ORIGINAL ARTICLE JGEB Vol. 6, No. 1, 2008 ISSN:1687-157X

Protective Role of Vitamin C Against Genotoxicity of Aluminium Sulphate in Vicia faba

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ABSTRACT

The genotoxic effect of aluminum sulphate at different concentrations on mitotic and meiotic activity in roots and flower buds and protein banding pattern in seeds of *Vicia faba* was evaluated. The protective role of Vitamin C against this genotoxicity was also investigated. Reduction in mitotic and meiotic activity was induced after different treatments with aluminum sulphate. A considerable frequency of chromosomal abnormalities was also recorded. The recorded values were increased by increasing concentration in almost all the different cytological tests. However, vitamic C successfully reduced the genotoxic effect of aluminum sulphate at the cytological level only. At SDS- protein level, aluminum sulphate shows main alterations in the M2 seed storage protein banding patterns expressed as disappearance of some bands, appearance of novel ones and changes in band's intensity.

Key Words: Aluminum sulphate, Vitamine *C, vicia faba*, genotoxicity.

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Journal of Genetic Engineering and Biotechnology, 2008, 6(1): 47-55

INTRODUCTION

The metal toxicity in plant has been known for long time *(Roy et al., 1989, Liu et al., 1995 and El-Ashry and Mohamed, 2006)*. Aluminium is the most frequent metallic element in the earth`s crust. Aluminium and its salts are widely used in household and in industry. It was found to increase protein synthesis, to decrease ribosomal RNA content and has the ability to induce chromosomal aberrations in plants *(Wallace and Anderson, 1984)*, sea urchins and mussels *(Pagano et al., 1996)*, mouse *(Manna and Das, 1972 and El-Sherbeny, 2001)* and human *(Yao et al., 1994)*. It is very important nowadays to search for protective substances that could minimize the toxic effects of different chemicals. However, Vitamins play a beneficial role against the mutagenicity of some chemicals *(Mohamed, 2002)*. Vitamin C (V. C) successfully reduced the clastogenic effect of many chemical agent and radiation *(Hoda et al., 1991)*. Therefore, the present investigation was undertaken to evaluate the mutagenic potential of aluminium sulpahte in *Vicia faba* and to test the antimutagenic efficiency of V. C in a trial to minimize the genotoxicity of aluminium sulphate.

MATERIALS AND METHODS

Vicia faba L. Var. Giza 2 was used in the present study and obtained from the Crop Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Aluminium Sulphate ($Al2(SO_4)_3$) Sarabkai M. Chemicals. India. Vitamin C (L- ascorbic acid) was purchased from Fluka. Aluminum sulphate doses of 1, 5, 10, 100, 150, 200 ppm were chosen for mitotic study while 100, 150, 200 ppm were used in meiotic and biochemical studies. The general dose used of Vitamin C was 100 mg/l.

Cytological procedures:

A. Mitotic study: Seeds of *Vicia faba* were grown until roots reached 1.5 – 3.0 cm in length, then seedling were divided and treated in six groups for duration of six hours as shown in (Table 1). The roots of all treatments were cut and fixed in 3 absolute ethyl alcohol: One glycial acetic acid (V/V) for 24 hrs, stained with feulgen and after squashing the slides were made permanent with dry ice, followed by mounted in Canada balsam *(Sharma and Sharma, 1980)*. The mitotic index and the percentage of chromosomal abnormalities were determined for each treatment group.
B. Meiotic study: Giza two seeds were soaked in freshly prepared solutions of aluminium sulphate (100, 150

and 200 ppm) for 6 hrs. A second group of seeds were soaked in solution of Vitamin C. A third group of seeds also soaked in the same concentrations of aluminium sulphate and Vitamin C simultaneously. Control seeds were soaked in distilled water. The seeds of all treatments were planted in pots. Three pots (6 plants / each) were used for each concentration. Flower buds were gathered after 40 days old, then fixed in carnoy's fluid and were examined using acetocarmine smear methods *(Sharma and Sharma 1980)*. Abnormalities, were scored in first and second meiotic divisions. All cytological data were statistically analyzed using T-test. Seeds of these experiments were grown in the field and M2 seeds were used for biochemical genetic analysis.

