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Arsenic toxicity in mice and its possible amelioration

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Abstract: Oral administration of arsenic trioxide(3 and 6 mg/kg body weight/d) for 30 d caused, as compared with vehicle control, dose-dependent significant reductions in body weight, absolute weight, protein, glycogen, as well as, total, dehydro and reduced ascorbic acid contents both in the liver and kidney of arsenic-treated mice. Succinic dehydrogenase(SDH) and phosphorylase only in the liver activities were significantly reduced in a dose-dependent manner. Acid phosphatase activity was significantly decreased in the liver of low dose arsenic-treated animals; however, significant rise in its activity was observed in high dose group. As compared with vehicle control, treatment also caused significant dose-dependent reductions in SDH, alkaline phosphatase and acid phosphatase activities in the kidney of mice. Vitamin E cotreatment as well as, 30 d withdrawal of arsenic trioxide treatment with or without vitamin E caused significant amelioration in arsenic-induced toxicity in mice. Administration of vitamin E during withdrawal of treatment also caused significant amelioration as compared from only withdrawal of the treatment. It is concluded that vitamin E ameliorates arsenic-induced toxicities in the liver and kidney of mice.

Keywords: arsenic toxicity; mice; oral administration; amelioration

Introduction

Chronic human poisoning from leaching of arsenicals into natural domestic water supplies has been a problem in several areas of the world(Tseng, 1999). The biggest arsenic calamity in the world is in West Bengal, India covering an area of about 37493 km^2 having about 24 million population. People of this area have higher arsenic content in their hair, urine and skin. Many peoples have arsenic skin lesions such as melanosis, leucomelanosis, keratosis, hyperkeratinosis, gangrene and skin cancer(Dhar, 1997).

Once absorbed, arsenic gets distributed in the liver, kidney, skin, lungs and spleen. The highest activity of ^{74}As per gram of tissue was found in the liver and kidney of mice (Cikrt, 1980). Arsenic induced hepatotoxicity (Reichl, 1991; Flora, 1997) and nephrotoxicity (Hirata, 1990; Flora, 1997) have been reported. Ramos (Ramos, 1995) reported that there is a positive correlation between arsenic concentration and lipid peroxidation levels in the liver and kidney of rats. Yamanaka and Okada(Yamanaka, 1994) also reported induction of lung specific DNA damage by metabolically methylated arsenics via the production of free radicals. The basic function of vitamin E in living organisms is its role as an antioxidant, that is, it protects all cell membrane lipids and unsaturated fatty acids against oxidative degradation(Odin, 1997).

The aim of the present investigation was to evaluate the effect of oral administration of 3 and 6 mg of arsenic trioxide/kg body weight/d for 30 d in the liver and kidney of mice. Its possible amelioration on vitamin E cotreatment and on 30 d withdrawal of treatment with and without vitamin E was also evaluated.

1 Materials and methods

1.1 Animals

Young inbred Swiss strain male albino mice (*Mus musculus*) weighing approximately 30—35 g were obtained from Cadila Health Care, Ahmedabad, India. All animals were fed with standard chow and water *ad-libitum* and maintained under laboratory condition.

1.2 Experimental design

100 such male mice were divided in 10 groups and caged separately. Animals of group Ⅰ and Ⅱ received tap water and vitamin E[2 mg/(0.2 ml olive oil · animal · d)] respectively for 30 d and served as vehicle and vitamin E controls respectively. Animals of group Ⅲ and Ⅳ were orally administered with 3 and 6 mg of arsenic trioxide/kg body weight/d for 30 d. In addition to arsenic trioxide treatment as mentioned for group Ⅲ and Ⅳ animals, group Ⅴ and Ⅵ animals were also administered with vitamin E [2 mg/(0.2 ml olive oil · d)] along with arsenic trioxide for 30 d. Possible amelioration of arsenic toxicity on 30 d withdrawal of treatment without (group Ⅶ and Ⅷ) and with vitamin E treatment (group Ⅸ and Ⅹ) was also evaluated(Table 1).

