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Prevention of Acetaminophen-Induced Mitodepression with Myrobalan (Fruit of *Terminalia chebula*) in *Allium cepa* Model

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ABSTRACT

Allium cepa bulbs were grown in pure tap water (group I), in seven concentrations of acetaminophen (7.81, 15.62, 31.25, 62.50, 125, 250, 500, and 1000 ppm) in the presence (group II) and absence of myrobalan (fruit of *Terminalia chebula*) at a fix concentration of 0.10 mg/mL. Parameters of study were mean root length, mitotic index, abnormal mitosis and chromosomal aberrations and morphology of root. Acetaminophen at all concentrations except 1000 ppm where roots did not grow at all, significantly inhibited root growth and declined mitotic index, effect appeared concentration dependent (group II). In the presence of myrobalan (group III) acetaminophen-induced mitodepression could be checked significantly. No morphological i.e. shape and color changes, abnormal mitosis and any type of chromosomal aberrations could be detected in any group. Probable protective role of myrobalan is discussed.

Keywords: *Allium cepa, Mitodepression, Acetaminophen, Myrobalan, Terminalia chebula*

Acetaminophen (paracetamol) has earned a prominent place as a common house hold analgesic which is available without prescription in several parts of the world [1]. Genotoxicity of acetaminophen is also on record and it is suggested that use of paracetamol may contribute to an increase in the total burden of genotoxic damage in man [2]. Ayurved (ancient Indian system of herbal medicine) recommends life time use of myrobalan alone or in Trifla (meaning three fruits: *T. chebula*, *T. belirica*, *Emblica officinalis*) to maintain general good health of human beings because it is safe and possesses antioxidant and antimutagenic properties [3-5]. Myrobalan alone does not exert any ill effects in *Allium* test [6]; however, acetaminophen does so [7-8].

Aim of this study was to find out whether myrobalan could reduce, nullify or intensify cytotoxic effect of acetaminophen in *Allium* root tip cells when administered simultaneously.

MATERIALS AND METHODS

Allium cepa

Dry healthy onion bulbs 1.5 to 2.0 cm in diameter were obtained from local market.

Test Herbal Drug

Myrobalan, dried young nuts of *Terminalia chebula* were procured from local herbal medicine shop, and were gently backed for few minutes and cooled. Swollen nuts were ground to a fine powder. A recent study (Sharma, 2003) revealed lack of mitostatic effect of myrobalan on mitosis in *Allium* test at 0.10 mg/mL [6]; therefore this concentration was selected for the present study.

Test Chemical

Acetaminophen (paracetamol) under trade name 'medimol' dispersible 500 mg B.P. tablets made by synchem laboratories, Baroda (Gujrat) was used. Each tablets was dissolved in known amount of luke warm tap water to prepare suspensions of known concentrations.

Experimental Design

Experiments were planned as per standard protocol for *Allium* test [9]. Onions were descaled and placed on, twelve test tubes filled with pure tap water, (Group I, controls). Second series of 12 test tubes bearing onions were filled with suspension of each concentration of acetaminophen (Group 2, acetaminophen exposed). Third series of test tubes bearing onions were also filled with different concentrations of acetaminophen suspen-

Table 1. Mean root length (MRL) of *Allium cepa* when exposed to acetaminophen alone or in combination with myrobalan (mean±SEM, n = 10)

S. No.	Concentration	Group of ONION bulbs					
		Gr I Control	GR II acetaminophen experimental	Gr. III acetaminophen + myrobalan	% Inhibition Gr I vs. Gr II	% Inhibition Gr I vs. Gr III	Difference Gr II vs. Gr III
1	Control 0.0	56.91±0.65					
2	15.62 ppm		51.08±0.59[a]	54.69±0.21[bc]	10.40%	3%	7.40%
3	31.25 ppm		48.81±0.27[a]	51.22±0.44[bc]	14.30%	9.98%	4.32%
4	62.50 ppm		44.11±1.71[a]	49.08±0.37[bc]	22.91%	13.58%	9.33%
5	125 ppm		37.98±0.50[a]	42.36±1.42[bc]	33.63%	25.66%	7.97%
6	250 ppm		32.83±1.00[a]	38.02±1.02[bc]	42.12%	33.92%	8.20%
7	500 ppm		27.51±0.66[a]	32.65±0.89[bc]	51.60%	42.62%	8.98%
8	1000 ppm		-	-	-	-	-

Statically significant based on 't' test at 5% level of significance. MRL = in mm.
[a] Control vs. Gr I (acetaminophen exposed)
[b] Control vs. Gr II (acetaminophen + myrabalan exposed)
[c] Gr II vs. Gr III
(-) No root growth at all

sion but each contained myrobalan powder at 0.10 mg/mL level (Group III, acetaminophen plus myrobalan exposed). All solutions were changed every 24 hrs. After 48 hr two onions out of twelve in each series with most poorly growing roots were removed. Same day i.e. after 48 hours distal 2 mm of five roots were cut off from five individual bulbs from each series, and fixed in acetic acid - ethyl alcohol (1:3 v/v) for chromosomal study. Every time fixation was done at a fix time 11:00 a.m.