Table 1: Treatment groups of Vitamin C and aluminium sulphate and abbrevations used in the text.

Plant group	Treatment for 6 hrs	Abbreviated in
Group 1	Tap water	Control (C)
Group 2	Vitamin C	Vit. C
Group 3	Aluminium sulphate	$Al_2 (SO_4)_3$
Group 4	Aluminium Sulphate	$Al_2 (SO_4)_3$+ Vit.C
	+ Vitamin C	Simultaneously
Group 5	Vitamin C for 3 hrs	Vit. C → $Al_2(SO_4)_3$
	then Aluminium Sulphate for 3hrs	Pre-treatment
Group 6	Aluminium sulphate for 3 hrs then Vitamin C for 3 hrs	$Al_2 (SO_4)_3$→ Vit. C post-treatment

Biochemical procedures :

Sodium dedocyl sulphate polyacrylamide gel electrophoresis (SDS- PAGE) was carried out according to *Laemmli (1970)* as modified by *Studier (1973)*. The dry M2 seeds of *Vicia faba* whose grandparent were previously soaked in aluminium sulphate solution and in aluminium sulphate + Vitamin C simultaneously were used to extract total protein. Alteration in seed storage protein profiles among the seed samples of the treated faba plants were compared with that of the control to measure the mutagenic potentiality of aluminium sulphate.

RESULTS

The ability of aluminium sulphate to affect the mitotic activity in root meristem cells of *Vicia faba* is shown in (Table 2). Mitotic activity expressed as mitotic index (MI) and was progressively inhibited as the concentration of aluminium sulphate increased. A highly significant decrease in MI appeared after treatment with the highest dose of aluminium sulphate (200 ppm) where it reached (42.00 + 0.29) compared with the control (85.02 + 2.29). When aluminium sulphate and Vitamin C were administered concurrently (Al2 $(So_4)_3$ + Vit C), the mitotic index depression was still observed but the intensity of reduction was much less in all treatments. The mitotic index was 68.96+ 0.46 with 200 ppm Al2 $(SO_4)_3$ + Vit C when compared with the same dose without Vitamin C (42.00 + 0.29). Pre-treatment with Vitamin C could not produce significant protective effect against the mitoinhibitory effect of aluminium sulphate with higher does. The mitotic index was 62.96+ 0.21 and 60.00 +0.16 with 150 and 200 ppm, respectively (Vit C→Al2 $(SO_4)_3$ compared with the same dose of aluminium sulphate alone (45.95+ 0.41 and 42.00 + 0.29). However, at the low dose of 1 ppm Al2 $(SO_4)_2$, Table 2, showed some improvement of mitotic index percentage compared with the control (83.09+ 0.24 and 85.02 + 2.29), respectively. When Vitamin C was given at the end of aluminium sulpahte treatment (Al2 $(SO_4)_3$→Vit C.), the improvement in division rate increased. Mitotic index was 72.98% after treatmcnt with 200 ppm (Al2 $(SO_4)_3$ → Vit C) compared with Al2 $(SO_4)_3$ alone (42.00%).

