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Can Genotoxic Effect be Model Dependent in *Allium* Test?-An Evidence.

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Abstract

Genotoxicity of peracetic acid (PAA) has been assessed in two models (protocols) of *Allium cepa* conducting two sets of experiments to know whether the results would be model dependent. One experiment was set as per Fiskesjo's model in which *Allium cepa* bulbs were grown in five concentrations of peracetic acid (0.039, 0.078, 0.156, 0.312 and 0.625 ppm) in tap water. Another experiment was set as per Rank and Nielson's model in which *Allium cepa* bulbs were first grown in tap water for 24 hours and were then further grown in the same concentrations of peracetic acid as in earlier model. Genotoxic effects of peracetic acid were assessed in both models using usual parameters i.e. shape, colour and length of root tips, mitotic index, chromosomal aberrations and cell death.

Magnitude of effect differed significantly in both models. More severe genotoxic effects could be seen in Fiskesjo's model. It is suggested that root primordial cells were in G0 state in Fiskesjo's model, which presumably lacked their defense system, hence were more prone to peracetic acid toxicity. Mitotically dividing root cells in Rank and Nielsen's model were equipped with antioxidant system and were more resistant to peracetic acid.

Keywords: *Allium* test; Root primordial cells; G0; Genotoxicity; peracetic acid

1. Introduction

During recent years two modified models of *Allium* assay are available for testing genotoxicity of substances. First model was suggested by Fiskesjo (1995) in which bulbs with dormant root primordial cells in G0 state were exposed to various concentrations of test substances. In other words, in this model G0-G1 transition was affected followed by other stages of cell cycle. Second model was proposed by Rank and Nielson (1993) in which *Allium* bulbs were initially grown in tap water for 24 hours then these bulbs with sprouting roots are grown in different concentrations of test substances. In this model all stages of cell cycle except G0 were exposed to test substances. On theoretical ground, it appeared that results may be model dependent hence present study was undertaken to test genotoxicity of a water disinfectant peracetic acid (PAA) in both the models.

2. Materials and Methods

2.1. Experiment-I Based on Fiskesjo's model (1995).

Allium cepa bulbs: Dry healthy onions (2n=16) of almost equal size i.e. 1.50 to 2.00 cm in diameter were obtained from local market.

Test chemical: Peracetic acid ($C_2H_4O_3$, MW 76.05) procured from National Chemicals, Vadodara, Gujrat, (India) was used. Different concentrations of PAA were prepared using tap water. Physicochemical analysis of tap water was done by standard procedures (APHA, 1998).

Experimental design: Outer pink brown scales and some of the brownish bottom plates of Allium cepa bulbs were removed carefully leaving the root primordia intact. For each concentration of peracetic acid, 12 glass tubes (330 ml capacity) were used. The tubes were filled with five concentrations (0.039 ppm, 0.078 ppm, 0.156 ppm, 0.312 ppm and 0.625ppm) of peracetic acid in tap water (Gr II). 12 tubes were filled with only pure tap water for controls (Gr I). Each descaled onion was placed on the top of each tube with root primordia downward in the liquid. Every day (after 24 hours) peracetic acid solutions and pure water were changed in both groups of tubes. After 48 hours two onions in each series with most poorly growing roots were removed and distal 2mm of five longest root tips were cut off from 05 individual bulbs and fixed in aceto-alcohol (1:3 v/v) for chromosomal study. These bulbs were discarded. Every time fixation was done at 11.00 AM. After 72 hours length of 05 longest roots in each series of each bulb was measured using a ruler. Mean length of roots for each bulb of each series was calculated. W. M. of root tips were also made to record their morphology.