Arsenic trioxide (analytical grade of Hi-Media Laboratories Pvt. Ltd., Mumbai, India) was dissolved in few drops of HCl and diluted with tap water. pH 7.0 of the solution was adjusted using 0.1 mol/L NaOH. Analytical grade olive oil and vitamin E(DL-tocopherol) were obtained from Figaro, Madrid, Spain and Hi-Media Laboratories Pvt.

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Ltd. Mumbai, India respectively. The doses of arsenic were based on LD_{50} value in mice (39.4 mg of arsenic trioxide/kg body weight) (Harrington, 1978). The effective dose of vitamin E was based on the earlier work in male mice (Chinoy, 1998). All treatments were given orally using a feeding tube attached to a hypodermic syringe.

Table 1 Experimental protocol

Group	Treatment	Duration of treatment, d	Autopsy, d
Ⅰ	Vehicle control	30	31st
Ⅱ	Vitamin E control[2 mg/(0.2 ml olive oil·animal·d)]	30	31st
Ⅲ	Low dose(3 mg/kg body weight) arsenic treated[(0.1 mg arsenic trioxide/(0.2 ml water·animal·d))	30	31st
Ⅳ	High dose(6 mg/kg body weight) arsenic treated[0.2 mg arsenic trioxide/ (0.2 ml water·animal·d)]	30	31st
Ⅴ	Low dose arsenic (0.1 mg arsenic trioxide/0.2 ml water·mouse·d) and vitamin E[2 mg/(0.2 ml olive oil·animal·d)] treated	30	31st
Ⅵ	High dose arsenic[0.2 mg arsenic trioxide/(0.2 ml water·mouse·d)] and vitamin E[2 mg/(0.2 ml olive oil·animal·d)] treated	30	31st
Ⅶ	Low dose arsenic[0.1 mg arsenic trioxide/(0.2 ml water·mouse·d)] for 30 d followed by 30 d of withdrawal	30 + 30	61st
Ⅷ	High dose arsenic [0.2 mg arsenic trioxide/(0.2 ml water·mouse·d)] for 30 d followed by 30 d of withdrawal	30 + 30	61st
Ⅸ	Low dose arsenic[0.1 mg arsenic trioxide/(0.2 ml water·mouse·d)] for 30 d followed by vitamin E treatment[2 mg/(0.2 ml olive oil·animal·d)] for 30 d	30 + 30	61st
Ⅹ	High dose arsenic[0.2 mg arsenic trioxide/(0.2 ml water·mouse·d)] for 30 d followed by vitamin E treatment[2 mg/(0.2 ml olive oil·animal·d)] for 30 d	30 + 30	61st

1.3 Morphological and biochemical analysis

Behavioural changes(activity status) in treated groups of mice were compared with the controls. To examine the effect of arsenic trioxide on body weight, each group of animals was weighed individually and means weight calculated. On completion of the treatment, the animals were sacrificed by cervical dislocation. The liver and kidney were isolated, blotted free of blood and weighed. The relative weights were calculated.

Total, dehydro-and reduced ascorbic acid contents were estimated by the method of Roe and Kuether(Roe, 1943). The glycogen and protein contents in the tissues were estimated by the method of Seifter(Seifter, 1950) and Lowry *et al*. (Lowry, 1951) respectively. The succinic dehydrogenase(SDH) activity was estimated by the method of Beatty *et al*. (Beatty, 1966) using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetra-zolium chloride(INT) as electron acceptor. Activities of acid and alkaline phosphatases were measured by the method as described by Bessey *et al*. (Bessey, 1945) and Sigma Technical Bulletin No. 104 respectively. The activity of phosphorylase was assayed by the method of Cori *et al*.(Cori, 1943). The inorganic phosphate released at the end of the reaction was estimated by the method of Fiske and Subbarao(Fiske, 1925). Tissue samples used for biochemical analysis were always from the same region of the organ.