The results in (Tables 2 and 3) showed a highly significant increase in the percentage of the mitotic and meiotic chromosomal abnormalities due to aluminium sulphate treatment. The frequency of total irregularities was found to be dose dependant. At all phases of mitotic division and in the 1st and 2nd meiotic divisions, the percentage of mitotic and meiotic abnormalities were increased compared with the corresponding control values. The maximum value of mean percentage of mitotic chromosomal abnormalities was reached (43.86%) with the highest concentration of aluminium sulphate (200 ppm) compared with control value of 1.23%. Treatment with Vitamin C alone induced a nonsignificant increase in the frequency of mitotic irregularities. When

Vitamin C was administered concurrently with aluminium sulphate as well as pre and post- treatment with Vitamin C, the treated roots showed in most cases a highly decrease in the means percentages of chromosomal abnormalities as compared with the corresponding values recorded after the same treatment with aluminium sulphate alone (Table 2). The decrease was more pronounced after concurrently treatment and post treatment than after pre-treatment. Results in (Table 3) revealed that the maximum value of means percentage of abnormal PMCs was reached 32.99% at the highest concentration (200 ppm) compared with the control value of 0.78%. A moderate decrease in the mean percentage of abnormal PMCs was observed after treatment with aluminium sulphate and Vitamin C concurrently compared with the corresponding values recorded after treatment with aluminium sulphate alone. Different types of chromosomal abnormalities in both mitotic and meiotic divisions after treatment with aluminium sulphate and after concurrently administration as well as pre and post- treatments with Vitamin C were represented in (Tables 2, 3 and Figure1).

Table 2: Mitotic index (MI), percentage of abnormal mitosis(abn) and typ of mitotic abnormalities in *Vicia faba* (Giza 2) root-tip meristems after root treatment for 6 hrs. with different concentrations of aluminium sulpahte + V.C administration.

Experimental doses (ppm)	% of MI ± S. E.	% of abn. ± S.E.	% of each type of abnormality relative to the number of abnormal mitosis					
			Frag.	Lagg.	Dist.	Stick	Micon	Bridg.
Control (C)	85.02±2.29	1.23±0.38	30.00	20.00	10.00	30.00	00.00	10.00
Vit. C	84.74±0.43	1.15±0.04	36.36	18.18	18.18	18.18	00.00	00.00
1 ppm	81.33±1.10	19.05±1.05	32.26	14.84	17.42	24.52	1.29	9.68
5 ppm	71.22±2.22	22.44±1.13	33.33	14.62	16.96	23.39	1.75	9.94
10 ppm	68.49±1.07	24.50±0.47	28.11	15.14	16.22	26.49	4.32	9.73
100 ppm	50.73±0.57	39.77±0.33	32.08	16.51	17.92	22.64	1.42	9.43
150 ppm	45.95±0.41	42.34±0.27	27.54	20.69	20.11	21.84	1.15	8.62
200 ppm	42.00±0.29	43.86±0.43	38.69	11.31	13.69	23.21	1.79	11.31
1 ppm+ Vit C	82.01±0.47	3.70±0.27	28.13	21.88	15.63	21.88	3.13	9.38
5 ppm+ Vit C	79.00±0.62	5.04±0.29	27.91	16.28	13.95	23.26	4.65	13.95
10 ppm+ Vit C	76.05±1.14	5.99±0.26	31.82	13.64	18.18	25.oo	2.27	9.o9
100 ppm+ Vit C	74.97±0.30	7.96±0.13	29.51	16.39	1667	22.95	3.28	8.20
150 ppm+ Vit C	72.94±0.31	8.95±0.47	30.77	16.92	20.00	24.61	1.54	6.15
200 ppm+ Vit C	68.96±0.46	10.10±0.06	29.73	15.57	20.27	22.97	2.76	6.76
Vit C → 1ppm	83.09±0.24	3.12±0.13	37.04	11.11	14.81	22.22	3.70	11.11
Vit C→ 5 ppm	81.98±0.42	6.26±0.15	28.57	17.86	19.64	23.21	3.57	7.14
Vit C→ 10 ppm	78.04±029	8.89±0.27	26.09	20.29	18.84	23.19	4.35	7.25
VitC→ 100 ppm	67.92±0.32	12.10±0.30	27.71	19.28	17.44	22.89	4.82	7.23
VitC→ 150 ppm	62.96±0.42	14.98±0.29	26.88	15.05	20.43	24.73	4.30	8.66
VitC→ 200 ppm	60.00±0.16	16.06±0.29	25.71	18.10	20.00	21.90	4.76	9.52
1 ppm→ Vit C	82.57±0.41	3.08±0.14	38.46	1923	15.38	23.08	00.00	3.85
5 ppm→ Vit C	79.79±0.56	5.98±0.19	29.79	12.77	38.46	25.53	2.13	8.61
10 ppm→ Vit C	77.00±0.29	7.98±0.34	30.00	16.67	18.33	21.67	5.00	8.33
100 ppm→Vit C	76.00±0.28	10.95±0.16	31.12	18.07	10.07	22.89	3.61	7.23
150 ppm→Vit C	79.99±0.31	13.90±0.20	28.70	18.52	19.44	23.15	3.70	6.48
200 ppm→Vit C	72.98±0.30	13.56±0.41	29.91	21.60	18.69	23.36	1.87	4.67

