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Determination of Potential Cytotoxic and Mutagenic Effects of Aluminum Acetate

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Abstract

In this study, both cytotoxic and mutagenic effects of aluminum acetate were evaluated by using *Allium cepa* root meristematic cells and Ames test. In *A. cepa* test, EC_{50} value was determined as 156.25 ppm and EC_{50}/2 (78.125ppm), EC_{50} (156.25 ppm), EC_{50}×2 (312.5 ppm) concentrations of aluminum acetate were used for root growth inhibition and mitotic index (MI) determination tests. *A. cepa* test results showed that MI significantly decreased with aluminum acetate concentration at each exposure time. Aluminum acetate showed a cytotoxic effect due to decreasing of MI%. It was also observed that aluminum acetate decreased significantly the percentages of prophase, metaphase, anaphase and telophase stages in all concentrations in each exposure time. The aluminum acetate was also investigated for its mutagenic effects at nontoxic concentrations (1000, 500, 250, 125, 62.5 µg/plate) by employing Ames test, as well. These results suggested that aluminum acetate has a weak mutagenic effect at 1000 µg/plate concentration.

Key words: Aluminum acetate, *Allium* test, Ames test, cytotoxicity, mutagenicity

INTRODUCTION

Living organisms are often exposed to various chemicals, which are widely used as food additives, cosmetics, pesticides, and industrial chemicals [1-3]. These chemicals or their derivatives cause mutagenicity, carcinogenicity or genotoxicity and they also cause serious environmental and health problems [4]. Therefore, investigations of such chemicals are important to determine their harmful effects for human health. Mutagenicity and cytotoxicity of various chemicals can be determined with different test systems. These test systems reliable, sensitive and can be carried out as they are very rapid and cheap [5-9]. Ames test is one of the widely used method to examine the toxic, mutagenic, carcinogenic effects of test substances used as particularly raw materials for medication [10]. Plant bioassays are sensitive, easy and cheap tools to perform the genotoxicity and cytotoxicity. *Allium cepa* test is reliable test system in order to evaluate effects of cytotoxic and genotoxic effects of various chemicals [11-13].

Aluminum acetate is a chemical, which is extensively used for medicinal purposes. This chemical can be used topically in the treatment of minor skin irritation (such as insect bites, contacting with poison ivy, oak and sumac plants, soaps, cosmetics, detergents, or skin rashes induced by jewelleries etc). Aluminum acetate can also be used in the treatment of draining lesions and helps to refresh and relax. Besides, it can be used for reducing sweating in the feet and swelling of minor injuries.

In this study we aimed to determine that cytotoxic and mutagenic effects of aluminum acetate by using *A. cepa* root meristematic cells and a short term mutation assay in *Salmonella typhimurium* with both TA98 and TA100 strains in the presence or absence of S9 mix, respectively. In our knowledge, the present study is the first research on cytotoxicity and mutagenecity of aluminium acetate by using both *Allium* and Ames test systems.

MATERIALS AND METHODS

Test organisms and chemicals

Dr. Bruce N. Ames (Californiya University Berkeley, CA., USA) supplied the S. typhimurium test strains TA98 and TA100. The test substance aluminum acetate was obtained from Sigma-Aldrich (CAS No. 289825) and dissolved in dimethyl sulphoxide (1% DMSO, purity 99%).

Determination of *A. cepa* root growth inhibition and Mitotic index (MI)

The root growth inhibition test protocol was carried out as described by Fiskesjo [11]. In order to determine the EC_{50} value of aluminum acetate, five different concentrations (100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm) were applied to clean and healthy onion roots. Aluminum acetate was dissolved in 1% DMSO. Concentration value reducing average root length by 50% for negative control value was determined as the effective concentration (EC_{50}). Determination of its possible toxic effects on roots at different concentrations; EC_{50}/2 (78,125ppm), EC_{50} (156.25 ppm), EC_{50}×2 (312.5 ppm) were tested by the *Allium* MI test.

