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A Study on the Cytological Effects of Myrobalan (Fruit of *Terminalia chebula*) in *Allium* Tests

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Abstract

The present study was aimed to find the cytogenetic effects of myrobalan using *Allium cepa* as a model system. The onion bulbs were grown in the suspension of myrobalan in tap water at various concentrations (0.01, 0.10, 1.0, 10.0 and 30 mg/ml) for 96 hours. The mean root length, the colour of growing roots as well as the mitotic index and chromosomal aberrations were observed in the presence of myrolaban, in order to assess the cytological effects of myrobalan. The colour of growing roots was not affected at 0.01 and 0.1 mg/ml myrobalan concentrations, while it appeared pale at 1 mg and 10 mg/ml and black at 30 mg/ml concentrations. The root length was not affected at low concentrations of myrobalan, however, concentrations 1 mg/ml and above inhibited root length. The mitotic index i.e., percentage of dividing root tip cells did not change at 0.01 and 0.10 mg/mlmyrobalan concentration, however, it decreased significantly at 1 mg/ml and no mitosis was observed at 10 mg/ml and above, as the cells were seen in interphase with nucleoli. Abnormal mitosis and chromosomal aberrations like sticky chromosomes, C–mitosis, laggards, multipolar anaphases, chromosome bridge, micronucleated cells and polykaryocytes were not seen in dividing and non–dividing cells among roots grown at various concentrations of myrobalan. The present finding reveals that myrobalan does not exert any cytotoxic effects in *Allium* model.

Key words: *Myrobalan, mitotic index, Allium cepa, root tip cells*, tannins

Introduction

Terminalia chebula Retz. (Combretaceae) is a component of well known herbal preparation 'Trifala', which means three fruits. It consists of *T. chebula* (Harar), *T. bellirica* (Belleric myrobalan) and *Emblica officinalis* (Amla). The fruits of *T. chebula* (myrobalan) are extensively used as adjuncts to other medicines [1]. In Ayurveda 'Trifala' or myrobalan alone is also recommended for life time use to maintain overall good human health [2], however, no experimental evidence is available in favour of lack of its genotoxic effect to strengthen this age old belief. Its antimutgenic and desmutagenic effect in microbial test system i.e., *E. coli* and *S. typhimurium* and cytotoxicity for

cultured human cancer cells has been shown [3–5]. *Allium* test is one of reliable model for screening drugs, chemical, pollutants and contaminants because root growth inhibition and adverse effects on chromosomes provide likely indication of toxicity [6,7], therefore, the present study was undertaken to find out cytogenetic effects of myrobalan on onion (*Allium cepa*) root tip cells to assess whether myrobalan has any effect on mitotic index or occurrence of chromosomal aberrations.

Experimental

Allium cepa

Dry healthy onions 1.5 to 2.0 cm in diameter were obtained from the local market.

Herbal Drug

Dry young fruits of *Terminalia chebula* (myrobalan) were purchased from the local market. The fruits were gently baked for few min in stainless steel container and after cooling, swollen nuts were grinded to obtain a fine powder and stored. A smooth suspension of powdered myrobalan was prepared in tap water at different concentrations. The recommended dose of myrobalan is 3–9 g/day for adults [2]. The experimental design is based on internationally accepted *Allium cepa* model [6]. The onions were descaled, leaving the root primordia intact. The samples containing myrobalan suspension or tap water in controls were taken and the descaled onion was placed on the top of each glass tube with root primordia dipping in the fluid. After 24 hr the test suspension and tap water were changed. After 48 hr, 2 onions out of 12 with most poorly growing roots were removed, distal 2 mm of five roots was cut off from five bulbs and were fixed in acetoalcohol (1:3 v/v) for the chromosomal study. The fixation was done at a fixed time in all experiments. After 96 hr, length of 05 root bundles (all the roots of a bulb) from each onion were measured. Physico–chemical analysis of water was performed using standard methods [8].

