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EFFECT OF VITAMIN C ON SERUM PROTEIN PROFILE IN MICE AFTER ALUMINUM SULPHATE INTOXICATION

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Key words: aluminum sulphate, total protein, liver enzymes, protein fractions, vitamin C

We tested the effect of aluminum sulphate on serum protein profile, liver enzyme activities, bilirubin levels, total protein content and the efficiency of vitamin C to reduce the adverse effect of aluminum sulphate. Mice were treated orally for 1, 2 and 3 weeks with three doses of aluminum sulphate 387.5, 775 and 1550 mg/kg b.wt. representing 1/16, 1/8 and 1/4 LD_{50}, respectively. Significant reduction in serum total protein content and protein fraction concentrations is noticed except serum albumin which recorded a significant increase in a dose-dependent relationship of aluminum sulphate through follow up of time. Liver function enzyme activities and bilirubin levels recorded significantly elevated levels with 1/4, 1/8 and 1/16 LD_{50} of aluminum sulphate at 1st, 2nd and 3rd weeks of treatment. Concurrent administration of vitamin C at a dose of 20 mg/kg b.wt. to healthy mice produced a significant increase in serum total protein content and protein fraction concentrations. No effect of vitamin C on bilirubin levels and liver function enzyme activities in healthy animals was observed. On the other hand, vitamin C ameliorated serum total protein content and protein fraction concentrations, bilirubin levels and liver function enzyme activities in mice when administrated with 1/4 LD_{50} of aluminum sulphate. Concerning the effect of different doses of aluminum sulphate within weeks, ANOVA test revealed significant variation among each group except normal control group which recorded insignificant change after 1st, 2nd, and 3rd week. In vitamin C-administrated healthy mice, only albumin concentration and bilirubin levels recorded a non significant variations within different weeks.

INTRODUCTION

Aluminum (Al) is environmentally ubiquitous element, found in every food product. Its usual human exposure is primarily dietary [Yokel, 2000]. Recent studies have suggest that even a slight impairment of renal function may increase the Al body burden significantly, which may lead to neurotoxicity, nephrotoxicity, osteodystrophy and hypochronic anemia [Tariq *et al*., 1999].

Moreover aluminum is a trivalent cation unable to undergo redox reactions and has been linked to many diseases such as dialysis, senile dementia and microcytic anemia even without iron deficiency. It has also been implicated in Alzheimer disease although this is controversial, because cell death due to oxidative injury is suspected to be a contributory factor in many neurotoxicity and neurodegenerative disorders [Abreo *et al*., 1999; Latha *et al*., 2002].

Toxicity of aluminum comes from substitution of Mg^{++} and Fe^{++} ions causing disturbances in intracellular signaling, excretory function and cellular growth. Neurotoxic action of Al probably comes from substitution of Mg^{++} ions in ATP, that finally influences function of every ATP-using enzymes. Toxicity of Al to skeletal system results in diminished resistance, thus tendencies to breaking, that resulting from lowering collagen synthesis and slowing down mineralization [Ochmanski & Barabasz, 2000].

On the other hand, Leonard & Gerber [1988] reported that epidemiological studies have provided clear evidence of a carcinogenic, mutagenic and teratogenic hazard of aluminum to man. Moreover, Ochmanski & Barabasz [2000] found high concentrations of Al in many neoplastic cells.

It is very important, nowadays to search for protective substances that could minimize the toxic effects of different chemicals. Vitamins play a beneficial role against the mutagenicity and genotoxicity of some chemicals. Vitamin C has been found to reduce the clastogenic effects of many chemical agents and radiation [Dhir *et al*., 1993; Odin, 1997]. In addition vitamin C is efficient in preventing oxidative stress induced cytotoxicity by aluminum [Ghaskadbi *et al*., 1992; Fahmy & Aly, 2000; Anane & Creppy, 2001].

Therefore, the present study was undertaken to evaluate the effect of different doses of aluminum sulphate on serum protein profile, liver enzyme activities, bilirubin levels and total protein content in mice and the efficiency of vitamin C to minimize the hazard effect of aluminum toxicity.

MATERIAL AND METHODS

Animals. Ninety mature male Swiss mice 9–12 weeks old, weighing 25–30 g were used in all experiments. The animals were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Cairo, Egypt and were maintained under controlled conditions of temperature and humidity. Mice received standard pellet diet (El-

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Table 1. Effect of different doses of aluminum sulphate on serum protein profile after 1, 2 and 3 weeks of the experiment.

