted due to the failure of the chromosomes to move to either of the poles. The percentage of laggards, fragments and bridges indicated the clastogenic effects of the mother tincture of *Terminalia bellerica* on *Vicia faba* cells.
The mother tincture of *Terminalia bellerica* behaved as a stathmokinetic agent [9] since its effects was on the spindle as evident by less number of abnormalities was found in prophase.
Thus the present study suggested that injudicious use of mother tinctures lead to several genetic deformities in bio-organisms and specific dose and duration of therapy would be useful in controlling diseases.

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