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ASSESSMENT OF CYTOTOXIC AND CHROMOTOXIC EFFECTS OF AMMONIUM ACETATE BY EMPLOYING PLANT BIOASSAY SYSTEM

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ABSTRACT
In the present study cytotoxic and chromotoxic effects of food preservative ammonium acetate was evaluated in the root meristem cells of *Vicia faba*. Cytotoxicity was evaluated by monitoring the effect of ammonium acetate on the cell division in actively dividing root tip cells while chromotoxicity was assessed by recording the frequency of aberrant cells and types of chromosomal aberrations in the treated roots. Roots were treated at room temperature in dark for 6, 12, 18 and 24h with 1, 2.5. 5 and 7.5 g/l solutions of ammonium acetate separately. The doses selected for evaluation were below LD50 dose of the compound. Results obtained indicated a mild cytotoxic and chromotoxic effects of the compound in the dose range tested.

KEYWORDS: cytotoxicity, chromotoxicity, ammonium acetate, *Vicia faba*.

INTRODUCTION
Food additives are synthetic chemicals substances which are added to food to preserve flavor or enhance its taste and appearance. With the increasing use of processed foods there has been a large scale increase in the use of food additives of varying levels of safety. There has been significant controversy associated with the risks and benefits of food additives. Some artificial food additives have been linked with cancer, digestive problems, neurological conditions, heart disease or obesity. Many food additives have been tested for their genotoxic effects in different test systems with positive results.[1-9] Food preservatives sodium benzoate and sodium sulfite was found to inhibit DNA synthesis and induce chromosomal aberrations in *Vicia faba* root meristems.[10] Sorbic acid and its potassium salt induced chromosome aberration and sister chromatid exchange in Chinese hamster cells.[11] Sodium and potassium sorbate has induced sister chromatid exchange and 6-thioguanine resistant mutation in Chinese hamster cells in vitro[11-12] Sodium nitrite induced chromosomal aberrations in Chinese hamster fibroblast cells and was mutagenic in the *Salmonella* microsome test system[13-14] Rencuzogullari et al.[15-16] have reported decrease in mitotic index in *Allium cepa* and human lymphocytes, due to the effect of sodium metabisulphite. Sodium phosphate (E 339) induced significant reduction in the frequency of dividing cells and induced chromosomal aberrations in dose dependent manner in pot Marigold root tips.[17] Micronuclei formation was recorded by many investigators following treatment with different food additives.[9. 18-22]

Ammonium acetate is a useful preservative (food additive) for processed foods, particularly meats. It is produced commercially by oxidation of acetaldehyde. It acts as a preservative used to prevent spoilage of food by inhibiting or preventing the growth of bacteria, fungi and other microorganisms. Also an acidity regulator used to change or maintain a pH which can be either acidic or alkaline. It is also used as a flavour enhancer to impart a sour or tangy taste. It is used in condiments, in the pickling of vegetables, bottled sauces, chutneys, snack foods, bread, cakes, cheese, crisps, dressings, mustards, seasonings, bakery items, ciders, soups and broths, preserved eggs, pre-cooked pasta and noodle products. Toxic effects of ammonium acetate has not been yet reported, however, induction of micronuclei formation in the treated root tip cells of *Vicia faba* has been reported earlier.[9] In the present study we report the cytotoxic and chromosome damaging potential of sodium acetate in the root meristem of *Vicia faba*.

MATERIALS AND METHODS
Vicia faba Linn. (2n = 12), commonly known as faba bean has been used as experimental material. Ammonium acetate (E 264, mol. formula CH_3COONH_4, CAS Registry No. 631-61-8, Central Drug House, Mumbai Cat. No. 010013) was used as test compound for evaluation. Solution of test compounds of five different strengths were prepared in distilled water.

Dosage of the test compound for genotoxicity study was determined by determining the LD50 concentration. LD50 was determined by exposing 100 healthy seeds, presoaked in distilled water for 24h, to a range of concentration of Ammonium acetate (1 g/l - 9 g/l) for 12 h. After treatment seeds were thoroughly washed in running tap water and left between folds of moist filter papers (Whatman No.1) for germination. Parallel control was set by soaking the seeds in distilled water for the same period. Number of seeds germinated in each treatment was counted and germination percentage was determined: LD50 value was determined by plotting the mean germination percentage of three replicated treatment. 5 doses of test compound below the LD 50 dose were selected for further study.