Parameters	Control (1)			¼ LD_{50} (2)			1/8 LD_{50} (3)			1/16 LD_{50} (4)			P <			LSD (weeks)		
	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	P 1	P 2	P 3	1 w (a)	2 w (b)	3 w (c)
Prealbumin	11.35 ± 0.02	11.53 ± 0.01	11.72 ± 0.03	6.66 ± 0.01	6.20 ± 0.01	5.40 ± 0.02	8.64 ± 0.04	8.28 ± 1.80	7.74 ± 0.04	9.72 ± 0.05	9.45 ± 0.03	9.10 ± 0.02	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Albumin	1.51 ± 0.05	1.53 ± 0.01	1.52 ± 0.01	5.57 ± 0.53	5.60 ± 0.32	6.20 ± 0.19	3.22 ± 0.19	3.51 ± 0. 20	5.05 ± 0.16	2.03 ± 0.01	2.26 ± 0.11	3.23 ± 0.20	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(c)	(c)	(a, b)
α-1-**Lipoprotein**	8.64 ± 0.01	8.56 ± 0.03	8.56 ± 0.05	6.17 ± 0.01	5.85 ± 0.02	5.31 ± 0.01	7.29 ± 0.01	6.80 ± 0.04	6.39 ± 0.01	8.19 ± 0.08	7.56 ± 0.04	7.29 ± 0.01	0.0001	0.0001	0.069			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
α-1-**Macroglobulin**	13.96 ± 0.05	13.78 ± 0.01	13.87 ± 0.05	8.83 ± 0.02	8.00 ± 0.01	7.56 ± 0.02	10.63 ± 0.05	10.09 ± 0.02	9.63 ± 0.03	13.42 ± 0.02	12.79 ± 0.01	12.16 ± 0.04	0.0001	0.0001	0.004			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
α-1-**Acid glycoprotein**	13.15 ± 0.05	13.78 ± 0.03	13.19 ± 0.04	9.00 ± 0.01	8.46 ± 0.03	7.56 ± 0.03	10.81 ± 0.07	10.27 ± 0.04	9.81 ± 0.01	10.09 ± 0.03	11.53 ± 0.02	10.99 ± 0.02	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2, 4)			(1,2, 3)						(b, c)	(a, c)	(a, b)
α-1-**Antitrypsin**	24.84 ± 0.18	24.41 ± 0.09	24.41 ± 0.08	17.75 ± 0.02	15.76 ± 0.05	14.86 ± 0.06	20.63 ± 0.03	18.64 ± 0.04	17.38 ± 0.05	23.42 ± 0.01	22.34 ± 0.04	21.89 ± 0.04	0.0001	0.0001	0.002			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Cholinesterase	16.11 ± 0.03	16.22 ± 0.06	16.13 ± 0.05	12.70 ± 0.02	11.89 ± 0.01	10.27 ± 0.09	14.32 ± 0.03	13.69 ± 0.02	12.97 ± 0.04	15.85 ± 0.02	15.04 ± 0.18	14.41 ± 0.01	0.0001	0.0001	0.0001			
LSD (groups)	(2,3, 4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Ceruloplasmin	8.64 ± 0.02	8.82 ± 0.02	8.73 ± 0.01	4.68 ± 0.02	4.59 ± 0.01	3.96 ± 0.04	5.94 ± 0.02	5.58 ± 0.01	4.95 ± 0..02	7.92 ± 0.02	7.38 ± 0.01	6.66 ± 0.03	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Haemopexin	11.62 ± 0.01	11.89 ± 0.02	11.53 ± 0.04	9.00 ± 0.03	8.28 ± 0.03	7.56 ± 0.04	10.00 ± 0.02	10.00 ± 0.01	9.45 ± 0.04	10.99 ± 0.02	10.81 ± 0.07	10.27 ± 0.08	0.0001	0.0001	0.001			
LSD (groups)	(2,3,4)			(1,3,4)			(2,3,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Haptoglobin	26.12 ± 0.23	26.03 ± 0.01	25.94 ± 0.02	19.00 ± 0.07	16.93 ± 0.07	14.32 ± 0.05	23.06 ± 0.05	22.07 ± 0.04	21.08 ± 0.08	25.13 ± 0.03	23.96 ± 0.02	23.15 ± 0.03	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Transferrin	27.74 ± 0.03	28.10 ± 0.07	27.47 ± 0.05	19.09 ± 0.05	17.11 ± 0.05	14.05 ± 0.02	22.70 ± 0.01	21.98 ± 0.04	21.89 ± 0.01	26.48 ± 0.04	25.19 ± 0.05	23.42 ± 0.01	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
β-1-**Lipoprotein**	26.48 ± 0.04	26.30 ± 0.02	26.75 ± 0.02	19.27 ± 0.05	17.83 ± 0.02	16.30 ± 0.01	22.61 ± 0.06	21.98 ± 0.02	21.62 ± 0.08	25.31 ± 0.07	25.04 ± 0.02	24.05 ± 0.02	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a, b)
Total protein	113.50 ± 1.29	112.70 ± 1.7	112.50 ± 2.08	80.50 ± 1.00	79.50 ± 1.91	76.75 ± 2.06	83.25 ± 3.90	82.0 ± 1.83	81.25 ± 0.96	92.25 ± 2.60	88.25 ± 2.27	88.25 ± 1.00	0.0001	0.522	0.366			
LSD (groups)	(2,3,4)			(1)			(1)			(1)						(-)	(-)	(-)

Data are means ± SD of five mice in each group. Serum protein profiles are expressed as μg/mL, serum albumin is expressed as mg/dL and serum total protein is expressed as mg/mL. Statistical analysis is performed by using two way analysis of variance (ANOVA) with interaction combined with post hoc (LSD). LSD is least significant difference between groups or weeks. P is level of significance, where $p< 0.05$ is significant. P1 is the significant difference between groups, P2 is the significant difference between durations and P3 is the significant difference between dose and duration.