1.4 Statistical analysis

For each parameter, at least 10 replicates were done. Results are expressed as mean ± *SEM*. The data were statistically analyzed using students *t*-test; $p < 0.05$ was considered as significant. Comparison of *p*-values were performed between group Ⅰ, group Ⅲ and Ⅳ (vehicle control vs. arsenic-treated) and between group Ⅲ, group Ⅴ, Ⅶ and Ⅸ (low dose arsenic-treated vs. low dose arsenic-treated plus vitamin E, withdrawal of treatment without vitamin E and with vitamin E) and between group Ⅳ and group Ⅵ, Ⅷ and Ⅹ (high dose arsenic-treated vs. high dose arsenic-treated plus vitamin E, withdrawal of treatment without vitamin E and with vitamin E). Comparisons were also made between withdrawal of treatment with and without vitamin E treatment.

2 Results

2.1 Clinical signs

Oral administration of arsenic trioxide for 30 d caused dullness, laziness, hair fall, and appearance of pigmented spots on the skin and hyper-keratinosis. Arsenic treatment also caused swelling of the feet; sometimes-gangrenous feet were also observed in high dose arsenic trioxide-treated mice. Only dullness was observed in mice co-treated with vitamin E and arsenic trioxide. Almost complete recovery occurred after 30 d of withdrawal of treatment with and without vitamin E.

2.2 Body weight

Arsenic trioxide treatment for 30 d caused dose-dependent, significant reduction in the body weight of mice as compared with the vehicle control. Co-treatment with vitamin E as well as withdrawal of treatment with or without vitamin E significantly ameliorates arsenic induced reduction in body weight as compared with arsenic-treated alone. However maximum recovery was observed in mice administered with vitamin E during withdrawal of the treatment. Administration of vitamin E during withdrawal of treatment also caused significant amelioration, as compared from only withdrawal of treatment, in body weight (Table 2).

2.3 Liver

Oral administration of arsenic trioxide for 30 d caused dose-dependent, significantly lower absolute liver weight of mice than that of vehicle control. Vitamin E co-treatment as well as withdrawal of treatment with and without vitamin E significantly ameliorates, as compared with arsenic alone

Table 2 Alterations in body weight of arsenic trioxide-treated and vitamin E co-treated mice

Groups of animals	Body weight, g	Change, %
Vehicle control	33.71 ± 0.55	—
Vitamin E control	35.14 ± 0.57	+4.24
Low dose		
Arsenic trioxide treated	23.65 ± 0.29[a]	−29.84
Arsenic trioxide + vitamin E treated	32.02 ± 0.20[b]	−5.01
30 d withdrawal	28.11 ± 0.26[b]	−16.66
30 d withdrawal + vitamin E treated	33.78 ± 0.14[bd]	+0.20
High dose		
Arsenic trioxide treated	20.66 ± 0.17[a]	−38.71
Arsenic trioxide + vitamin E treated	29.19 ± 0.13[c]	−13.40
30 d withdrawal	28.11 ± 0.26[c]	−25.42
30 d withdrawal + vitamin E treated	31.88 ± 0.23[cd]	−5.40

Notes: values are mean ± *SEM*; $N = 10$; values in parentheses indicates percent change from vehicle control; [a] $p < 0.05$ as compared with control; [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated; [d] $p < 0.05$ as compared with withdrawal of the treatment

treated groups, in absolute liver weight. Significantly higher relative liver weight was observed in arsenic-treated mice. As compared with arsenic trioxide-treated, withdrawal of treatment significantly ameliorates arsenic-induced changes in relative liver weight. However, vitamin E co-treatment as well as vitamin E treatment during withdrawal of treatment significantly reduces the relative liver weight (Table 3).