Healthy and equal sized *Vicia faba* seeds were selected. Dry seeds were soaked for 18 h in distilled water and allowed to germinate between two layers of moist cotton at room temperature. When radicals appeared, the testa was removed to avoid fungal contamination. Fifty seedlings with newly emerged roots 1.00–2.00 cm in length were selected for treatment. 20 seedlings were selected for each treatment and transferred into container (10 cm tall, 30–20 cm diameter) containing one of the solutions viz. 1 g/l, 2.5 g/l, 5 g/l, 7.5 g/l of ammonium acetate and treated for 6, 12, 18 and 24 hours. The negative and positive control groups were suspended over the distilled water and solution containing 0.2% EMS for the same periods respectively. The solutions of test compounds were put in containers covered with aluminum paper with holes which permitted the roots to be exposed. The experiments were set in the dark. Twenty five primary roots from each treatment group were harvested based on uniformity of size after the end of treatment periods. The roots were rinsed in distilled water to remove traces of adhering treatment solution. The roots were fixed in Carnoy's fixative (3: I-ethanol: glacial acetic acid) for 24 hours followed by washing and storage in 70% ethanol. The solutions were freshly prepared before use. For slide preparation and microscopic examination, the root tips were hydrolyzed in 1 N HCl at 60°C for 10 min. After hydrolysis the roots were stained in 1.5% acetocarmine. 1 mm of the mitotic zone from well-stained root tips were cut on a clean slide and squashed under a cover glass in a drop of acetic acid (45%). The slides were scored under a light microscope for number of dividing and non-dividing cells and number of cells with different types of chromosomal aberrations.

Mitotic Index (MI%) was determined by counting the number of mitotic cells among the total amount of scored cells per seedlings. 1000 cells from each root tip and a total of 5000 cells were screened for dividing and non-dividing cells. Mean MI% in control, positive control and treated root tips was determined. Twenty five microscopic fields in each slide, selected randomly, were screened carefully and the total number of abnormal cells was recorded. The mean percentage of abnormal cells was calculated for each treatment. Different stages of mitotic division were examined and different types of anomalies were recorded and statistically analyzed separately for each treatment.

Data were statistically analysed by Graph Instat 3 computer based statistical software. Significance of differences between control and treatments were evaluated by Student's t-test. Analysis of variance (ANOVA) was done to test significant differences between concentrations of test compounds and period of treatments.

RESULTS

Results obtained after treatment of roots with 1.0, 2.5, 5.0 and 7.50 g/l solutions of ammonium acetate for 6, 12, 18 and 24 h are shown in Tables 1-4. Increase in concentration of ammonium acetate as well as durations of treatment led to decrease in mitotic activity in the root meristem of treated roots as evident by reduction in mitotic indices. When mean of mitotic indices recorded in the ammonium acetate treated root tips were compared with that of control to test significance of difference, it was observed that 1.0 g/l treatment of ammonium acetate resulted in non-significant reduction in mitotic index at all the treatment periods. 2.5, 5.0 and 7.5 g/l of treatment induced significant reduction in mitotic indices at all the durations of treatment except 6 h. The reduction in MI (%) in treated roots ranged from 2.4% at lowest concentration and shortest treatment duration to 54% at highest concentration and longest treatment duration when compared to control roots. Statistically highly significant variation both between treatment durations ($p<0.01$) and concentrations ($p<0.001$) was recorded in two way ANOVA test (Table 2). Inhibition of mitotic activity in the root meristem of plants is often used for tracing cytotoxicity of chemical substances. The lowering of mitotic index has been attributed to the inhibition of DNA synthesis.[28,29] at S-phase or a blocking in the G2-phase of the cell cycle thus preventing the cell from entering mitosis.[30] Inhibition of the enzyme DNA polymerase, which is necessary for the synthesis of DNA as well as other enzymes directly involved with the formation and assembly or orientation of spindle fibers are also reasons for antimitotic effect of the chemical compounds. Any of the reasons stated above might be the cause of reduction of mitotic index, as observed in the present study.