Kahira Company for Oil and Soap, Egypt) containing 17% protein, 11% fat, 47% carbohydrate, 2.5% minerals, 4.5% fiber, 6.5% ash, and 11.5% water.

Chemicals. All chemicals used were of high analytical grade and originated from different companies: Merck, Germany; Sigma, USA and El-Nasr Pharmaceutical Chemical Company, Egypt. Aluminum sulphate $Al_2 (SO_4)_3$ was obtained form Sarabkai M. Chemicals, India.

Doses and treatment. Oral LD_{50} of aluminum sulphate for mice is 6200 mg/kg b.wt. [Leonard & Gerber, 1988]. Aluminum sulphate was dissolved in bidistilled water and administered by gavage daily for 1, 2 and 3 weeks as a clear solution at three different concentrations 1550, 775 and 387.5 mg/kg b.wt. representing 1/4, 1/8 and 1/16 LD_{50} of the compound, respectively.

Vitamin C was dissolved in bidistilled water and administered by gavage as a clear solution in a dose of 20 mg/kg b.wt. [O'Brien & Luo, 1997] daily for the same duration to evaluate its side effect.

A combined dose of 1/4 LD_{50} of aluminum sulphate and 20 mg/kg b.wt. vitamin C were administered to another group of mice by the same way to evaluate the role of vitamin C in enhancement of the toxic effect of aluminum sulphate.

Blood samples were taken 24 h after the last treatment and then centrifuged at 3000 xg to separate serum.

Experimental design. Animals were divided into six main groups each of 15 mice. Group 1: served as control. Groups 2, 3, 4: received 1/4, 1/8 and 1/16 LD_{50} of aluminum sulphate, respectively. Group 5: received an oral dose of 1/4 LD_{50} of aluminum sulphate in a combination with a dose of 20 mg/kg b.w. vitamin C. Group 6 received the same dose of vitamin C.

Each main group was divided into three sub-groups for evaluating the effect of time after daily administration of aluminum sulphate and vitamin C for 1, 2 and 3 weeks.

Preparation of anti-mice sera in rabbits. Complete Freund's adjuvant and incomplete Freund's adjuvants were used to prepare polyspecific antisera directed against mice serum proteins. Rabbits weighing 1.5–2.0 kg were immunized against mice serum proteins to obtain antiserum, according to the method mentioned by Harlo & Lane [1988].

Two-dimensional (crossed) immunoelectrophoresis. Crossed immunoelectrophoresis was the combination of electrophoretic separation of mice serum proteins (antigens) in agarose gel followed by electrophoresis in the second dimension in an antibody-containing gel according to the method of Clark & Freeman [1968] and Fouad *et al.* [1983].

From the position of albumin and transferrin in the electrophoretic diagram the other proteins were identified, as shown in Figure 1, according to their electrophoretic mobility [Weeke, 1970]. Albumin peak in the electrophorogram was separated but could not be measured because its high peak value. So, it was estimated colorimetrically using the method of Spencer & Price [1977].

Table 2. Effect of different doses of aluminum sulphate on liver function parameters after 1, 2 and 3 weeks of the experiment.

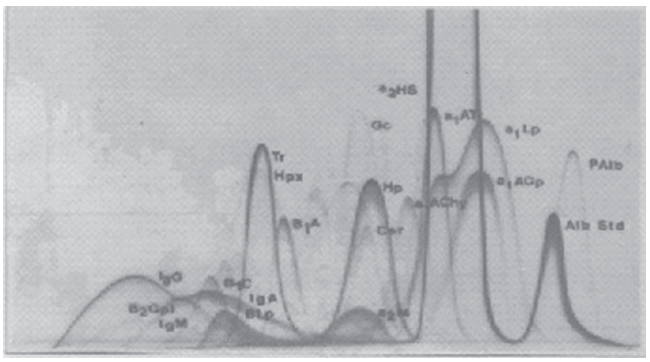
Parameters	Control (1)			¼ LD_{50} (2)			1/8 LD_{50} (3)			1/16 LD_{50} (4)			P <			LSD (weeks)		
	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	P 1	P 2	P 3	1 w (a)	2 w (b)	3 w (c)
Bilirubin	51.75 ± 0.95	52.0 ± 0.82	51.50 ± 1.29	185.50 ± 2.10	191.75 ± 4.85	205.50 ± 7.04	175.20 ± 1.70	177.50 ± 1.75	184.67 ± 3.39	139.37 ± 0.73	152.80 ± 5.22	167.81 ± 2.58	0.0001	0.0001	0.0001			
LSD (groups)		(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)					(b, c)	(a,c)	(a,b)
AST	48.85 ± 1.02	49.00 ± 1.45	48.90 ± 2.81	59.05 ± 3.49	63.35 ± 2.82	69.11 ± 2.66	54.77 ± 1.52	58.84 ± 2.34	63.03 ± 3.40	52.84 ± 1.48	54.39 ± 2.22	59.57 ± 1.43	0.0001	0.0001	0.0001			
LSD (groups)		(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)					(b, c)	(a,c)	(a,b)
ALT	25.69 ± 1.12	25.54 ± 1.14	25.31 ± 2.01	29.26 ± 1.15	33.84 ± 1.11	41.81 ± 1.88	28.91 ± 1.05	33.26 ± 2.31	39.78 ± 1.75	27.00 ± 1.07	32.67 ± 1.07	38.02 ± 1.31	0.0001	0.0001	0.055			
LSD (groups)		(2,3,4)			(1,4)			(1)			(1,2)					(b, c)	(a,c)	(a,b)
ALP	180.13 ± 2.83	180.84 ± 3.94	180.77 ± 2.30	194.28 ± 3.60	202.05 ± 5.61	209.49 ± 4.68	191.06 ± 2.67	198.37 ± 4.88	207.01 ± 5.34	189.16 ± 1.76	196.88 ± 3.82	205.80 ± 7.03	0.0001	0.0001	0.0001			
LSD (groups)		(2,3,4)			(1,4)			(1)			(1,2)					(b, c)	(a,c)	(a,b)