The whole mounts (fixed material was dehydrated in series of alcohol mixtures, cleared in xylene and mounted in DPX) of 05 mm long root tips from experimental and control onions were prepared. The fixed material was squashed in 2% acetocarmine. Four fields from each slides were observed to cover 50 cells in each field from each root tip. The mitotic index was calculated as percentage of the dividing cells. The slides were also observed for the mitotic arrest, chromosome fragments, abnormal orientation, lagging chromosomes and polyploidy etc.

All experiments were done thrice. Statistical analysis was performed using students ‘t’ test and probability level of less than 5 % was considered significant.

Results

Physicochemical properties of tap water

The water analysis showed normal physico–chemical properties (Table 1).

Shape and colour of roots

Myrobalan exposure at 0.01 and 0.1 mg/ml did not cause any change in colour of roots, however, at 1.0 and 10 mg/ml concentration of myrobalan, the colour of roots turned pale and black at

30 mg/ml (Table 2). The morphology of root tips was not affected at any of these concentrations of myrobalan.

Root length

Myrobalan concentration at 0.01 and 0.10 mg/ml did not inhibit the growth of roots, however, 78% and 92% inhibition in root growth was observed at 1.0 and 10 mg/ml and above concentration of myrobalan (Table 3).

Mitotic index

Myrobalan treatment at 0.01 and 0.10 mg/ml concentrations did not affect mitotic index (MI) in root–tip cells, but 1 mg/ml concentration significantly lowered MI. At 10 and 30 mg/ml myrobalan concentration all cells appeared in interphase with well differentiated nucleoli and no mitosis was observed (Table 4).

Abnormal mitosis and chromosomal aberrations

The analysis of large number of metaphases and anaphases did not revealed any type of abnormal mitosis or chromosomal aberrations (Table 5).

Discussion

Myrobalan is widely used in Ayurvedic medicines, however, studies related to its cytogenetic effects are limited. In the present study, the cytogenetic effect of myrobalan were evaluated on *Allium* test model. The results show no significant change in root length or colour of root tips at low concentration of myrobalan, however, at higher concentrations these parameters were affected.

Myrobalan treatment at low concentrations showed no significant change in mitotic index, however, at higher concentration (1 mg/ml), MI was lowered. At higher concentrations of myrobalan (10 and 30 mg/ml) mitosis was not observed. It is possible that a high concentrations of any chemical may have an inhibitory or stimulatory effect on the cell cycle, as has been shown for caffeine in *Drosophila prosaltans* [9] and for *Alpinea mutans* and *Pogostemun heyeanus* extracts in *Allium* root tip cells [10].

From the alkaloids isolated from periwinkle plant (*Vinca rosea; Catharanthus rosens*), two compounds vinblastine sulfate and vincristine sulfate have been shown to be useful in the chemotherapy of malignancy [11]. These compounds act by inhibiting mitosis. Further they bind to tubulin and prevent the formation of the mitotic spindle. As myrobalan also show similar property of lowering mitotic cell division it is suggested that myrobalan may be a potential drug in cancer treatment.

The desmutagenic and antimutagenic action of *T. chebula* was found against some of the mutagenic agents such as UV, N–methyl–N'–nitrosoguanidine, benzo(a)pyrine and 3–amino–1–methyl 5H–pyrido indole in *E. coli* and *Salmonella typhimurium* [3]. Myrobalan has been shown to exert antimutagenic activity against sodium azide and 4–nitro–o–phenylenediamine [4]. The tannins isolated from various plants including *T. chebula* were found to exert selective cytotoxic effect

against human tumor cell lines [12,13]. Antimutagenicity of hydrolyzable tannins from fruit pulp of *T. chebula* in *S. typhimurium* against 4–nitro–o–phenylenediamine and 4–nitroquinoline–N–oxide has also been shown [5].