The *A. cepa* test was performed according to Fiskesjo [11]. 10 ppm methyl methane sulfonate (MMS) (positive control), 1% DMSO (negative control) and EC_{50}/2 (78.125ppm), EC_{50} (156.25 ppm), EC_{50}×2 (312.5 ppm) concentrations of aluminum acetate were applied to onion roots for 24, 48 and 72 h. At the end of the exposure periods, roots were cut and treated immediately in a chilled Carnoy's fixative (ethanol: acetic acid = 3:1) for 24 h and kept at 4 °C overnight. Then the roots were transferred to 70% alcohol and kept in refrigerator. After roots have been removed from 70% ethanol, they were hydrolyzed using 1N HCl in water bath at 60 °C for 7 minutes. After hydrolysis process, roots have been soaked in dH_2O for 15 min. Roots were stained with Feulgen stain for an hour at room temperature [14]. Five slides were examined for each concentration and 1,000 cells/per slide were counted. Mitotic index (MI) was evaluated with following formulation. MI% = Divided cell number/Total cell number x 100 [11].

Ames test

The cytotoxic doses of aluminum acetate were determined by Dean *et al.* [15]. In the present study, aluminum acetate was investigated for its mutagenic effects at nontoxic concentrations (62.5, 125, 250, 500, and 1000 µg/plate). Ames test was carried out as a standard plate incorporation test with TA98 and TA100 strains of *S. typhimurium* in the presence or absence of S9 mixes [16]. Spontaneous control, solvent control and positive control were also applied. The test substance was dissolved in DMSO. DMSO was used also for solvent control. Sodium azide (SA) for TA100 and 4-nitro-o-fenilendiamine (NPD) for TA 98 were used as positive controls in the absence of S9 mix. 2-aminoanghtracene (2AA) for TA100 and 2-aminofluorene (2AF) for TA98 were also used as positive controls in the presence of S9 mix. The revertant bacterial colonies on each plate were counted.

Statistical analyzes

The data of root length, MI and mitotic phases were analyzed statistically using SPSS 18.0. Dunnett-t test (two tailed). The level of statistical significance was in all cases $p \leq 0.05$. Dunnett-t test was also used to validate the mutagenic action.

RESULT AND DISCUSSION

Assessment of the genotoxicity potential of chemical substances is important to provide useful information about genotoxicity and mutagenicity [17]. *A. cepa* test is sensitive and easy method in order to determination the toxicity of different chemicals and detect abnormalities in mitotic cells. It is very important to determine EC_{50} value in order to detect toxic and genotoxic effects of various chemicals on *A. cepa's* chromosomes and nuclear division [11]. EC_{50} value exhibited to be a useful parameter for choosing the test concentrations for the genotoxicity tests. The present study determined the cytotoxic capacity of aluminum acetate using *A. cepa* test. 156.25 ppm concentration of aluminum acetate was decreased the root length by about 50% (1.58%), compared to the negative controls (DMSO at 1%). These results also indicated that there was a dose-dependent decrease. Thus, EC_{50} determination test showed the significantly cytotoxic effects ($p \leq 0.05$) of aluminum acetate (Table 1).

Table 1. *A. cepa* root growth inhibition test results

Test Substance	Concentration (ppm)	Mean ± SD	Inhibition (%)
Control	-	3.35±0.17	-
Negative Control (1% DMSO)	-	3.19±0.14	-
Positive Control	10	0.71±0.12*	-
Aluminum acetate	156.25	1.58±0.18*	50.47
	312.5	1.45±0.14*	54.55
	625	1.33±0.12*	58.31
	1250	1.32±0.07*	58.62
	2500	1.08±0.19*	66.14
	5000	1.04±0.25*	67.40
	10000	0.79±0.11*	75.24

* Means difference is significant at the $p \leq 0.05$ level. (Dunnett t test 2-sided), SD: Standard Deviation