Data are means ± SD of five mice in each group. Liver function enzymes are expressed as U/mL while, bilirubin level is expressed as mg/L. Statistical analysis is performed by using two way analysis of variance (ANOVA) with interaction combined with post hoc (LSD). LSD is least significant difference between groups or weeks. P is level of significance, where $p<0.05$ is significant. P1 is the significant difference between groups, P2 is the significant difference between durations and P3 is the significant difference between dose and duration.

TABLE 3. Effect of vitamin C on serum protein profile after 1, 2 and 3 weeks of the experiment.

Parameters	Control (1)			Vitamin C (2)			1/4 LD_{50} (3)			1/4 LD_{50} + Vitamin C (4)			P <			LSD (weeks)		
	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	P_1	P_2	P_3	1 w (a)	2 w (b)	3 w (c)
Prealbumin	11.35 ± 0.02	11.53 ± 0.01	11.72 ± 0.03	11.89 ± 0.05	12.43 ± 0.02	14.23 ± 0.01	6.66 ± 0.01	6.20 ± 0.01	5.40 ± 0.02	9.28 ± 0.08	10.09 ± 0.02	11.08 ± 0.05	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Albumin	1.51 ± 0.01	1.53 ± 0.02	1.52 ± 0.01	1.79 ± 0.02	1.79 ± 0.05	1.77 ± 0.01	5.57 ± 0.53	5.60 ± 0.32	6.20 ± 0.19	3.07 ± 0.15	2.88 ± 0.13	1.79 ± 0.02	0.0001	0.459	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						-	-	-
α-1-**Lipoprotein**	8.64 ± 0.01	8.56 ± 0.03	8.56 ± 0.05	8.55 ± 0.03	8.55 ± 0.04	10.18 ± 0.02	6.17 ± 0.01	5.85 ± 0.02	5.31 ± 0.01	7.47 ± 0.02	8.11 ± 0.01	8.82 ± 0.05	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(c)	(c)	(a,b)
α-1-**Macro-globulin**	13.96 ± 0.05	13.78 ± 0.01	13.87 ± 0.05	14.05 ± 0.03	15.04 ± 0.01	15.58 ± 0.05	8.83 ± 0.02	8.00 ± 0.01	7.56 ± 0.02	12.79 ± 0.02	13.42 ± 0.01	14.05 ± 0.02	0.0001	0.008	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(c)	(c)	(a,b)
α-1-**Acid glycoprotein**	13.15 ± 0.05	13.78 ± 0.03	13.19 ± 0.04	14.05 ± 0.04	14.95 ± 0.04	15.67 ± 0.04	9.00 ± 0.01	8.46 ± 0.03	7.56 ± 0.03	11.98 ± 0.02	13.15 ± 0.02	13.60 ± 0.02	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a)	(a)
α-1-**Antitrypsin**	24.84 ± 0.18	24.41 ± 0.09	24.41 ± 0.08	24.86 ± 0.04	27.02 ± 0.07	31.26 ± 0.06	17.75 ± 0.02	15.76 ± 0.05	14.86 ± 0.06	21.80 ± 0.02	22.25 ± 0.02	23.06 ± 0.05	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Cholinesterase	16.11 ± 0.03	16.22 ± 0.06	16.13 ± 0.05	16.75 ± 0.02	17.65 ± 0.04	18.91 ± 1.08	12.70 ± 0.02	11.89 ± 0.01	10.27 ± 0.09	11.62 ± 0.04	13.78 ± 0.03	16.75 ± 0.04	0.0001	0.0001	0.0001			
LSD (groups)	(2,3, 4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Ceruloplasmin	8.64 ± 0.02	8.82 ± 0.02	8.73 ± 0.01	8.64 ± 0.09	10.64 ± 0.04	11.62 ± 0.03	4.68 ± 0.02	4.59 ± 0.01	3.96 ± 0.04	7.38 ± 0.02	7.92 ± 0.01	9.59 ± 0.05	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Haemopexin	11.62 ± 0.01	11.89 ± 0.02	11.53 ± 0.04	13.15 ± 0.04	14.05 ± 0.05	14.95 ± 0.01	9.00 ± 0.03	8.28 ± 0.03	7.56 ± 0.04	8.46 ± 0.05	10.81 ± 0.04	12.34 ± 0.03	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Haptoglobin	26.12 ± 0.23	26.03 ± 0.01	25.94 ± 0.02	27.92 ± 0.05	29.00 ± 0.05	32.16 ± 0.08	19.00 ± 0.07	16.93 ± 0.07	14.32 ± 0.05	19.81 ± 0.01	22.97 ± 0.04	25.58 ± 0.03	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Transferrin	27.74 ± 0.03	28.10 ± 0.07	27.47 ± 0.05	32.43 ± 0.05	29.10 ± 0.03	32.14 ± 0.09	19.09 ± 0.05	17.11 ± 0.05	14.05 ± 0.02	20.36 ± 0.05	23.15 ± 0.02	25.22 ± 0.07	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a)	(a)
β-1-**Lipoprotein**	26.48 ± 0.04	26.30 ± 0.02	26.75 ± 0.02	27.47 ± 0.05	29.63 ± 0.04	31.17 ± 0.04	19.27 ± 0.05	17.83 ± 0.02	16.30 ± 0.01	22.43 ± 0.04	23.69 ± 0.03	25.31 ± 0.02	0.0001	0.0001	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(b, c)	(a, c)	(a,b)
Total protein	113.50 ± 1.29	112.70 ± 1.7	112.50 ± 2.08	125.25 ± 3.30	130.50 ± 1.73	142.00 ± 6.21	80.50 ± 1.00	79.50 ± 1.91	76.75 ± 2.06	94.75 ± 0.95	98.75 ± 2.50	100.40 ± 1.50	0.0001	0.022	0.0001			
LSD (groups)	(2,3,4)			(1,3,4)			(1,2,4)			(1,2,3)						(c)	(c)	(a, b)

