

Cytogenetical effect of *Mentha piperita* extract on the somatic chromosomes in *Vicia faba*.

Monika Asthana¹ & Avnish Kumar²

^{1,2}Dept. of Biotechnology, School of Life Science, Khandari Campus, Dr. B.R. Ambedkar University, Agra (U.P.)

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Abstract

Mentha pipertia is a medicinal plant that has been used in folk medicine since time immortal. *Mentha piperita* depicts antimicrobial, antiseptic action and used for treating nausea, flatulence, sickness, vomiting, stomachache etc. Extract of this plant was tested against *Vicia faba* to study scientific rationale behind use and its effect on somatic chromosomes. Extract have been prepared in the laboratory. Then presoaked seeds of *Vicia faba* in water were treated in different concentrations (5-50%) of extract for 24 hrs of duration. The germinating seeds both treated and controlled having root tips of about 0.5 to 1 cm in length were fixed in acetic alcohol (1:3) fixative for 8-12 hrs and then preserved in 70% alcohol. Detailed studies were conducted on the clastogenetic effects and chromosomal behaviour during cell-division.

The observation revealed that the extract had a strong mitostatic effect on *Vicia faba*. It induced chromosomal abnormalities during mitotic cycle such as sticky chromosomes, fragmentation of chromosomes, chromatin bridges, lagging chromosomes unequal segregation at anaphase and polyploidy. There was gradual increase in the total percentage of abnormalities with increase in concentration from 5 to 50% and duration from 4 hrs to 12 hrs. The results of the present findings indicate that *Mentha piperita* is more drastic than other common plants and injudicious use of drug lead to several genetic deformities in bio-organism which are said to be safe and potentially with no side effects.

Key words:

Ayurvedic, somatic chromosomes, clastogenic effects, mitostatic.

Pharmacologists, toxicologist and phytochemist over other allopathic drugs due to their comparatively less or no side effects (World Ayurved Foundation 2012). 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

Introduction

Ayurveda is India's traditional natural system of medicine. These drugs have come into lime light of

synthetic drugs like antibiotics, analgesics, antihelminitics etc [Baliga & Rao, 2010]. *Mentha piperita* L. Vern. Hindi- Paparaminta, Phudina, English- Peppermint (Fam Labiatae) are commonly used medicinal plants. *Mentha piperita* depicts antimicrobial [Iskan *et al.*, 2002], antiseptic, deodorant, stimulant and carminative action. Generally used as an external applicant in congestive headaches, rheumatism, neuralgia etc and for allaying nausea, flatulence, sickness, vomiting and as an infants cordial. This plant has been cultivated in several parts of Indian subcontinent and medicines from it like "Pudin Hara®" and "Itch Guard®" are available in market [Shah & Mello, 2004].

It is, therefore, considered of interest to investigate the mitostatic and clastogenic effect of these drugs on somatic cell division and their chromosomal behaviour in the root-tip cells of *Vicia faba*.

Material and Method

The extract of *Mentha piperita* have been prepared by grinding 100 gm. dried plant (leaves, seeds, flowers etc.) 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 colored labeled bottle. Different concentrations of this extract 5%, 10%, 20%, 30%, 40%, 50% used in this study were prepared. Somatic effects have been studied through following two methods:

Root-tip treatment:

Healthy seeds of *Vicia faba* were 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 *Mentha piperita* for 4, 8, 12 and 24 hrs. duration. The root tips were fixed in acetic alcohol at the time of maximum mitotic index and their morphological

and clastogenic effects on somatic chromosomes have been studied.

Seed-soaked treatment:

Pre-soaked seeds (6 to 12 hrs) in water of test plants were treated in different concentrations (5% to 50%) of *Mentha piperita* for 24 hrs. duration. The germinating seeds both treated and controlled having root tips of about 0.5-1cm length were fixed in acetic alcohol (1:3) fixative for 8–12 hrs and then preserved in 70% alcohol [Sarbhoy 1980]. Important stages of mitotic division were photo-graphed 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 *Mentha piperita* had a strong mitostatic effect on *Vicia faba* root as evident by the mitotic index (MI) which decreases with the increases in duration from 4 to 12 hrs and concentration from 5% to 50% in the test plant (table 1). The reduction in MI has been suggested due to turbagenic or physiological changes induced by the extract in nuclear chromatin and chromosomal anomalies resulting in deficiency of nucleic acid and associated proteins or due to inhibition in respiration and energy generating ATP synthesis.

The extracts used in this study resemble with other medicinal plants regarding the ability to inhibit cell-division, for instance water extract of *Pulicaria crispa*, *Anastatica hierochuntica* L. [Shehab and Adam, 1983], active constituents of *Peganum harmala* [Abderrhman, 1998], extract of castor seed [Borah and Talukdar, 2002]. In seed-soaked treatment the abnormalities were found less in number (table-2). This revealed that the meristem cells were affected more severely in root-tip treatment.

The stickiness of chromosomes (Figure 1A) was important that have been considered due to loosening of nucleic acid from the protein architecture of the chromosomes or due to depolymerization of DNA [Darlington, 1942] and partial dissolution of nucleoprotein. At higher concentration the stickiness of cells were very common. Similar, effect was previously recorded by Haroun and Shehri [2001], for *Calotrois procera* extract.

The chromatin bridge (Figure 1B) formed because of stickiness between chromosomes, and also due to inversions and high frequency. Similar, effect was previously recorded by Ayse Nihal Gomurgen [2000].