Arsenic trioxide treatment also caused dose-dependent, significant reduction in protein, glycogen, as well as, total, dehydro and reduced ascorbic acid content as, compared with that of vehicle control. Vitamin E co-treatment as well as withdrawal of treatment with and without vitamin E caused significant amelioration in arsenic-induced changes, as compared with arsenic-treated alone. However maximum recovery was observed in withdrawal plus vitamin E-treated animals. Administration of vitamin E during withdrawal of treatment caused, as compared with only withdrawal of treatment, significant amelioration in all these parameters (except dehydro ascorbic acid) (Table 4).

Table 3 Effect of arsenic trioxide in liver weight of mice

Groups of animals	Liver weight, g			
	Absolute weight, g	Change, %	Relative weight, g /100 g body weight	Change, %
Vehicle control	3.16 ± 0.04		9.37 ± 0.07	
Vitamin E control	3.22 ± 0.04	+1.89	9.17 ± 0.05	−2.13
Low dose				
Arsenic trioxide treated	2.47 ± 0.02[a]	−21.83	10.44 ± 0.06[a]	+11.41
Arsenic trioxide + vitamin E treated	2.92 ± 0.02[b]	−7.59	9.12 ± 0.02[b]	−2.66
30 d withdrawal	2.78 ± 0.02[b]	−12.02	9.89 ± 0.09[b]	+5.54
30 d withdrawal + vitamin E treated	2.67 ± 0.02[bd]	−15.50	7.90 ± 0.04[bd]	−15.6
High dose				
Arsenic trioxide treated	2.21 ± 0.03[a]	−30.06	0.69 ± 0.06[a]	+14.08
Arsenic trioxide + vitamin E treated	2.69 ± 0.02[c]	−14.87	9.22 ± 0.02[c]	−1.60
30 d withdrawal	2.56 ± 0.03[c]	−18.98	10.19 ± 0.03[c]	+8.75
30 d withdrawal + vitamin E treated	2.48 ± 0.02[cd]	−21.51	7.78 ± 0.03[cd]	−16.9

Notes: values are mean ± *SEM*; $N = 10$; values in parentheses indicates percent change from vehicle control: [a] $p < 0.05$ as compared with control; [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated; [d] $p < 0.05$ as compared with withdrawal of the treatment

Table 4 Effect of arsenic trioxide on biochemical parameters in liver of mice

Groups of animals	Parameters				
	Protein[1]	Glycogen[2]	Total ascorbic acid[3]	Dehydro ascorbic acid[3]	Reduced ascorbic acid[3]
Vehicle control	21.02 ± 0.44	1692 ± 21	80.20 ± 0.09	3.29 ± 0.18	4.77 ± 0.21
Vitamin E control	21.59 ± 0.29	1710 ± 18	79.10 ± 0.15	2.88 ± 0.17	5.11 ± 0.21
Low dose					
Arsenic trioxide treated	11.58 ± 0.23[a]	1248 ± 17[a]	54.60 ± 0.15[a]	2.25 ± 0.10[a]	3.13 ± 0.19[a]
Arsenic trioxide + vitamin E treated	14.82 ± 0.35[b]	1309 ± 13[b]	60.70 ± 0.15[b]	2.81 ± 0.15[b]	3.25 ± 0.18[b]
30 d withdrawal	18.39 ± 0.19[b]	1388 ± 8[b]	66.50 ± 0.17[b]	2.72 ± 0.16[b]	4.52 ± 0.19[b]
30 d withdrawal + vitamin E treated	21.54 ± 0.19[bd]	1502 ± 14[bd]	75.90 ± 0.12[bd]	1.71 ± 0.13[bd]	5.88 ± 0.19[cd]
High dose					
Arsenic trioxide treated	7.46 ± 0.29[a]	966 ± 18[a]	35.40 ± 0.11[a]	1.14 ± 0.09[a]	2.16 ± 0.18[a]
Arsenic trioxide + vitamin E treated	9.66 ± 0.14[c]	1208 ± 16[c]	42.20 ± 0.19[c]	1.98 ± 0.13[c]	2.24 ± 0.08[c]
30 d withdrawal	13.47 ± 0.13[c]	1117 ± 14[c]	45.60 ± 0.11[c]	1.79 ± 0.13[c]	2.77 ± 0.17[c]
30 d withdrawal + vitamin E treated	17.18 ± 0.14[cd]	1426 ± 15[cd]	59.10 ± 0.16[cd]	1.96 ± 0.31[cd]	3.95 ± 0.27[cd]