2.2. Experiment-II Rank and Nielson's model (1993).

Descaled bulbs were grown in 70 glass tubes filled with pure tap water for 24 hour. 10 bulbs with poor root growth were removed. Remaining 60 bulbs were divided in to two groups. Group I consisted of 10 bulbs for further growth in pure water served as controls. Group II consisted of 50 bulbs for further growth, 10 in each of five same concentrations of peracetic acid as used in experiment I. Both groups of *Allium cepa* were allowed to grow for next 48 hours. Exposure time 48 hours equals to two cell cycles (Kihlman, 1971). After 72 hours five longest root tips were fixed in acetoalcohol for scoring chromosomal aberrations after measuring their length for mean root length. W.M. were also prepared for morphology.

2.2.1. Storing-squashing-staining

24 hour fixed tips were stored in 70% alcohol. Tips were warmed in N-HCl and then stained and squashed in ready to use stain carmine obtained from CDH Pvt. Ltd. New Delhi. Four fields were observed per slide to cover about 200 cells. Total 2000 cells of 10 slides were

Table 1. Physico-chemical properties of Tap water (Aug-Dec 2008).

01	Turbidity	90 NTU
02	pH value	7.2
03	Colour	Colourless
04	Total Alkalinity	90 mg/L
05	Carbonates	8 mg/L
06	Bicarbonates	140 mg/L
07	Hardness	162 mg/L
08	Chloride	52 mg/L
09	BOD (5 days at 20°C)	5 mg/L
10	COD	9 mg/L
11	Fluoride	0.49 mg/L
12	Nitrite	Nil
13	Dissolved oxygen	5.2 mg/L
14	Calcium	120 mg/L

Table. 2 Mean Root Length of *Allium cepa**

S.No.	Groups and Concentrations of PAA	E-1 after 72 hr of cultivation in PAA	E-2 at 72 hr when 24 grown bulbs were cultivated in PAA	% change from controls in E-1	% change from controls in E-2	Difference between % changes in E-1 & E-2
1	0.00 ppm C	5.86±0.15	5.86±0.15	Control value is taken 100%	Control value is taken 100%	
2	0.039 ppm E	4.60±0.18[a]	5.78±0.20[b]	21.50%	1.36%	20.14 S
3	0.078 ppm E	4.50±0.13[a]	5.22±0.27[ab]	23.20%	10.92%	12.28 S
4	0.156 ppm E	4.40±0.16[a]	5.13±0.31[ab]	24.91%	12.45%	12.46 S
5	0.312 ppm E	3.09±0.14[a]	4.11±0.32[ab]	47.26%	29.86%	17.40 S
6	0.625ppm E	0.54±0.05[a]	2.45±0.34[ab]	90.78%	55.19%	32.59 S

** Fiskesjo's model (E-1) and in Rank and Nielson's model (E-2). (Mean ± SEM, n=30, p=2.04)*

"a" = stastically significant based on 't' test at 5% level of significance when control vs all experimental groups in each experement were compared. "b" = When groups between E-1 and E-2 were compared. S=Significant, C=Control, E=Experimental

observed for each concentration in both experiments. Mitotic index was calculated as percentage of dividing cells. Slides were also observed to find out mitotic arrest, chromosome fragments, abnormal orientation, micronuclei, polyploidy, cell death etc. Experiments were done thrice;'t' test was applied at 5% level of significance.

3. Results

Result of present experiments have shown the following facts:

Physicochemical analyses of tap water: Details are shown in Table-1.

Mean Root Length (MRL, Table-2): When *Allium cepa* bulbs were grown with root primordial cells in G0 i.e. in Fiskesjo's model, growth of roots was found to be significantly inhibited at all the concentrations of peracetic acid. When sprouted roots i.e. growing roots (Rank and Nielson's model) were further grown in peracetic acid solutions significant inhibition also occured at last four concentrations. More severe inhibition was found in Fiskesjo's model.

Mitotic Index (MI, Table-3): *Allium cepa* bulb with root primordial cell in G0 i.e.in Fiskesjo's model, their mitotic index i.e. percentage cells in mitosis declined at all concentrations progressively. When sprouted roots (in Rank and Nielson's model) were grown in percetic acid solutions, the mitotic index declined at all concentrations except at lowest concentration of PAA. More severe effect was found in Fiskesjo's model.