The antimutagenic activity of six chinese medicinal herbs were tested on *Salmonella* and it was found that tannins and catechin compounds were responsible for the inhibition of mutagenecity caused by benzo(a) pyrine [14]. However, studies by Ohtsuka *et al.* [15] showed that the antimutagenic effect of chinese medicines was due to saponins and flavonoids. The high concentration of flavonoids in green tea has been shown to be responsible for the antimutagenic and the anticarcinogenic property of tea extract [15]. As myrobalan possesses a large number of compounds such as steroids, saponins, tannins, anthroquinone, phenolic compounds, flavonoids, terflavins etc. [16–19] it is possible that tannins, saponins and flavonoids, which are the principal components in *T. chebula* may be responsible for the antimutagenic effect as well as the occurrence of no chromosomal aberrations in root tip cells.

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References

[1] Anonymous. The Wealth of India, Raw Materials, CSIR, New Delhi, 1996, p.171.

[2] Bramhaverchasva. Jadi Butiyon Dwara Swasthya Sanrakshan, 9th edition. Yug Nirmana Yojna Gayatri Tapobhumi, Mathura, 1997.

[3] Jain AK, Shimoi K, Nakamura Y, Tomita I, Kada T, Curr Sci 1987; 56: 1266.

[4] Grover IS, Bala S. Indian J Exp Biol 1992; 30: 339–341.

[5] Kaur Simran, Grover IS, Singh M, Kaur S. Mutation Res. 1998; 419: 69.

[6] Fiskesjo G. *Allium* test. In: Ottare S, Atterwill CK, Editors. Methods in Molecular Biology. Humana Press Inc Totowa NJ USA: 119–127.

[7] Fiskesjo G. *Allium* test for screening chemicals; evaluation of cytological parameters. In : Wang W, Gorsuch WJ, Hughes JS. Editors. Plants for environmental studies. Lewis Publishers CRC NY : 308.

[8] APHA. Standard methods for examination of water and wastewater. 20th edition. Washington DC, 1998.

[9] Itoyama MM, Bicudo HEMC, Cordeiro JA. Brazilian J. Genet. 1997; 20: 655.

[10] Dias FL, Takahahi CS. Rev Brasil Genet 1994; 17: 175.

[11] Bruce AC, Carmen JA, Gregory AC, Paul C. Antineoplastic agents. In: Molinoff PB, Ruddon RB, Editors. Goodman and Gilman the pharmacological basis of therapeutics. 9th edition. McGraw Hill 1996: 1257.

[12] Kashiwada Y, Nonaka G, Nishioka I, Chang JJ, Lee KH. J Nat Prod 1992; 55: 1033.

[13] Lee SH, Ryn SY, Choi SU, Lee CO, No Z, Kim SK, Ahn JW. Arch. of Pharmacol Res 1995; 18: 118.

[14] Horikama K, Mohri T, Tanaka Y, Tokiwa H. Mutagenesis 1994; 9: 523.

[15] Ohtsuka M, Fukuda K, Yano H, Kojiro M. Jap. J. Can. Res.1995; 86: 1131.

[16] Chetty KM, Rao KN. Indian J of Forestry 1989; 12: 117.

[17] Bhaumik T, Joshi PC, Dev AK, Kundu AB. Bull. Medico-Ethnobotanical Res 1989; 10: 190.

[18] Lin T, Nonaka G, Nishioka I, Ho F. Chem. Pharmaceutical Bull. 1990; 38: 3004.

[19] Reddy VRC, Kumari R, Reddy SV, Anzeem MA, Prabhakar MG, Appa Rao AVN. Fitoterapia 1990.

Table 1. Physico–chemical properties of tap water.

S.No.	Properties	Mean ± SEM
01	Turbidity	0.92 ± 0.06 NTU
02	pH Value	7.45 ± 0.08
03	Colour	Colourless
04	Total alkalinity	45.20 ± 3.41 mg/l
05	Carbonates	8.00 ± 0.85 mg/l
06	Bicarbonates	136.10 ± 3.81 mg/l
07	Hardness	267.3 ± 44.12 mg/l
08	Chlorides	46.72 ± 5.22 mg/l
09	BOD	5.14 ± 1.70 mg/l
10	COD	8.25 ± 4.20 mg/l
11	Fluoride	0.30 ± 0.02 mg/l
12	Nitrite	Nil
13	Dissolved oxygen	7.2 ± 0.1 mg/l