The chromosome damaging ability of ammonium acetate was measured in terms of number of abnormally dividing cells and percent abnormal cells and the data collected are shown in Table 3. EMS (positive control) treatment resulted concentration and duration of treatment related highly significant increase in percentage of abnormal cells. Treatment of roots with different concentrations of ammonium acetate for 6, 12, 18 and 24 h resulted in concentration and duration of treatment related increase in the aberration rate as compared to negative control. However, statistically significant increase was recorded

only after 18h (5 and 7.5 mg/l) and 24h (2.5, 5 and 7.5 mg/l) of treatment. Microscopic examination of the squashes of treated root tips showed the presence of fragments of chromosomes, disturbed metaphase, disturbed ana-telophase and bridge at anaphase and telophase. Disturbed metaphase and disturbed ana-telophase were most common types of mitotic abnormalities recorded. Chromosome and chromatid bridges were observed only in roots treated with 7.5 mg/l for 24h. Chromosome fragments were also found in very low frequency (Table-4).

Table 1 Effect of Ammonium acetate treatments on the cell division in the root meristem of *Vicia faba L*

Concentration (gm/l)	Period of treatment	Number of cells observed	Number of actively dividing cells	Mitotic Index (MI) % (Mean ± SE)
Control		1496	376	25.13 ± 2.56
EMS 0.2%	6 h	1322	158	11.95 ± 1.15**
1 g/L		1378	325	23.58 ± 2.45
2.5 g/L		1396	316	22.63 ± 2.30
5 g/L		1402	302	21.54 ± 2.45
7.5 g/L		1445	282	19.51 ± 1.90*
EMS 0.2%	12 h	1304	134	10.28 ± 1.44**
1 g/L		1476	321	21.74 ± 2.25
2.5 g/L		1378	256	18.57 ± 1.40**
5 g/L		1372	231	16.83 ± 1.80**
7.5 g/L		1356	202	14.89 ± 1.65**
EMS 0.2%	18 h	1356	124	9.14 ± 1.10**
1 g/L		1366	286	20.93 ± 1.86
2.5 g/L		1342	239	17.80 ± 1.82**
5 g/L		1422	222	15.61 ± 1.20**
7.5 g/L		1387	202	14.56 ± 1.55**
EMS 0.2%	24 h	1287	102	7.93 ± 1.30**
1 g/L		1346	276	20.50 ± 1.92
2.5 g/L		1365	227	16.63 ± 1.75**
5 g/L		1340	207	15.44 ± 1.90**
7.5 g/L		1356	185	13.64 ± 1.44**

*, ** Statistically significant at p<0.05 and 0.01 respectively in Student's t-test
1000 cells per root tip and total 5000 cells have been scored in each case.

Table 2. Two-way analysis of variance (ANOVA) of mitotic index showing significant variation between treatments as well as periods of treatment

Sources of variation	df	Mean Square	F-value
Between periods	2	5.678	8.36
Between treatment	5	14.248	20.98
Residual	10	0.679	

*** significant at p<0.001.

Table 3: Induction of mitoic abnormality by ammonium acetate treatments on the cell division in the root meristem of *Vicia faba L.*

Concentration (gm/l)	Period of treatment	Number of cells observed	Number of actively dividing cells	Number of abnormally dividing cells	% Abnormal cells (% that of observed cells)
Control		1496	376	3	0.15
EMS	6 h	1322	158	28	2.12***
1 g/L		1378	325	3	0.15
2.5 g/L		1396	316	4	0.14
5 g/L		1402	302	5	0.14
7.5 g/L		1445	282	6	0.28
EMS	12 h	1304	134	34	2.61***
1 g/L		1476	321	4	0.20
2.5 g/L		1378	256	5	0.22
5 g/L		1372	231	5	0.29
7.5 g/L		1356	202	6	0.44
EMS	18 h	1356	124	39	2.88***

1 g/L		1366	286	5	0.29
2.5 g/L		1342	239	6	0.37
5 g/L		1422	222	7	0.49*
7.5 g/L		1387	202	9	0.65*
EMS		1287	102	34	2.64***
1 g/L		1346	276	6	0.37
2.5 g/L	24 h	1365	227	8	0.59*
5 g/L		1340	207	10	0.75**
7.5 g/L		1356	185	13	0.96**

* and ** differ significantly from the control in Student's t-test at p<0.05 and 0.01 respectively.

Table 4 Types of chromosomal aberration and their numbers recorded after treatment of roots of Vicia faba with different concentrations of ammonium acetate for different periods.