Morphology (shape-colour of tips, Table-4): Only at highest concentration of PAA in Fiskesjo's model, bulb like formation occurred at tips. No alteration could be seen in another i.e. Rank and Nielson's model.

Cytological effects (aberrations, Table-5): These were seen only at last two higher concentration of PAA in both models. More severe effects were seen of Fiskesjo's model where even cell death could be observed at highest concentration. No aberrations were seen in controls.

Inference: Results indicate that growing cells in Rank and Nielson's model, where G0 cells were not present were more resistant towards genotoxic effects of PAA.

4. Discussion

The results of the present experiments clearly show an important fact that root cells of *Allium cepa* gave different responses to peracetic acid in two models.

In Fiskesjo's model *Allium cepa* bulbs had their root primordial cells in G0 state. Ascorbate free radiacals stimulate onset of cell proliferation i.e. quiescency-proliferative shift (G0-G1-S-M) in onion root primordia

Table 3. Mitotic Index of *Allium cepa* root tip cells*

S.No.	Groups and Concentrations of PAA		E-1 after 72 hr of cultivation in PAA	E-2 at 72 hr when 24 grown bulbs were cultivated in PAA	% change from controls in E-1	% change from controls in E-2	Difference between % changes in E-1 & E-2
1	0.00 ppm	C	43.90±0.41	43.90±0.41	Control value is taken 100%	Control value is taken 100%	
2	0.039 ppm	E	38.72±0.26^{a}	43.20±0.30^{b}	11.79%	1.59%	10.2 S
3	0.078 ppm	E	34.47±0.66^{a}	41.69±0.31^{ab}	21.48%	5.03%	16.45 S
4	0.156 ppm	E	32.00±0.73^{a}	39.81±0.36^{ab}	27.10%	9.31%	17.79 S
5	0.312 ppm	E	26.84±1.80^{a}	37.36±0.43^{ab}	38.86%	14.89%	23.97 S
6	0.625 ppm	E	8.91±0.41^{a}	27.82±0.45^{ab}	79.70%	36.62%	43.08 S

** Fiskesjo's model (E-1) and in Rank and Nielson's model (E-2). (Mean ± SEM, n=30, p=2.04)*

"a" = stastically significant based on 't' test at 5% level of significance when control vs all experimental groups were compared. "b" = when groups between E -1 and E- 2 were compared. S=Significant, C=Control, E=Experimental

Table 4. Morphology of Allium cepa root tips*

S. No.	Groups and Concentrations of eracetic Acid	Morphology						
		Shape of root tips				Colour of root tips		
		Normal	Abnormal			Normal	Abnormal	
		Straight	Crochet hooks	Bulbs	Broken tip	White	Pale yellow	Dark yellow
1.	0.00 ppm	Yes	No	No	No	Yes	No	No
	C-1							
	C-2	Yes	No	No	No	Yes	No	No
2.	0.039 ppm	Yes	No	No	No	Yes	No	No
	E-1							
	E-2	Yes	No	No	No	Yes	No	No
3.	0.078 ppm	Yes	No	No	No	Yes	No	No
	E-1							
	E- 2	Yes	No	No	No	Yes	No	No
4.	0.156ppm	Yes	No	No	No	Yes	No	No
	E-1							
	E-2	Yes	No	No	No	Yes	No	No
5.	0.312ppm	Yes	No	No	No	Yes	No	No
	E-1							
	E-2	Yes	No	No	No	Yes	No	No
6.	0.625ppm	**NO**	No	**YES**	No	Yes	No	No
	E-1							
	E-2	Yes	No	No	No	Yes	No	No

* *72 hours cultivation in peracetic acid (E-1) and at 72 hours when 24 hours grown roots were further grown for 48 hours in peracetic acid (E-2)*
C-1 & C-2 = Control, E-1 & E-2 = Experimental