Table 3: Total pollen mother cells (PMCs) examined, percentage of total abnormalities, percentage of abnormal PMCs in the first and second meiotic division and types of abnormalities in meioses of Vicia faba plant after seed soaking treatment of *Vicia faba* seeds for 6 hrs. in Vitamin C, different concentrations of aluminium sulphate and of aluminium sulphate + Vitamin C simultaneously.

Treatments	Total examined PMCs	% of abn. PMCs ± S. E.	1st meiotic division		2nd meiotic division		% of each type of abnormalities				
			Total PMCs	%of abn. PMCs	Total PMCs	% of abn. PMCs	Frag.	Lagg.	Dist.	Stick	Brid.
Control	3450	0.78 ± 0.004	1850	0.81	1600	0.75	29.63	22.22	18.52	14.81	14.81
Vit C	3240	0.68 ± 0.0004	1815	0.66	1425	0.70	31.82	22.73	18.18	13.64	13.64
100 ppm	3007	29.02 ± 0.77	1490	27.32	1517	30.65	22.48	20.99	19.61	17.66	19.27
150 ppm	3011	32.02 ± 0.29	1481	31.33	1530	32.68	21.99	21.06	20.33	18.78	17.84
200ppm	2940	32.99 ± 0.34	1370	33.21	1570	32.81	22.58	21.34	19.90	19.07	17.11
100ppm + Vit C	3120	10.06 ± 0.31	1510	9.93	1610	10.19	23.25	21.66	20.06	19.11	15.92
150ppm + Vit C	3340	12.01± 0.53	1617	12.18	17.23	11.84	23.19	21.95	19.70	16.96	18.20
200 ppm + Vit C	3211	14.01 ± 0.46	1499	13.81	1712	14.19	24.22	22.44	19.33	17.78	16.22