Period of treatment	Dose	Total cells observed	No. of Abnormal cells	Types and Number of Aberrant cells			
				Fg	DM	DA+T	Br
6 h treatment	Control	1496	3	0 (0.000)	2 (0.133)	1 (0.066)	0 (0.000)
	EMS	1322	28	5 (0.378)	3 (0.226)	5 (0.378)	8 (0.604)
	1 g/L	1378	2	0 (0.00)	2 (0.145)	0 (0.00)	0 (0.00)
	2.5 g/L	1396	2	0 (0.00)	2 (0.0143)	0 (0.00)	0 (0.00)
	5 g/L	1402	2	0 (0.00)	2 (0.207)	0 (0.00)	0 (0.00)
	7.5 g/L	1445	4	1 (0.069)	3 (0.207)	0 (0.00)	0 (0.00)
12 h treatment	EMS	1304	34	3 (0.230)	2 (0.153)	6 (0.459)	8 (0.612)
	1 g/L	1476	3	0 (0.00)	3 (0.203)	0 (0.00)	0 (0.00)
	2.5 g/L	1378	3	0 (0.00)	3 (0.218)	0 (0.00)	0 (0.00)
	5 g/L	1372	4	1 (0.072)	3 (0.218)	0 (0.00)	0 (0.00)
	7.5 g/L	1356	6	2 (0.147)	4 (0.294)	0 (0.00)	0 (0.00)
18 h treatment	EMS	1356	39	4(0.294)	3 (0.221)	7 (0.515)	8 (0.589)
	1 g/L	1366	4	0 (0.00)	3 (0.219)	1 (0.073)	0 (0.00)
	2.5 g/L	1342	5	0 (0.00)	3 (0.223)	3 (0.223)	0 (0.00)
	5 g/L	1422	7	1 (0.070)	2 (0.140)	4 (0.280)	0 (0.00)
	7.5 g/L	1387	9	2 (0.144)	2 (0.144)	4 (0.288)	1 (0.072)
24 h treatment	EMS	1287	34	5(0.388)	6 (0.466)	6 (0.466)	8 (0.626)
	1 g/L	1346	5	1 (0.074)	3 (0.222)	1 (0.074)	0 (0.00)
	2.5 g/L	1365	6	2 (0.146)	2 (0.146)	2 (0.146)	0 (0.00)
	5 g/L	1340	9	2 (0.148)	3 (0.223)	2 (0.148)	2 (0.148)
	7.5 g/L	1356	11	2 (0.147)	4 (0.294)	2 (0.147)	3 (0.213)

EMS 0.2% positive control.

Fg. Fragments, DM. Disturbed metaphase, DA+T Disturbed anaphase +Telophase, Br Bridge at anaphase and Telophase.

DISCUSSION

Inhibition of mitotic activity in the root meristem of plants is often used for tracing cytotoxicity of chemical substances. The lowering of mitotic index has been attributed to the inhibition of DNA synthesis[23,24] at S-phase or blocking in the G2-phase of the cell cycle thus preventing the cell from entering mitosis.[25] Inhibition of the enzyme DNA polymerase, which is necessary for the synthesis of DNA as well as other enzymes directly involved with the formation and assembly or orientation of spindle fibers are also reasons for antimitotic effect of the chemical compounds. Any of the reasons stated above might be the cause of reduction of mitotic index, as observed in the present study.

In the ammonium acetate treated root tip cells mitotic abnormality such as disturbed metaphase and anaphase were commonly observed. This indicates that the compound mainly acts as spindle poison. Presence of chromosome fragments (although not frequently) indicate mild clastogenic effect.

CONCLUSION

The decrease in mitotic index and increase in the frequency of chromosomal aberrations following ammonium acetate treatments in root tip cells of *Vicia faba*, indicate mild cytotoxic and chromotoxic effects of the compound in the dose range tested. Ammonium acetate is the ammonium salt of acetic acid. In aqueous solutions ammonium acetate dissociate into the acetate anion (CH_3COO^-) and the ammonium cation (NH_4^+). The acetate anion and/or ammonium cation thus generated might have caused disturbances in the assembly of spindle apparatus and induced mild clastogenic effect.