Notes: values are mean ± *SEM*; $N = 10$; [1] mg/100 mg fresh tissue weight; [2] μg/100 mg fresh tissue weight; [3] mg/ 100 mg fresh tissue weight; [a] $p < 0.05$ as compared with control; [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated; [d] $p < 0.05$ as compared with withdrawal of the treatment

Dose-dependent, significantly lower succinic dehydrogenase and phosphorylase activities were observed in the liver of arsenic-treated mice than that of vehicle control. Alkaline phosphatase activity was significantly higher in the liver of high dose arsenic and vitamin co-treated mice as well as, withdrawal of treatment with and without vitamin E treatment. As compared with vehicle control, acid phosphatase activity was significantly lowered in low dose arsenic-treated group while it was significantly higher in high dose group. Vitamin E co-treatment as well as withdrawal of treatment with and without vitamin E caused significant recovery in arsenic-induced changes. Almost control levels were observed in withdrawal plus vitamin E-treated mice. Administration of vitamin E during withdrawal of treatment significantly ameliorates, as compared with withdrawal without vitamin E, succinic dehydrogenase, acid phosphatase and phosphorylase (in high dose group) activities in the liver of mice (Table 5).

Table 5 Effect of arsenic trioxide on enzymatic changes in the liver of mice

Groups of animals	Parameters			
	Succinic dehydrogenase[1]	Alkaline phosphatase[2]	Acid phosphatase[2]	Phosphorylase[3]
Vehicle control	21.95 ± 0.39	4.10 ± 0.16	9.06 ± 0.14	322.23 ± 11.23
Vitamin E control	22.51 ± 0.31	4.13 ± 0.14	10.73 ± 0.18	331.19 ± 8.01
Low dose				
Arsenic trioxide treated	8.85 ± 0.12[a]	4.38 ± 0.10	8.08 ± 0.21[a]	302.00 ± 10.30
Arsenic trioxide + vitamin E treated	13.68 ± 0.21[b]	4.13 ± 0.10	7.87 ± 0.25	263.94 ± 12.69[b]
30 d withdrawal	16.75 ± 0.16[b]	4.10 ± 0.14	10.44 ± 0.28[b]	362.83 ± 10.8[b]
30 d withdrawal + vitamin E treated	19.96 ± 0.22[bd]	4.37 ± 0.15	8.98 ± 0.25[bd]	329.77 ± 12.81
High dose				
Arsenic trioxide treated	7.34 ± 0.29[a]	4.35 ± 0.22	11.36 ± 0.29[a]	260.27 ± 13.14[a]
Arsenic trioxide + vitamin E treated	10.79 ± 0.13[c]	5.32 ± 0.22[c]	6.64 ± 0.28[c]	190.39 ± 11.08[c]
30 d withdrawal	14.53 ± 0.17[c]	5.72 ± 0.16[c]	12.13 ± 0.21[c]	403.42 ± 12.95[c]
30 d withdrawal + vitamin E treated	17.71 ± 0.71[cd]	5.70 ± 0.15[c]	10.10 ± 0.29[cd]	287.24 ± 13.98[cd]