Data are means ± S.D. of five mice in each group. Serum protein profiles are expressed as μg/mL, serum albumin is expressed as mg/dL and serum total protein is expressed as mg/mL . Statistical analysis is performed by using two way analysis of variance (ANOVA) with interaction combined with post hoc (LSD). LSD is least significant difference between groups or weeks. P is level of significance, where $P \leq 0.05$ is significant. P_1 is the significant difference between groups, P_2 is the significant difference between durations and P_3 is the significant difference between dose and duration.



PA1b	= Prealbumin	ChE	= Cholinesterase
A1b	= Albumin	Cer	= Ceruloplasmin
α1-Lp	= α1-Lipoprotein	Hpx	= Haemopexin
α1-M	= α1-Macroglobulin	Hp	= Haptoglobin
α1-AGP	= α1-Acid glycoprotein	TR	= Transferrin
α1AT	= α1-Antitrypsin	β-Lp	= β-Lipoprotein

FIGURE 1. 2D-Immunoelectrophoresis on Cellogel sheets.

Biochemical assays. Serum total protein was estimated according to the method of Bradford [1976]. Determination of bilirubin content was carried out by the method of Henry [1974]. Alanine and aspartate aminotransferases were assayed according to the method of Bergmeyer & Bernt [1974]. Alkaline phosphatase was estimated according to the method of Belfield & Goldberg [1971].

Statistical analysis. The statistical significance of the results was determined by two-way analysis of variance (ANOVA) combined with post-hoc (least significant difference) test (SPSS-computer program).

RESULTS

Table 1 showed a significant reduction in prealbumin level at 1/4, 1/8 and 1/16 LD_{50} of aluminum sulphate after 1st, 2nd and 3rd weeks of administration, recording the lowest level at the 3rd week using 1/4 LD_{50} of aluminum sulfate. On the other hand, albumin showed a significant increase using different doses of aluminum sulphate as compared to the control mice, recording the highest level at the 3rd week using 1/4 LD_{50} of aluminum sulphate. Significant reduction in the level of α-lipoprotein, α-1-macroglobulin, α-1-acid glycoprotein, α-1-antitrypsin, cholinesterase, ceruloplasmin, haemopexin, haptoglobin, transferrin, β-lipoprotein and total protein content was noticed in a dose-dependant manner with follow up of time. Hence, protein fractions were affected by doses of aluminum sulphate, time as well as the interaction between doses and duration.

Liver function enzyme activities AST, ALT and ALP and bilirubin levels showed a significantly elevated level with all doses of aluminum sulphate after different durations recording the highest level at the 3rd week using 1/4 LD_{50} (Table 2). A significant difference between weeks, doses of aluminum sulphate and a significant effect of the interaction between them were observed as well.

Vitamin C administrated to healthy mice showed a significant increase in prealbumin, α-lipoprotein, α-1-macroglob-

Table 4. Effect of vitamin C on liver function parameters after 1, 2 and 3 weeks of the experiment.