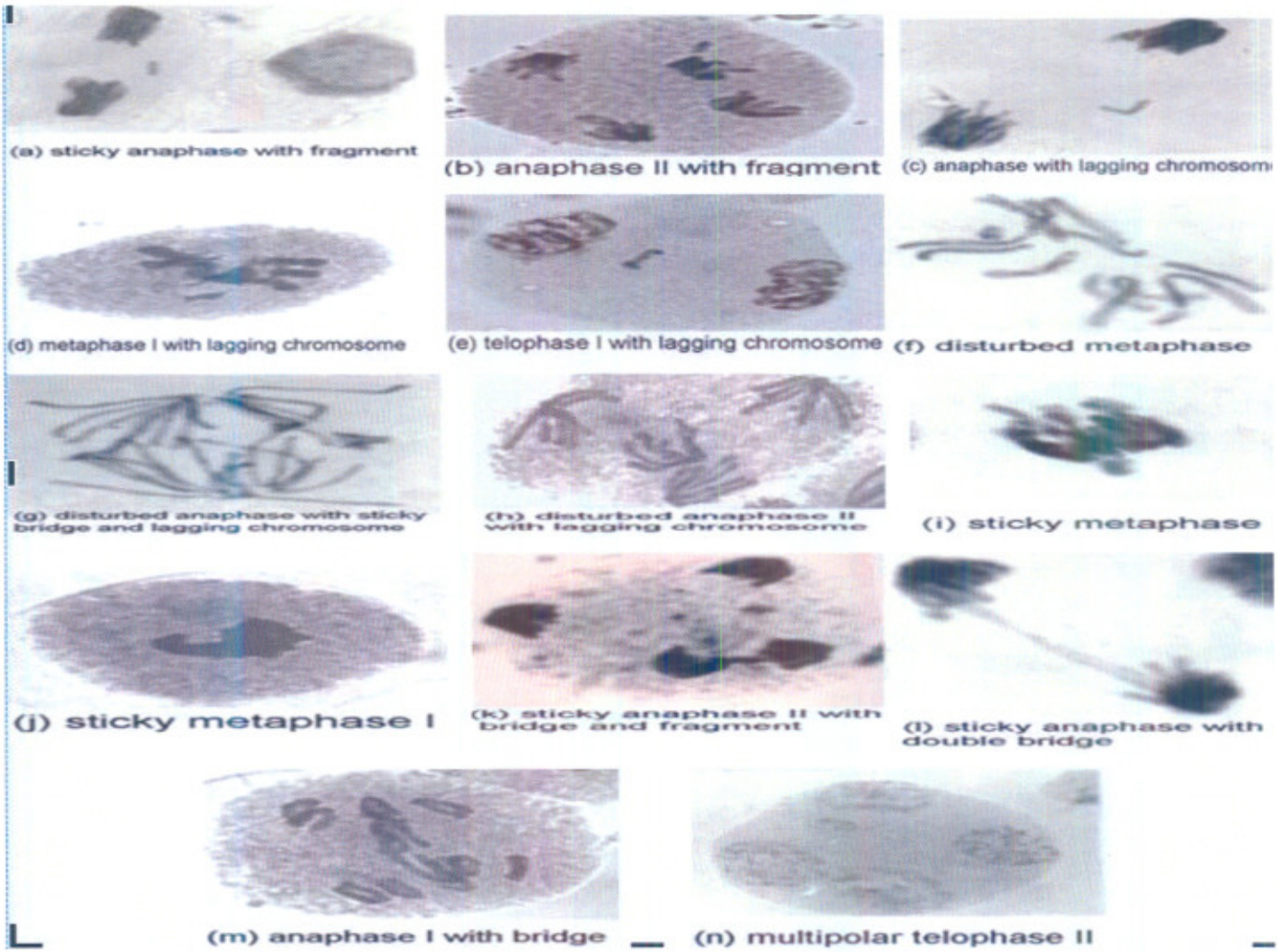

Figure 1: Types of abnormalities in mitotic and meiotic cells of *Vicia faba* after treatment with different concentrations of aluminium sulphate and with aluminium sulphate + Vitamin C concurrently.