Table 2 Morphology of *Allium cepa* root tips following cultivation in myrobalan suspension**.**

		Morphology of root tips					Colour of root tips	
S. No.	Concentration of myrobalan suspension (mg/ml)	Abnormal			Normal	Normal	Abnormal	
		Crochet hooks	Bulb	Broken tips	Straight	White	Pale	Dark Brown/Black
01.	0.00	No	No	No	Yes	Yes	No	No
02.	0.01	No	No	No	Yes	Yes	No	No
03.	0.10	No	No	No	Yes	Yes	No	No
04.	1.00	No	No	No	Yes	No	Yes	No
05.	10.00	No	No	No	(–)	No	Yes	No
06.	30.00	No	No	No	(–)	No	No	Yes

Roots were cultivated in myrobalan suspension for 96 hr. (–) indicate very minute roots.

Table 3. Root length of *Allium cepa* after cultivation in different concentrations of myrobalan suspension.
Values are Mean ± SEM, n = 16

S. No.	Concentration of myrobalan mg/ml	Mean Root–length mm	% change in comparison to controls
1.	0	35.50 ± 4.42	100 %
2.	0.01	36.50 ± 2.66 (NS)	2.8 % increase (NS)
3.	0.1	33.33 ± 2.90	6.1 % inhibition (NS)
4.	1.0	7.66 ± 1.47[a]	78.4 % inhibition
5.	10.0	2.83 ± 0.43[a]	92.0 % inhibition
6.	30.0	2.75 ± 0.69[a]	92.2 % inhibition

p values, [a] (> 0.05) level as compared to controls, NS – non significant

Table 4. Mitotic index of *Allium cepa* root tip cells cultivated in different concentrations of myrobalan.

Concen-tration of myrobalan (mg/ml)	Prophase	Metaphase	Anaphase	Telophase	Total number of cells observed	Mitotic Index
0.00	1104.10 ± 7.29	161.55 ± 1.29	161.55 ± 1.29	168.18 ± 2.12	4965.83 ± 18.29	32.12 ± 3.95
0.01	1045.50 ± 3.58	156.26 ± 1.24	142.18 ± 1.69	246.22 ± 2.66	5138.77 ± 15.2	30.94 ± 2.90
0.10	818.20 ± 2.42	154.20 ± 1.06	118.02 ± 1.27	229.33 ± 1.51	5186.91 ± 14.67	25.44 ± 1.35
1.00	96.41 ± 1.23	101.15 ± 1.82	30.03 ± 1.18	95.67 ± 1.44	5138.67 ± 13.87	6.2 ± 0.69[a]
10.0	Nil	Nil	Nil	Nil	5845.81 ± 13.31	Nil
30.0	Nil	Nil	Nil	Nil	5706.87 ± 16.37	Nil

Values are Mean ± SEM, n = 20, p values, [a] <0.05 level as compared to controls,
NS – non significant

Table 5. Cytological effects in *Allium cepa* root tip cells cultivated in different concentration of myrobalan suspension.

Conc. (mg/ml)	Number of counted cells Meta.+Ana.	Normal Meta.	Normal Ana.	Sticky Chromo-somes	C–Mitosis	Vagrant (lagging) Chromo-some	Multi-polar Ana-phases	Bri-dges	Frag-ments	MNC Micro nucleo-cytes	Poly–Karyo-cytes
0.00	806.92	50.05	49.95	–	–	–	–	–	–	–	–
0.01	775.93	52.35	47.65	–	–	–	–	–	–	–	–
0.10	734.99	56.64	43.36	–	–	–	–	–	–	–	–
1.00	647.30	65.38	34.62	–	–	–	–	–	–	–	–
10.0	Nil	Nil	Nil	–	–	–	–	–	–	–	–
30.0	Nil	Nil	Nil	–	–	–	–	–	–	–	–

(–) indicate no microscopic effect.