Parameters	Control (1)			Vitamin C (2)			1/4 LD_{50} (3)			1/4 LD_{50} + Vitamin C (4)			P <			LSD (weeks)		
	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	1 w	2 w	3 w	P_1	P_2	P_3	1 w (a)	2 w (b)	3 w (c)
Bilirubin	51.75 ± 0.95	52.0 ± 0.82	51.50 ± 1.29	50.25 ± 0.96	52.75 ± 0.50	51.50 ± 1.29	185.50 ± 2.10	191.75 ± 4.85	205.50 ± 7.04	81.95 ± 0.83	80.56 ± 0.66	74.75 ± 1.89				(c)	(-)	(a)
LSD (groups)		(3,4)		(3,4)			(1, 2, 4)			(1, 2, 3)			0.0001	0.007	0.0001			
AST	48.85 ± 1.02	49.00 ± 1.45	48.90 ± 2.81	49.22 ± 2.02	49.26 ± 2.93	50.00 ± 2.30	59.05 ± 3.49	63.35 ± 2.82	69.11 ± 2.66	50.86 ± 1.71	57.36 ± 2.09	61.57 ± 2.41				(b, c)	(a, c)	(a, b)
LSD (groups)		(3,4)		(3,4)			(1, 2, 4)			(1, 2, 3)			0.0001	0.0001	0.0001			
ALT	25.69 ± 1.12	25.54 ± 1.14	25.31 ± 2.01	26.00 ± 1.12	25.60 ± 1.80	25.21 ± 2.39	29.26 ± 1.15	33.84 ± 1.11	41.81 ± 1.88	27.78 ± 1.01	32.84 ± 1.17	37.21 ± 2.15				(c)	(a, c)	(a, b)
LSD (groups)		(3,4)		(3)			(1, 2, 4)			(1, 3)			0.0001	0.0001	0.059			
ALP	180.13 ± 2.83	180.84 ± 3.94	180.77 ± 2.30	180.84 ± 3.24	180.25 ± 4.19	179.70 ± 2.53	194.28 ± 3.60	202.05 ± 5.61	209.49 ± 4.68	190.69 ± 4.47	196.71 ± 3.26	201.51 ± 5.81				(c)	(-)	(a)
LSD (groups)		(3,4)		(3,4)			(1, 2, 4)			(1, 2, 3)			0.0001	0.039	0.0001			

Data are means ± SD. of five mice in each group. Liver function enzymes are expressed as U/mL while, bilirubin level is expressed as mg/L. Statistical analysis is performed by using two way analysis of variance (ANOVA) with interaction combined with post hoc (LSD). LSD is least significant difference between groups or weeks. P is level of significance, where $P \leq 0.05$ is significant. P_1 is the significant difference between groups, P_2 is the significant difference between durations and P_3 is the significant difference between dose and duration.

ulin, α-1-acid glycoprotein, α-1-antitrypsin, cholinesterase, ceruloplasmin, haemopexin, haptoglobin, transferrin, β-lipoprotein and total protein content. Amelioration in the level of serum protein fraction concentrations and total protein content was observed after different durations of vitamin C administration with 1/4 LD_{50} of aluminum sulphate recording 48.46, 290.00, 40.58, 46.79, 45.10, 33.59, 40.17, 64.49, 41.46, 43.41, 40.66, 33.68 and 21.02%, respectively at the 3rd week of administration, which recorded the highest percentage value of enhancement (Table 3). Therefore, protein fractions were significantly affected by the dose of vitamin C administered with 1/4 LD_{50} of aluminum sulphate, duration and the interaction of dose and time.

Vitamin C administrated to healthy mice showed an insignificant change in liver function enzyme activities and bilirubin level as compared to the control group (Table 4). The same enhanced manner and the same effect of dose, time and interaction between them were observed in AST, ALT, ALP and bilirubin levels at the different durations of vitamin C administration with 1/4 LD_{50} of aluminum sulphate recording the highest percentages value of improvement at the 3rd week amounting to 14.44, 13.80, 4.46 and 253.88%, respectively (Table 4).

DISCUSSION

The present study revealed a significant reduction in prealbumin level at the different doses of aluminum sulphate after 1st, 2nd and 3rd weeks of administration and recorded the lowest significant level using the highest concentration and the longest treatment. Swain & Chainy [1997] reported that aluminum treatment constitutes one of the factors for the mechanism of tissue injury. Therefore, the production of prealbumin by the hepatocytes decreases [Princen *et al*., 1981; Dickson *et al*., 1982]. Based on this finding, the reported reduction in prealbumin level may be due to a decrease in prealbumin gene transcriptional. Moreover, the increase in the rate of degradation of prealbumin is considered to be an important factor that determines the decrease in prealbumin level.

In addition, Leonard & Gerber [1988] found reduction in ribosomal RNA content to bind to nuclear chromatin in aluminum exposure. Thus, the reduction in prealbumin level may be due to a decrease in the mechanism of protein synthesis .

On the other hand, albumin level showed a significant increased level at all doses of aluminum sulphate after different durations, recording the highest significant level at the 3rd week using 1/4 LD_{50}. In accordance with the present results, Scornik & Botbol [1976] and Leonard & Gerber [1988] stated that aluminum was found to increase some protein fractions, thus the biosynthesis of excretable serum albumin may be increased. The authors added that the decrease in albumin degradation rate may be an important factor that determines the increased level of albumin. Moreover, Hoch-Ligetti [1953] and Ochmanski & Barabasz [2000] reported that disturbance in the enzyme system of liver metabolism after aluminum exposure may be at least in part responsible for the protein disequilibrium. Panduro *et al.* [1986] stated that changes in serum α-fetoprotein and albumin concentrations were closely correlated with the rate of gene transcription and the increase in albumin level may be due to an increased rate of transcription and not switch to α-fetoprotein.

In contrast to the present results, Fouad *et al.* [1983] and Dinarello [2000] found a decrease in albumin concentration in response to tissue injury such as inflammatory reactions. The authors attributed this reduction to the acute phase response and decreased hepatic albumin synthesis, although the liver is producing increasing amounts of a variety of proteins.