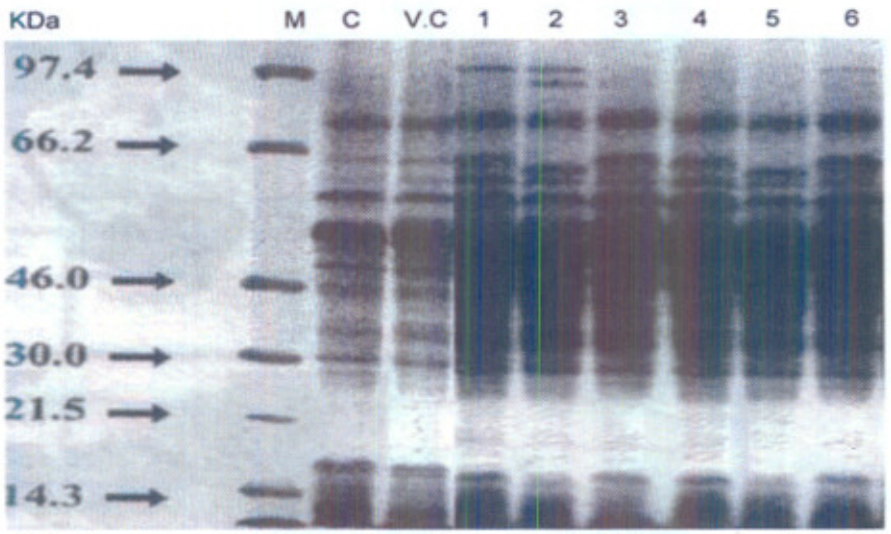

Figure 2: Electrophoretic banding patterns of *Vicia faba* after seed treatment with different concentrations of aluminium sulphate and with aluminium sulphate + Vitamin C concurrently.
M = marker, C = control, V. = Vitamin c, 1 = 100 ppm, 2= 150 ppm 3= 200 ppm, 4 = 100 ppm +V.C, 5 = 150ppm, 6 = 200 ppm+

Table 4: Effect of different concentrations of aluminium sulpahte and aluminium sulphate + Vitamin C concurrently on protein bands of mitotic and meiotic cells of *Vicia faba* plant.

Sample	**C**	**V. C**	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**
Rows	**(mol. W)**	**(mol. W)**	**(mol. W)**	**(mol. W)**	**(mol. W)**	**(mol. W)**	**(mol. W)**	**(mol. W)**
1			102.591	103.259				101.486
2	80.155		83.340					
3				94.900				
4		79.637	79.293	79.293	79.293	79.293	79.293	79.293
5	86.439	68.439						68.439
6			67.702		67.996	67.702	66.109	
7	62.762	62.762	62.762	62.762	62.762	62.762	62.762	62.762
8	57.556	57.806		58.816	58.813		58.182	
9	50.001	50.218		50.218		50.546	50.001	
10	42.233	42.233	42.233	42.233	42.233	42.233	42.233	42.233
11	39.236	39.236						
12	37.900	37.900						
13	32.012		34.383	34.383	34.383	34.383	34.383	34.383
14	31.943	31.943	31.943	31.943	31.943	31.943	31.943	31.943
15	28.676	28.676	28.676	28.676	28.676	28.676	28.676	28.676
16			26.005	26.005	26.005	26.005	26.005	26.005
17			23.337	23.337	23.337	23.337	23.337	23.337
18	16.940	16.940	16.940	16.940	16.940	16.940	16.940	16.940
19	15.978	15.978	15.978	15.978	15.978		15.978	15.978
Sum no of bands	**13**	**13**	**13**	**14**	**12**	**11**	**13**	**12**

- C is the control
- 1 to 6 are the plant groups tested as mentioned in the materials and methods section.

Chromosomal fragments (Figure 1 a and b) were a common type of abnormalities observed during both mitotic and meiotic cell divisions after all treatments. Other chromosomal abnormalities included in the present investigation were laggards and disturbed phases (Figure 1 c-h). Chromosomal stickiness was also observed on both mitotic and meiotic divisions Figure1 (I-l). Considerable frequency of bridges Figure1 (g,k,l and m) was observed in anaphase and telophase stages after all treatments. Among the cytological aberrations appeared in a considerable frequency was the induction of micronulei (Figure 1). (N). In addition to the capability of aluminium sulphate to induce cytological aberrations, denoting its mutational potential, it is also induced obvious alteration in the electrophoretic profiles of the M2 seed proteins of *Vicia faba* L. (Figure 2 and Table 4). The changes in protein banding patterns did not show the dose response relationship, but they were more pronounced at the high molecular weight region than the middle and lower regions. With regard to Vitamin C, only few alterations were noticed in bands intensity. In (Table 4 and Figure 2) the appearance of novel protein bands, the disappearance of other bands as well as change in bands intensity in comparison to control profile represent the major changes in the electrophoretic banding patterns after aluminium sulphate treatment (lanes 1- 3). With regarded to the interaction between aluminium sulphate and Vitamin C (lanes 4- 6), the same protein pattern alterations scored due to aluminium sulphate treatment were still recorded. The increase in bands intensity in the present study after aluminium sulphate treatment was also observed. Table 4 shows the presence of about 19 protein bands. Sex out of them, which having molecular weights of 62.672, 42.233, 31.943, 28.676, 16.940 and 15.978 KDa were common in all treatments of *Vicia faba* used in the present study.