After 72 hrs total lengh of the 05 root bundles in each series of each onion was measured to record mean root length.

Squashing and Observations of Slides

Root tips were squashed using N-HCl and 2% aceto-carmine stain. Four fields from each slide were observed to cover 50 cells in each i.e. total 200 cells per slide, 3000-4000 cells were observed for each group of onion. Mitotic index was calculated as percentage of dividing cells. Slides were also observed to find out mitotic arrest, chromosomal aberrations, fragments, abnormal orientation, lagging chromosomes and polyploidy etc.

Statistics

Experiments were repeated five times. Students't' test was applied at 5% level of significance.

RESULTS

Mean Root Length

All tested concentrations of acetaminophen (except at 1000 ppm where roots did not grow at all) caused significant inhibition in the growth of roots (Gr. II) in comparison to control (Gr. I). A comparison between Gr. II and Gr. III (acetaminophen + myrobalan) revealed that myrobalan could check acetaminophen induced root growth inhibition at all concentrations (Table 1).

Morphology, Color and Shape of Root Tips

Morphology i.e. color and shape of *Allium cepa* root tips cultivated in all test concentrations of acetaminophen alone (Gr. II) or acetaminophen plus myrobalan (Gr. III) did not reveal any change from controls (Gr. I) (Table 2).

Table 2. Morphology of *Allium cepa* root tips following 72 hrs exposure to acetaminophen alone or in combination with myrobalan.

S. No.	Groups	Morphology i.e. shapes of root tips				Color of root tips		
		Abnormal			Normal	Normal	Abnormal	
		Crochet hooks	Bulb	Broken tips	Straight	White	Pale	Dark brown / black
	Gr - I control	No	No	No	Yes	Yes	No	No
	Gr. II Only acetaminophen solution							
1	15.62 ppm	No	No	No	Yes	Yes	No	No
2	31.25ppm	No	No	No	Yes	Yes	No	No
3	62.50 ppm	No	No	No	Yes	Yes	No	No
4	125 ppm	No	No	No	Yes	Yes	No	No
5	250 ppm	No	No	No	Yes	Yes	No	No
6	500 ppm	No	No	No	Yes	Yes	No	No
7	1000 ppm	-	-	-	-	-	-	-
	Gr III Exp acetaminophen + myrobalan 0.1 mg/mL							
1	15.62 ppm	No	No	No	Yes	Yes	No	No
2	31.25 ppm	No	No	No	Yes	Yes	No	No
3	62.50 ppm	No	No	No	Yes	Yes	No	No
4	125 ppm	No	No	No	Yes	Yes	No	No
5	250 ppm	No	No	No	Yes	Yes	No	No
6	500 ppm	No	No	No	Yes	Yes	No	No
7	1000 ppm	-	-	-	-	-	-	-

(-) No growth at all

Table 3. Mitotic index (MI) of *Allium cepa* root tip cells when exposed to acetaminophen alone or in combination with myrobalan (mean±SEM, n=10)

S. No.	Concentration	Group of ONION bulbs					
		Gr I Control	GR II acetaminophen experimental	Gr. III acetamino-phen + myrobalan	% Inhibition Gr I vs. Gr II	% Inhibition Gr I vs. Gr III	DifferenceGr II vs. Gr III
1	Control 0.0	46.17±1.29					
2	15.62 ppm		39.64±1.25[a]	44.12 ± 1.52[bc]	14.12%	4.44%	9.68%
3	31.25 ppm		30.31±0.62[a]	38.19 ± 1.40[bc]	34.35%	17.28%	17.07%
4	62.50 ppm		24.81±0.60[a]	32.95 ± 0.66[bc]	46.26%	28.63%	17.63%
5	125 ppm		20.22±0.80[a]	29.70 ± 0.70[bc]	56.20%	35.67%	20.53%
6	250 ppm		17.38±0.67[a]	24.67 ± 0.54[bc]	62.35%	46.56%	15.79%
7	500 ppm		12.61±0.54[a]	19.25 ± 0.71[bc]	72.68%	58.30%	14.38%
8	1000 ppm		-	-	-	-	-

Statically significant based on 't' test at 5% level of significance.
[a] Control vs. Gr I (acetaminophen exposed)
[b] Control vs. Gr II (acetaminophen + myrabalan exposed)
[c] Gr II vs. Gr III
(-) No root growth at all

Mitotic Index

In comparison to controls (Gr. I), all test concentrations of acetaminophen (Gr. II) significantly lowered mitotic index i.e. percentage of dividing cells. This mitodepression effect appears dose dependent. Even in the presence of myrobalan (Gr. III) acetaminophen exterted its inhibitory action on mitosis but effect was found significantly less pronounced indicating preventive action of myrobalan against acetaminophen (Table 3).

Abnormal Mitosis

No chromosomal aberrations and abnormal mitosis could be seen in root tip cells of onions in any groups (Table 4).

DISCUSSION

Earlier workers observed cytotoxicity, spindle disturbing effect and chromosomal effects of acetaminophen (paracetamol) in animal, human and plant cells [10-19]. Results of the present study reveal cytotoxicity i.e. mitodepression mitostatic (low mitotic index to no cell growth at all) but no chromosomal effect. This descripency might be due to use of comparatively lower concentrations in the present study of acetaminophen than those used by other workers.