The fragments (Figure 1C) were noticed either due to terminal breaks in the chromosome or failure of chromosome thread to rejoin [Wolf and Borslet, 1954]. The lagging chromosome (Figure 1D) 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 [Wuu & Grant, 1966]. 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 Ayurvedic drugs on *Vicia faba* cells.

The polyploid cells were found only by the treatment of *Mentha piperita*. Polyploid cells (Figure 1E) were arisen due to diplochromosomes or due to non-disjunction and unequal separation of chromosomes. [Baquar & Khan, 1971]. Such alterations in chromosome may leads to a haplo-insuiciency in function and are often severe enough to cause death of the cell.

The injudicious dose of drug's effects was on the spindle and it also observed less number of abnormalities in prophase. Thus the present study suggested that injudicious use of drugs lead to

several genetic deformities in bio-organisms and specific dose and duration of therapy would be useful in controlling diseases.

Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

"Author MA" designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. "Author AK" managed the experimentations, analyses of the study and the literature searches. All authors read and approved the final manuscript.

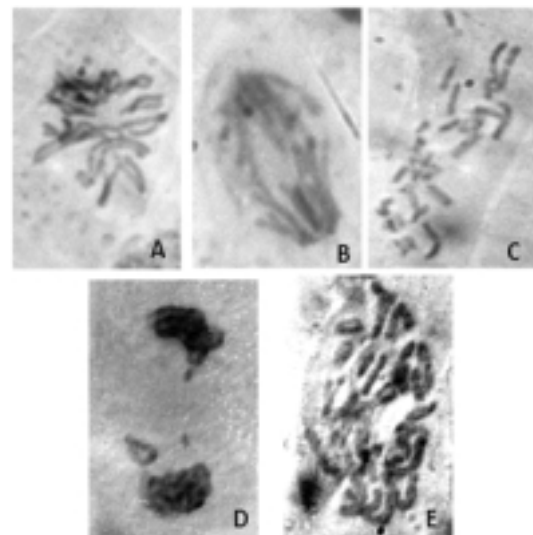


Figure 1: Effect of different doses of *Mentha piperita* extract on meiotic chromosome of *Vicia faba*- E. Polyploid cell D. Lagging chromosome A. Sticky chromosome B. chromatin bridges. C. Fragmentation

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

Du.	Conc. %	TOC	MI	Cond SC	Frag	CB	La	Tot % Ab
4hrs	Control	812	14.37	8.94	-	-	-	0.94
	5	810	14.28	9.76	0.92	0.58	-	11.26
	10	825	14.02	10.35	1.03	0.99	-	12.35
	20	821	13.95	11.02	1.15	1.05	-	13.22
	30	805	13.51	11.98	1.28	1.10	-	14.36
	40	809	13.26	12.25	1.46	1.25	-	14.96
	50	803	13.08	13.03	1.68	1.38	0.95	18.56
8hrs	Control	820	14.61	1.36	-	-	-	1.36
	5	815	14.20	9.98	1.03	0.72	-	11.73
	10	804	13.98	11.79	1.21	1.03	-	14.03
	20	812	13.63	12.25	1.38	1.26	-	14.89
	30	821	13.45	13.03	1.65	1.29	-	16.48
	40	817	13.06	13.50	1.73	1.38	0.95	18.79
	50	809	12.98	14.28	1.90	1.53	0.46	22.38
12hrs	Control	815	14.33	1.39	-	-	-	1.39
	5	810	13.99	13.06	1.25	1.16	-	15.47
	10	819	13.63	13.98	1.50	1.32	-	16.80
	20	806	13.42	14.50	1.76	1.53	-	17.79
	30	800	13.01	14.33	1.83	1.75	-	19.14
	40	813	12.82	15.28	1.95	1.82	-	20.26
	50	805	12.63	13.96	2.01	1.98	-	23.31
24hrs	Control	810	14.62	0.95	-	-	-	0.95
	5	790	13.50	10.00	0.99	0.73	-	11.72
	10	802	13.25	10.55	1.10	0.96	-	12.61
	20	783	13.03	10.75	1.40	1.15	-	13.25
	30	809	12.78	12.03	1.55	1.25	-	14.81
	40	807	12.41	11.28	1.80	1.40	-	17.13
	50	795	12.02	11.50	1.95	1.63	0.62	19.13

Note: Du. = Duration; Con % = Concentration in percentage; TOC = Total observed cell; MI= Mitotic Index; Cond SC = Condensed and sticky chromosomes; Frag = Fragments; CB = Chromatin bridges; La = Laggards; Tot % Ab = Total percentage of abnormalities

Table 2: Somatic chromosomal abnormalities (%) induced by different concentration of *Mentha piperita* (seed-soaked treatment of 24 hrs duration) in *Vicia faba*

Conc %	TOC	MI	Cond SC	Frag	CB	Tot % Ab
Control	779	12.52	0.25	-	-	0.25
5	803	11.43	4.25	-	0.53	4.78
10	788	10.95	4.81	-	0.63	5.44
20	750	10.68	5.17	-	0.77	5.94
30	765	10.25	5.93	-	0.95	6.88
40	737	10.09	6.51	0.97	1.23	9.72
50	790	9.78	7.52	1.56	1.57	13.18
Mean	772.16	10.53	5.69	0.42	0.94	7.63

Note : Conc % = Concentration in percentage; TOC = Total observed cell; MI = Mitotic Index; Cond SC = Condensed and sticky chromosomes; Frag = Fragments; CB = Chromatin bridges; La = Laggards; Tot % Ab = Total percentage of abnormalities

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