DISCUSSION

The present investigation deals with the genotoxic effect of aluminium sulphate on *Vicia faba* plants. The protective role of Vitamin C against this genotoicity was also evaluated. The forgoing data revealed that aluminium sulphate has a pronounced effect on mitotic index, total percentage of abnormalities in mitotic and meiotic divisions, types of abnormalities in somatic and germ cells and protein profiles of *Vicia faba* plants. This indicated that aluminium sulphate caused a toxic effect in chromosomes of *Vicia faba* plants. One of the important effects of aluminium sulphate was in general a highly significant reduction in mitotic index in *Vicia faba* root tip meristems. This inhibition of mitotic activity was almost concentration dependant. In this respect *Roy et al. (1989)* were observed that aluminium sulphate has a cytotoxic effect on root tip cell of *Allium sativum* during a time course study and during recovery. The end point considered were mitotic and frequencies of aberrant cells and micronuclei induced. Confirming results of *Deufel (1951) on Vicia faba and Oehkers (1943)* on *Oenothera* numerous studies have shown that aluminium salts can inhibit cell division and produce chromosomal aberrations in plants. Inhibition of cell division in root tip menistems as a primary effect of aluminium has been reported *(Wojciechowska and Kocik, 1983). Johnson and Jackson (1964)* suggested that the blocking of all divisions could result from interaction of aluminum with phosphorus or from inhibition of uptake and transport of *Ca and Al. Matsumoto et al. (1976)* suggested that Al could bind to DNA of Pea cell and inhibit its synthesis.

Both mechanisms may prevail. Moreover the results in the present investigation showed that Vitamin C minimized the genotoxicity of aluminium sulphate especially in post-treatment. This indicated that Vitamin C had a protective effect at the cytological level only. This protective mechanism may be due to the effect of Vitamin C on the defense system of organisms and protection of cells and tissues against harmful exo-and endogenous factors *(Andrew, 1997).* Also Vitamin C well known to minimize the formation of mutagenic conjugation of pesticide with them *(Hoda and Sinha, 1992). Farghaly (2003)* showed that Vitamin C successfully reduced the clastogenic effects of many chemical agents and radiation as it may scavenge harmful species, free radicals of electrophiles which damage DNA and other cell targets. These protective effects of Vitamin C showed that our results were similar to the results obtained by other workers on the antigenotoxcity of chemicals. *Hassan (1996)* noticed that black cumin and garlic extracts had antimutagenic effects against sodium azaid. El-Shiekh *(1999)* also found that Vitamin B-complex had the ability to minimize the incidence of mitotic inhibition and clastogenicity by modification and detoxification of bavistin fungicide. *Also Hassan (2001)* reported that the frequenciues of mitotic abnormalities induced by fenobucarb insecticide decreased after treatment with stimofort and nutrileaf foliar fertilizer. He concluded that the two fertilizers have the ability to minimize the effect of the insecticide.