MI and mitotic phase studies were carried out for 24, 48 and 72 h. An increase or decrease in levels of MI can be an indicator of the cytotoxicity of the agent examined [18]. Lower MI may indicate the toxic effect of test compounds and higher MI may result from cell division induction. The effect of aluminum acetate on the MI (%) and mitotic phases of *A. cepa* root meristem cells was show in Table 2. All concentrations of aluminum acetate decreased the MI compared to negative control at each exposure time and the effect on MI was dose-dependent. The highest MI percentage value was obtained from 24 h applications of 78.125 ppm with a score of 63.76± 17.85. The lowest MI percentage value was determined at 312.5 ppm at 72 h with a score of 6.48± 2.33 compared to other concentrations. As a result of *A. cepa* test, aluminum acetate showed cytotoxic effect to *A. cepa* root tips. As a result of *A. cepa* test, aluminum acetate caused dose-dependent inhibition of mitotic index in all treatment periods. The highest applied concentration of aluminum acetate (312.5 ppm) caused more than 50% decrease in the MI for all treatment periods. Furthermore, 156.25 ppm concentration of aluminum acetate caused over 50% decrease in MI for 72 h treatment periods.

Table 2. The effects of aluminum acetate aqueous extract on mitotic index and mitotic phases of *A. cepa* root meristem

Concentration (ppm)	Treatment period	Counting cell number	MI± SD
Negative Control	24 h	5366	87.13±21.05
MMS (10µg/ml)		5091	38.90± 9.36*
78.125		5402	63.76± 17.85*
156.25		5467	55.34± 14.09*
312.5		5505	41.22± 12.87*
Negative Control	48 h	5217	84.06±23.41
MMS (10µg/ml)		5143	35.59± 8.34*
78.125		5472	55.29± 7.06*
156.25		5484	48.52± 18.85*
312.5		5443	24.34± 5.56*
Negative Control	72 h	5218	81.09±19.24
MMS (10µg/ml)		5083	30.19± 6.35*
78.125		5371	47.35± 14.48*
156.25		5556	37.74± 9.75*
312.5		5337	6.48± 2.33*

Mitotic Phases (%) ± SD			
Prophase	Metaphase	Anaphase	Telophase
82.5±24.22	1.79±0.32	1.54±0.32	1.51±0.25
36.86±6.45*	0.56±0.22*	0.75±0.17*	0.74±0.20*
62.03±16.24*	0.46±0.16*	0.63±0.25*	0.64±0.15*
54.07±14.58*	0.45±0.08*	0.39±0.16*	0.43±0.09*
40.21±11.25*	0.33±0.17*	0.30±0.41*	0.38±0.12*
79.96±18.84	1.73±0.44	1.34±0.34	1.04±0.36
33.50±10.25*	0.63±0.12*	0.74±0.21*	0.72±0.23*
53.63±9.66*	0.63±0.28*	0.59±0.32*	0.44±0.18*
47.09±17.34*	0.65±0.21*	0.37±0.19*	0.41±0.16*
24.08±6.60*	0.14±0.05*	0.06±0.02*	0.07±0.02*
78.18±21.65	1.38±0.38	1.00±0.29	0.54±0.19
28.80±3.68*	0.56±0.12*	0.49±0.13*	0.53±0.18*
45.78±7.99*	0.61±0.17*	0.51±0.17*	0.45±0.12*
37.74±10.32*	0.58±0.13*	0.42±0.11*	0.39±0.9*
6.48±2.48*	0.52±0.11*	0.33±0.12*	0.10±0.03*

* Means difference in significant at the at the level of $p \leq 0.05$, Dunnet-t test (2-sided) SD: Standart deviation

Aluminum acetate was showed a cytotoxic effect due to decreasing of MI%. Inhibition of mitotic activities is used for evaluated of cytotoxic chemicals. There are some possible mechanisms for chemically decreased MI in plant cells. One possible mechanism is that decrease in MI could be due to blocking of G1 suppressing DNA synthesis [19]. Another possible mechanism is a blocking of G2 preventing the cell

from entering mitosis [20]. The lowering of the MI might have been achieved by the inhibition of DNA synthesis at the S-phase. All concentrations of aluminum acetate changed the percentage of particular phases in comparison to the control. Aluminum acetate decreased the percentages of prophase, metaphase, anaphase and telophase stages, significantly at all the concentrations exposed for 24, 48 and 72 h periods. Percentages of mitotic phases at different concentrations of aluminum acetate were as the values obtained from MI%. There were statistically significant differences between negative control and the other groups ($p \leq 0.05$). These changes observed in mitotic stages can be associated with inhibition of DNA synthesis in S phase, depending on applied concentration of the chemical [21].