Concerning α-lipoprotein and β-lipoprotein level, a significant reduction was noticed with different doses of aluminum sulphate after 1st, 2nd and 3rd weeks of administration, as compared to the control group. The reduction in α- and β-lipoproteins may be due to a decrease in the synthesis of lipid moiety (since, α-lipoprotein is more concerned with HDL while β-lipoprotein is concerned with LDL and hormones). Moreover, the synthesis of the apoprotein could also be decreased, the conjugation step which binds the lipid moiety to the protein moiety is curtailed, the release of lipoproteins into the serum is inhibited as well as lipoproteins may be rapidly withdrawn from the circulation (metabolized by the liver) than released into it [Feingold, 1989a].

Lukiw *et al*. [1998] reported that brain gene transcription in the presence of trace amounts of ambient aluminum impairs mammalian brain DNA, genetic information and brain-immunological signals. In addition, Campbell *et al*. [1999] and Tariq *et al*. [1999] reported that aluminum sulphate causes cell death due to oxidative injury caused by increased free radicals that could be a contributory factor in many neuro- and nephrotoxicity. Thus, as a result of neurological toxicity, the different immunological signals mediated by cytokines that stimulate lipid synthesis and lipoproteins release could be disturbed and represent a deleterious part of the acute phase response [Feingold *et al*., 1989b].

With regard to α-1-macroglobulin, a significant reduction was noticed in a dose dependent relationship of aluminum sulphate with follow up of time. Milland *et al*. [1990] reported that the reduction in serum α-1-macroglobulin is due to a decrease in the mRNA level. However Princen *et al*. [1981], Dickson *et al*. [1982] and Fouad *et al*. [1983] referred the reduction in α-1-macroglobulin in response to chemical toxicity, *i.e*. to decrease in its production by hepatocytes up to 50%. In addition, the capacity for acute phase response of serum α-1-macroglobulin is significantly reduced and this may be due to dysfunction in protein synthesis mechanism, that in turn leads to the reduction in the level of total protein content and its fractions [Alexandrakis *et al*., 2001].

The present results showed a significant decrease in serum α-1-acid glycoprotein, α-1-antitrypsin and cholinesterase. In accordance with the present results, Onda [1977] suggested the significant reduction in α-1-acid glycoprotein and α-1--antitrypsin to the suppression of mitosis of the hepatocyte. This explanation is supported by Leonard & Gerber [1988] that aluminum sulphate inhibited mammalian cell division. Milland *et al*. [1990] and Motta *et al*. [2001] reported that the responses of acute phase α-1-acid glycoprotein, α-1-antitrypsin and cholinesterase are greatly reduced in response to tissue injury. Moreover, in accordance to the present findings Mittra *et al*. [2001] found an inhibition in serum cholinesterase activity post aluminum phosphide poisoning.

Contradictory to the present results Fouad *et al*. [1983]

found an increase in serum α-1-acid glycoprotein and α-1--antitrypsin in response to chemical poisoning and tissue injury such as inflammation.

The present results indicated also a significant reduction in serum ceruloplasmin in response to different doses of aluminum sulphate after different durations. In agreement with the present results, Pierre *et al*. [1988] and Milland *et al*. [1990] reported that acute-phase response of ceruloplasmin is greatly reduced in inflammatory tissue. In contrast to the present finding, Princen *et al*. [1982] and Dickson *et al*. [1982] found that the production of ceruloplasmin increased substantially by liver hepatocytes in response to tissue injury.

The obtained results showed a significant reduction in serum haemopexin, haptoglobin and transferrin in a dose-dependent manner of aluminum sulphate through follow up of time.

As a matter of fact, haptoglobin, haemopexin and transferrin are the parameters concerned with carriage of free hemoglobin and its liberated catabolic component porphyrin. Haemopexin is particularly specified for heme carriage and recycling, while transferrin is iron binding and transport protein [Ganong, 1999; Vittori *et al*., 1999].

In accordance to the present finding, Vittori *et al*. [1999] found that decreased serum haptoglobin concentration in aluminum citrate treated rats, supports the assumption of haemolytic nature of anemia. The authors added that erythropoiesis impairment induced by aluminum may be a combined effect of direct action on circulating erythrocytes and interference with the cellular iron metabolism in erythroid progenitors. Moreover, Ganchev *et al*. [1998] and Gonzalez--Revalderia *et al*. [2000] reported that in low-level aluminum intoxication, ferritin and transferrin were decreased. Ochmanski & Barabasz [2000] added that aluminum causes erythropoietin production, inhibition of hem-synthesizing enzymes and symptoms of anemia due to binding to transferrin.

The present results are concerned with a significant decrease in serum total protein content in aluminum intoxication and recording severe significant reduction in a dose-dependant relationship and through follow up of time. The dramatic increase in protein degradation is thought to be the single most important factor determining the decrease in total protein content [Scornik & Botbol, 1976].

The basis for such a decrease seems to be an increase in the activity of lysosomal endopeptidases and cathepsins A and D or cathepsins B1 and D [Stein *et al*., 1971; Suleiman *et al.*, 1980].