REFERENCES

1. Nagao MM, Honda Y, Seino T, Yahagi T Kawachi. Mutagenicities of protein pyrolates. Cancer Let, 1977; 2: 335-339.
2. Ishidate M. Jr, Odashima S. 1977. Chromosome test with 134 compounds on Chinese hamster cells in vitro-a screening for chemical carcinogens. Mutat Res. 1977; 48: 337-353.
3. Matsuoka A, Hayashi M, Ishidate Jr. Chromosomal aberration test on 29 chemicals combined with S9 mix in vitro. Mutat Res, 1979; 66: 277-290.
4. Luca D, Luca V, Cotor F, Raileanu L. In vivo and in vitro cytogenetic damage induced by sodium nitrite. Mutat Res. 1987; 189: 333-339.
5. Mukherjee A, Giri AK, Talukder G, Sharma A. 1988. Sister chromatid exchanges and micronuclei formations induced by sorbic acid and sorbic acid-nitrite in vivo in mice. Toxicol. Let, 1988; 42: 47-53.
6. Kumar Palani L, Paneerselvam N. Cytogenetic studies of food preservative in *Allium cepa* root meristem cells. Medicine Biology, 2007; 14: 60-63.
7. Pandey R M, Santhosh U. Impact of food additives on mitotic chromosomes of *Vicia faba* L. Caryologia, 2007; 60: 309-314.
8. Turkoglu Sifa, Evaluation of genotoxic effects of Sodium propionate, Calcium propionate and Potassium propionate on the root meristem cells of *Allium cepa*. Food and Chemical Toxicology, 2008; 46: 2035-2041.
9. Kumari Nishi, Abha, Jha Anand M. Genotoxicity testing of food additives by employing *Vicia* MN assay. Journal of Phytology, 2012; 4(5): 42-45.
10. Njagi G D, Gopalan HN. Cytogenetic effects of the food preservatives-sodium benzoate and sodium sulphite on *Vicia faba* root meristems. Mutat Res, 1982; 102: 213-219.
11. Hasegawa MM, Nishi Y, Ohkawa Y, Inui N. 1984. Effects of sorbic acid and its salts on chromosome aberrations, sister chromatid exchanges and gene mutations in cultured Chinese hamster cells. Food. Chem. Toxicol., 1984; 22: 501-507.
12. Abe S, Sasaki M. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst, 1977; 58: 1635-1643.
13. Ishidate M. Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A. Primary mutagenicity screening of food additives currently used in Japan. Food. Chem. Toxicol, 1984; 22: 623-636.
14. AkÝn A, SŸmer S. The mutagenic effects of sodium nitrite and monosodium glutamate used as food additives demonstrated by the *Salmonella* microsome test system. Microbiol. Bul, 1991; 25: 94-107.
15. Rencuzogullari E, Kayraldiz A, Ila HB, Cakmac T, Topaktas M. 2001a. The cytogenetic effect of sodium metabisulphite, a food preservative in root tip cells of *Allium cepa* L. Turkish J. Biol, 2011a; 25: 361-371.
16. Rencuzogullari, E., Ila HB, Kayraldiz A, Topaktas M. Chromosome aberrations and sister chromatid exchange in cultured human lymphocytes treated with sodium metabisulphite, a food preservative. Mutat Res, 2001b; 490: 107-112.
17. Romeo-Cristian M, Gabriela Capraru. 2008. Influence of sodium phosphate (E339) on mitotic division in *Calendula officinalis* L. Analele Stiintifice ale Universitatii, Alexandru Ioan Cuza, Sectiunea Genetica si Biologie Moleculara, TOM IX, 2013; 81-84.
18. Luca D, Luca V, Cotor F, Raileanu L. 1987. In vivo and in vitro cytogenetic damage induced by sodium nitrite. Mutat Res, 1987; 189: 333-339.
19. Munzer M, Guigas C, Renner HW 1990. Re-examination of potassium sorbate and sodium sorbate for possible genotoxic potential. Food. Chem. Toxicol, 1990; 28: 397-401.
20. Meng Z, Zhang L. Cytogenetic damage induced in human lymphocytes by sodium bi sulphite. Mutation Res, 1992; 298: 63-69.
21. Gomurgen A N. Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. Cytologia, 2005; 70: 119-128.
22. Turkoglu Sifa. Evaluation of genotoxic effects of Sodium propionate, Calcium propionate and Potassium propionate on the root meristem cells of *Allium cepa*. Food and Chemical Toxicology, 2008; 46: 2035-2041.
23. Schneiderman MH, Dewey WC, Highfield DP. Inhibition of DNA synthesis in synchronized Chinese hamster cell treated in G1 with cycloheleximide. Exp. Cell Res, 1971; 67: 147–155.
24. Sudhakar R, Gowda N, Venu G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa. Cytologia, 2001; 66: 235–239.
25. Van't Hoff J. The action of IAA and kinetin on the mitotic cycle of proliferative and stationary phase excised root meristem. Exp. Cell Res, 1968; 51: 167.