In many studies, *A. cepa* test were used in order to determine cytotoxic effects of various chemical substances. Cytotoxicity of sodium metabilsülfit [22], boric acid [23] potassium metabisulfite and potassium nitrate [24] were investigated by using *A. cepa* test and their results showed that abnormal cells percentage increases and MI decreases as statistically significant in *A. cepa* L. root tip cells. Srivastava and Mishra [25] used *A. cepa* and *Vicia faba* tests in order to determine cytogenetic effects of atrazine. Test results showed that this pesticide reduced MI significantly depending on treatment concentration in both two-test systems. Saxena et al. [6] determined cytogenetic effects of carbofuran on meristem cells in root tip of *A. cepa* and *A. sativum*. It was determined that carbofuran caused a reduction on MI due to concentration increase and enhance chromosome abnormalities. These studies are similar to our study in terms of identifying reduction in MI depending on concentration increase.

Ames test is accepted one of the reliable methods particularly to examine toxic, mutagenic, carcinogenic effects of substances which were used as especially raw materials for medication [26, 27]. Five different concentrations of aluminum acetate (1000, 500, 250, 125, 62.5 µg/plate) were applied for Ames test. In the same time, plates containing positive control mutagens were also tested, and they displayed very significant increases in the spontaneous mutation rate in two strains tested. According to Ames test result, all concentrations were found not to be mutagenic on both TA98 and TA100 strains of *S. typhimurium* in the presence or absence of S9 mix except that 1000 µg/plate (Table 3).

Table 3. Mutagenicity of Aluminum acetate on TA98 and TA100 strains of *S. typhimurium*.

Agent	Amount (µg/plate)	Number of His$^+$ Revertants/plate Mean ± SD*			
		TA98		TA100	
		- S9	+ S9	- S9	+ S9
Aluminium acetate	1000	38.40±3.36	36.60±2.07	128.00±3.81	210.40±20.61*
	500	36.40±2.51	33.00±6.12	123.40±3.05	168.00±14.73
	250	33.80±3.27	31.60±3.13	117.00±3.54	161.20±17.24
	125	27.20±1.10	31.00±1.22	107.00±2.74	156.00±19.16
	62.5	25.60±2.88	26.80±4.60	99.40±2.97	155.40±11.46
Negative Control (DMSO)	100	23.00±2.00	24.60±2.41	94.80±7.29	117.60±5.03
SA	10			2724.00±83.32*	
2AA	5				2190.00±96.18*
2AF	200		967.40±28.65*		
NPD	200	1289.60±44.80*			

* Means statistically significant at the level of $p \leq 0.05$, SD Standard deviation, SA: Sodium azide, 2AA: 2-aminoanthracene, 2AF: 2-aminofluorene, NPD: 4-nitro-o-phenylenediamin

The number of spontaneous revertant colonies for *S. typhimurium* TA98 in the presence and absence of S9 fraction were evaluated statistically and found as 24.60±2.41 and 23.00±2.00, respectively. These values for *S. typhimurium* TA100 determined as 117.60±5.03 and 94.80±729. The highest statistically significant ($p \leq 0.05$) mutagenic response was found as 210.40±20.61 in TA 100 with S9 mix. Most of the results, whether increasing relatively to the negative control group, were not statistically significant ($p \leq 0.05$) in all examined strains, except 1000 µg/plate doses of alumin-um acetate in TA100 with S9 mix. This concentration of aluminum acetate was found statistically significant for revertant bacterial colonies in TA100 with S9 mix.

In conclusion, root growth inhibition test and *A. cepa* MI study showed that aluminum acetate was cytotoxic at the applied concentrations. According to the Ames test result, all concentrations were not mutagenic on *S. typhimurium* TA98 and TA100 strains in the presence or absence of S9 mix except that 1000 µg/plate of aluminum acetate. These results suggested that aluminum acetate has a weak mutagenic effect at 1000 µg/plate concentration.

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