Table 5. Cytological effects of peracetic acid in *Allium cepa* root tip cell *

S. No.	Treatment	% Microscopic effects (2000 cells/group)									
		At Metaphase		At Anaphase					Other observation		
		N	SC	N	LC	MPA	CB	F	MNC	PKC	CD
in	0.00 ppm										
	C-1	+	-	+	-	-	-	-	-	-	-
	C-2	+	-	+	-	-	-	-	-	-	-
2.	0.039ppm										
	E-1	+	-	+	-	-	-	-	-	-	-
	E- 2	+	-	+	-	-	-	-	-	-	-
3.	0.078ppm										
	E-1	+	-	+	-	-	-	-	-	-	-
	E-2	+	-	+	-	-	-	-	-	-	-
4.	0.156ppm										
	E-1	+	-	+	-	-	-	-	-	-	-
	E-2	+	-	+	-	-	-	-	-	-	-
5.	0.312ppm										
	E-1	54.60±0.26%	45.40±0.38%	49.10±0.30%	-	-	50.90±0.45%	-	-	-	-
	E-2	74.10±0.40*%	25.90±0.65*%	79.60±0.54*%	-	-	20.40±0.35*%	-	-	-	-
6.	0.625ppm										
	E-1	38.50±0.58%	61.50±0.28%	21.50±0.30%	-	-	78.50±0.62%	-	-	-	+
	E-2	59.40±0.31*%	40.60±0.42*%	70.30±0.46*%	-	-	29.70±0.34*%	-	-	-	-

* *Feskisjo's model (E-1) and in Rank and Nielson's model (E-2) (n=30, p=2.04)*
(+) = observed, present LC = Lagging chromosome MNC = Micronucleated cells '*' = E-1 vs E-2, significant
(-) = Not seen, absent MPA = Multipolar anaphase PKC = Polykaryocytes
N = Normal CB = Chromosomal bridge CD = Cell death
SC = Scattered F = Fragment C-1 & C-2 = Control

(Liso *et al*., 1988). Ascorbate controls activity of propyl hydroxylase, an enzyme inhibition of which results in abnormal cells and in delayed cell cycle progression in onion roots (Detullio *et al*., 1999). Probably lycorine like activity of peracetic acid i.e. initial inhibition in the biosynthesis of ascorbic acid among G0-G1 transition cells in Fiskesjo's model can prevent cell cycle (Arrigoni *et al*., 1975). Peracetic acid has been found to damage DNA directly (Buschini *et al*., 2004) hence cell death and severe inhibition of mitosis in Fiskesjo's model at higher concentrations could have been due to such direct genotoxic effect of PAA on root primordial cells on onions. PAA-induced damage to DNA and RNA polymerase can inhibit cell proliferation as has been observed following hydroquinoline exposure to *Allium cepa* (Ferrero and Torre, 1986). Possibility of PAA-induced initial disturbances in nutrient sensing like phenomenon by quiescent center of root apical meristem can not be ruled out as cells need nutrients to divide (Dennis and Nigel, 2006).

Antimicrobial activity of PAA was considered similar to other peroxides and oxidising agents (Block, 1991). The release of active oxygen is basis of its disinfectant activity (Liberti and Notarnicola, 1999). It has been suggested that oxidation of sulfhydryl roups in proteins and enzymes cause maembrane damage (Kitis, 2004).

PAA has been suggested to cause oxidative damage to DNA (Tutmi *et al*., 1973) PAA was also shown to inactivate catalase,which decompose hydrogen peroxide formed in the oxidative reactions. The activities of antioxidant enzymes-catalase, superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase (Acharya *et al*., 2008) may provide protection among growing root cells.

It appears that already growing root cells in Rank and Nielson's model were fully equipped the antioxidant machinery, which could have reduced genotoxicity of PAA. In root primordial cells in G0 state could not synthesiz their defense component, hence were found much more prone to PAA toxicity.

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