Ochmanski & Barabasz [2000] suggested that aluminum salts may bind to DNA, RNA and inhibit them, reducing protein synthesis. Also the reduction in serum total protein content may be due to an increase in its excretion resulting from tissue damage, since aluminum causes nephrotoxicity [Tariq *et al*., 1999].

Recently Crichton *et al*. [2002] found that aluminum reduced protein content in mammalian cells.

In contrast to the present findings Miller & Levine [1974] reported that aluminum was found to increase protein synthesis.

Concerning liver function enzyme activities AST, ALT and ALP and bilirubin levels, their significantly elevated levels were observed after different durations of aluminum sulphate treatments and at all doses, recording the highest level at the 3rd week using 1/4 LD_{50}. The activities of transaminases can serve as an index of the metabolic "aerobity" degree or a relative role of aerobic and anaerobic pathways in the energy metabolism of different animals, since AST can provide Kreb's cycle intermediates which, in turn, favour the succinate production, while ALT can be correlated to lactate production through transforming alanine to pyruvate, these observations led to express a supposition about a regulatory role of AST in oxidative metabolism, while ALT participates in the regulation of glycolysis [Zayed, 1998]. The diminution of AST was more manifested than that of ALT, demonstrating that, although, the latter is more specific for liver cells, yet it is less sensitive than AST in detecting liver cell damage [Ghanem *et al*., 1970a,b].

Anane & Creppy [2001] reported that free radicals were elaborated by aluminum sulphate toxicity leading to the accumulation of calcium in the mitochondria and resulted in irreversible damage to its membrane, leading to discharge of its enzymes content to the circulation [Farber *et al*.,1981].

Based on this finding, AST and ALT enzyme activities are sub-cellular localized in the mitochondria and cytoplasm [Van Noorden & Frederiks, 1992] and their elevated levels in serum may result from discharge of the enzymes from mitochondrial membrane to the circulation. Moreover, Suolinna *et al*. [1989]; El-Demerdash [2004] and Yosef [2004] stated that aminotransferases enzymes can be considered as a marker enzyme for cell toxicity and their serum elevated level after aluminum salt administration give an additional support for aluminum cytotoxicity.

The increased serum level of ALP and bilirubin after aluminum sulphate administration may be related to proliferation of bile ductules and canaliculi [Mansy *et al*., 1990] and damage in nuclear membrane by the elaborated free radicals which lead to the discharge of the enzyme through the plasma membrane, since ALP is localized mainly in the nucleus and supernatant fraction of the cell [Ghanem *et al*., 1970a,b; Campbell *et al*., 1999; Anane & Creppy, 2001] and/or may be due to aluminum acting as a signal on gene expression, so the transcription of DNA specific sequence into messenger RNA is repressed. Gene repression is an effective way for inhibiting enzyme activity [Hoek *et al*., 1997; Ochmanski & Barabasz, 2000].

Our data confirmed the medical importance and the curative effects of vitamin C in amelioration of serum protein fraction concentrations, total protein content, liver function enzyme activities and bilirubin level when added with 1/4 LD_{50} of aluminum sulfate. Previous reports indicated that vitamin C (ascorbic acid) prevents lipid peroxidation and protects antioxidant system, so prevents accumulation of free radicals and their hazardous action [Anane & Creppy, 2001; Ramanathan *et al*., 2002; Yosef, 2004].

Also vitamin C plays an important role against mutagenicity and successfully reduces the clastogenic effects [Odin, 1997; Fahmy & Aly, 2000].

Furthermore, ascorbic acid reduces blood pressure, increases hemoglobin concentration, reduces irreversibly sickled cells, abolishes the erythrocyte osmotic fragility and increases resistance of the cells to lysis in children suffering from sickle cell anemia [Jaja *et al*., 2002].

It also inhibits proliferation of leukemic cells, non enzymatic glycation and peroxidation in diabetic rats and protects

kidney from damage during hyperglycemia [Qian *et al*., 2000; Zhong *et al*., 2001].

Ochmanski & Barabasz [2000] reported that aluminum salts may bind to DNA and RNA and inhibit such enzymes as hexokinase, acid and alkaline phosphatases, phosphodiesterase and phosphooxydase and protein synthesis. Based on these findings, administrations of vitamin C with 1/4 LD_{50} of aluminum sulphate in the present results showed an enhanced level of all protein fraction concentrations, total protein content and liver function tests, that may result from vitamin C stimulation of the protein synthesis mechanism through breaking down the binding of aluminum with DNA and RNA. Thus administration of vitamin C to healthy normal animal stabilized and protected the normal biological conditions of the body and hence promoted protein synthetic machineries [Anane & Creppy, 2001], which is consistent with the present findings of a significant increase in serum total protein content and protein fraction in vitamin C-treated normal group, while it preserved normal liver enzymes.

CONCLUSIONS

In conclusion, all doses of aluminum sulphate showed a drastic effect on serum protein fraction concentrations, total protein content, liver function enzymes and bilirubin levels recording the most severe action at the third week of 1/4 LD_{50}. Vitamin C plays a protective role against aluminum sulphate toxicity causing amelioration in all parameters under investigation. On the other hand, vitamin C administrated to healthy animal increased total protein content and protein fraction concentrations and preserved normal liver function parameters.

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