In this study, cytological observations revealed that aluminium sulphate induced a highly significant increase in the percentage of the mitotic and meiotic chromosomal abnormalities. The frequency of total irregularities was found to be dose dependant with aluminium sulphate alone, non significant with Vitamin C alone and a highly decreased when Vitamin C administrated concurrently with aluminium sulphate as well as pre and post- treatments with Vitamin C. This obtained data proved the safety of Vitamin C and revealed its ability to counteract the cytotoxicity of aluminium

sulphate. In this investigation different types of abnormalities were induced in both mitotic and meiotic divisions after treatment with aluminium sulphate and after concurrently administration as well as pre and post- treatments with Vitamin C. Chromosomal fragments, laggards, disturbed phases, stickiness, bridges were observed in the two divisions while, micronuclei were observed in mitotic division only.

Chromosomal fragments were the dominating type of abnormalities observed in mitotic and meiotic divisions after all treatments, the induction of these abnormalities indicates the mutagenic capacity of aluminium sulphate and it might lead to micronuclei formation. The occurrence of laggards and disturbed phases indicated that aluminium sulphate completely or partially affect the spindle apparatus. Laggards could be attributed to irregular orientation of chromosomes *(Patil and Bahat, 1992)*. Disturbed phases may be due to inhibition in the respiratory pathway resulting in low energy production necessary for chromosome movement *(Armbruster et al., 1991)*. The induction of chromosomal stickiness on both mitotic and meiotic divisions may be due to physical interference with chromosome condensation, which cause many chromosomes to adhere to one another resulting in stickiness *(Mc Gill et al., 1974)*. Bridges were also observed after the different treatments. It may be attributes to stickiness of chromosomes *(Kabarity et al., 1974)* or due to the formation of dicentric chromosomes as a result of breakage and reunion *(Tomkins and Grant, 1972)*. Micronulei appeared in a considerable frequency, it may originate from lagging chromosomes or from centric fragments. Micronuclei are true mutagenic aspects which lead to loss of the genetic material and have been regarded as an indication of the mutagenicity of their inducers *(Ruan et al., 1992)*.

In the present study, the genotoxicity of aluminium sulphate has been measured by its ability to induce obvious alterations in the electrophoretic profiles of the M2 seed proteins of *Vicia faba* L. Changes in seed proteins electrophoretic profiles have been attributed to the occurrence of either gene mutations or induction of cytological aberrations *(Hassan et al., 2002, Mohmed 2005 and Abdel- Hamied 2005)*. *Abdel-Salam et al. (1993)* attributed such observation to the higher sensitivity of genes encoding protein bands in this region to mutagenicity than the genes encoding protein bands in the other two regions. *El-Sherbeny et al. (2002)*, *Soliman (2003)* and *Shehab et al. (2004)* attributed these changes to mutational events, where some of the electrophoretic bands disappeared due to the loss of some genetic material or changes in gene sequences. On the other hand Vitamin C induced only few alterations in bands intensity indicating negligible mutagenic potentiality of this Vitamin and that vitamic C dose not antigonize the effect of aluminium sulphate at biochemical level. The increase in band intensities in the present study after aluminium sulphate treatment could be due to gene mutation at the regulatory system that control the concerned structural genes *(Abdel-Salam et al., 1993)* and or / gene duplication that produced by induction of breaks, laggards and micronuclei *(Abdel-Hamied, 2001 and 2005)*.

The disappearance of some bands in this study could be attributed to the loss of some genetic material due to induction of laggards, breaks and micronuclei. This conclusion was in agreement with that reported by *Abdel-Salam et al. (1993)*, *Hassan (2000)*, *Shehab et al. (2004)* and *Abdel Hamied (2000, 2005)*. Appearance of novel bands may be explained on the basis of band subfraction which could be attributed to cytological anomalis that lead to gene duplication followed by occurrence of a point mutation that encoded for the fractionated band. This lead also to the presence of two bands one of them with the original molecular weight while the other with an altered one *(Mohamed et al., 2003, Shehab et al., 2004 and Abdel-Hamied et al., 2005)*.

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