A careful persual of results indicate two findings for discussion, firstly probable mechanism of action of acetaminophen in root tip cells for lowering mitosis and secondly probable protective action of myrobalan.

Acetaminophen can bind irreversibly to DNA, can cause DNA break, can inhibit both replicative DNA synthesis and repair DNA synthesis [1]. It can block DNA replication by inhibiting deoxyribonucleotide (n NTP) synthesis in several mammalian cell types [18]. Also, acetaminophan is topoisomerase II poison [20]. It

Table 4. Cytological effects of different concentrations of acetaminophen alone or in combination with myrobalan suspension in tap water.

S. No.	Groups	Treatments	Number of counted cells Metaphase + Anaphase	Microscopic effects in percent									
				Normal Metaphase	Normal Anaphase	Sticky chromosomes	C-mitosis	Vagrant (lagging) chromosome	Multipolar Anaphases	Bridges	Fragments	MNC	Polykaryotes
1	Gr. I	Control (tap water)	1000	+	+	-	-	-	-	-	-	-	-
2	Gr. II	15.75 ppm Aceta.	1000	+	+	-	-	-	-	-	-	-	-
	Gr. III	15.75 ppm Aceta. + myrobalan	1000	+	+	-	-	-	-	-	-	-	-
3	Gr. II	31.25 ppm Aceta.	1000	+	+	-	-	-	-	-	-	-	-
	Gr. III	31.25 ppm Aceta. + myrobalan	1000	+	+	-	-	-	-	-	-	-	-
4	Gr. II	62.50 ppm Aceta.	1000	+	+	-	-	-	-	-	-	-	-
	Gr. III	62.50 ppm Aceta. + myrobalan	1000	+	+	-	-	-	-	-	-	-	-
5	Gr. II	125 ppm Aceta.	1000	+	+	-	-	-	-	-	-	-	-
	Gr. III	125 ppm Aceta. + myrobalan	1000	+	+	-	-	-	-	-	-	-	-
6	Gr. II	250 ppm Aceta.	1000	+	+	-	-	-	-	-	-	-	-
	Gr. III	250 ppm Aceta. + myrobalan	1000	+	+	-	-	-	-	-	-	-	-
7	Gr. II	500 ppm Aceta.	1000	+	+	-	-	-	-	-	-	-	-
	Gr. III	500 ppm Aceta. + myrobalan	1000	+	+	-	-	-	-	-	-	-	-

+ = Present, - = Absent, Aceta. = Acetaminophen, MNC = Micronucleate cells

can increase membrane permeability, can deplete cellular glutathione level and can cause lipid peroxidation and can bind with proteins [10, 21]. During late G1 restriction point gate opens in the presence of complex molecules at promotors of essential cell cycle genes and unrepaired DNA does not allow cells to go beyond G1 stage [22]. It appears that acetaminophen might have interfered in some complex manner at DNA and/or gene level resulting in mitodepression- mitostatic condition in *Allium* root tip cells.

Cytotoxicity of N-acetyl-p-benzoquinone imine (NAPQI) a common intermediate of paracetamol in cultured rat hepatocytes could be prevented fully by the addition of N-acetylcysteine, GSH or ascorbate during the exposure period [10]. Cytotoxicity of acetaminophen was prevented by curcumin, a natural constituent of *Curcuma longa* in rat hepatocytes [23]. Curcumin protected against paracetamol induced lipid peroxidation, increased cellular GSH and lowered LDH leakage. Crude extract of seaweed, *Sargussum polycysturn* (brown alga) could afford protection against acetaminophan induced lipid peroxidation through their free radical scavenging property [24]. It is likely that acetamonophen induced peroxidative damage could result in the decline in mitosis but if myrobalan possesses antioxidant properties it can reduce toxic effects of acetaminophen. Infact myrobalan has been shown to exert antioxidant and free radical scavenging activities [4, 25-26].

Unrepaired DNA does not allow cells to go beyond G1 stage [22] and acetaminophen may damage DNA [2]. It is likely that acetaminophen induced DNA damage could have been remedied by myrobalan because it is known to exert antimutagenic activity in bacteria against direct acting mutagens sodium azide and 4-nitro-o-phenylene diamine, [27] later this property was attributed to tannins [28].

The *Allium* root cells also posses certain enzymes, the mixed function oxidases like that of mammalian hepatocytes that can activate promutagens to mutagens [9]. In case of metal toxicity detoxification in root cells takes place in the cytoplasm and cell wall within 12-24 hr. which was held responsible for the mitotic activity at low concentration exposures [29]. Similar action towards acetaminophen at low concentrations in the present case can not be ruled out.

Individual plant components like sulfhydryl and flavonoid compounds, gallic acid, ellagic acid, mucic acid, citric acid, reducing sugars and tannins can modulate effects of many genotoxicants [30], myrobalan possesses many of such compounds [31], which can be held responsible for reducing cytotoxic effects of acetaminophen in *Allium cepa* root tip cells in the present case.

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