Notes: values are mean ± *SEM*; $N = 10$; [1] μg formazon formed/100 mg fresh tissue weight/15 min; [2] μmol of *p*-nitrophenol released/100 mg fresh tissue weight/30 min; [3] μg phosphorus released/100 mg fresh tissue weight/15 min; [a] $p < 0.05$ as compared with control; [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated; [d] $p < 0.05$ as compared with withdrawal of the treatment

2.4 Kidney

Oral administration of arsenic trioxide caused, as compared with vehicle control, significant, dose dependent reduction in absolute weight (Table 6), protein, glycogen, as well as total dehydro and reduced ascorbic acid contents in the kidney (Table 7). Activities of succinic dehydrogenase, acid and alkaline phosphatase were also reduced significantly in kidney of arsenic-treated mice (Table 8). Co-treatment with vitamin E, as well as, 30 d withdrawal of treatment with and without vitamin E treatment significantly ameliorates arsenic-induced changes. Maximum recovery was observed in 30 d withdrawal of treatment with vitamin E. Administration of vitamin E during withdrawal of treatment was significantly different when compared with withdrawal of treatment without vitamin E (Tables 6—8).

Table 6 Effect of arsenic trioxide in kidney weight of mice

Groups of animals	Kideney weight, g			
	Absolute weight, g	Change, %	Absolute weight, g/100 g body weight	Change, %
Vehicle control	1.73 ± 0.02		5.20 ± 0.66	
Vitamin E control	1.77 ± 0.01		5.21 ± 0.52	
Low dose				
Arsenic trioxide treated	1.41 ± 0.02[a]	− 18.49	4.86 ± 0.12	− 6.53
Arsenic trioxide + vitamin E treated	1.61 ± 0.08[b]	− 6.93	5.36 ± 0.23[b]	+ 3.07
30 d withdrawal	1.80 ± 0.03[b]	+ 4.04	5.80 ± 0.04[b]	+ 11.53
30 d withdrawal + vitamin E treated	1.83 ± 0.04[b]	+ 5.78	5.54 ± 0.23[b]	+ 6.53
High dose				
Arsenic trioxide treated	1.21 ± 0.02[a]	− 30.05	4.32 ± 0.12[a]	− 16.92
Arsenic trioxide + vitamin E treated	1.51 ± 0.03[c]	− 12.71	5.03 ± 0.19[c]	− 3.26
30 d withdrawal	1.53 ± 0.03[c]	− 11.56	4.93 ± 0.20[c]	− 5.19
30 d withdrawal + vitamin E treated	1.77 ± 0.03[c]	− 2.31	5.53 ± 0.16[c]	+ 6.34

Notes: values are mean ± *SEM*; $N = 10$; values in parentheses indicates percent change from vehicle control; [a] $p < 0.05$ as compared with control: [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated

Table 7 Effect of arsenic trioxide on biochemical parameters in the kidney of mice

Groups of animals	Biochemical parameters				
	Protein[1]	Glycogen[2]	Total ascorbic acid[3]	Dehydro ascorbic acid[3]	Reduced ascorbic acid[3]
Vehicle control	28.4 ± 0.1	1054 ± 26	102.6 ± 0.5	7095 ± 0.60	3.12 ± 0.35
Vitamin E control	28.6 ± 0.1	1037 ± 40	102.1 ± 0.8	7.31 ± 0.65	2.73 ± 0.30
Low dose					
Arsenic trioxide treated	21.7 ± 0.1[a]	983 ± 14[a]	96.4 ± 0.9[a]	5.41 ± 0.40[a]	1.72 ± 0.25[a]
Arsenic trioxide + vitamin E treated	24.5 ± 0.1[b]	975 ± 5	98.6 ± 0.4[b]	5.99 ± 0.24	1.95 ± 0.15[b]
30 d withdrawal	25.2 ± 0.1[b]	998 ± 11[b]	99.6 ± 0.3[b]	6.52 ± 0.20[b]	2.02 ± 0.17[b]
30 d withdrawal + vitamin E treated	26.8 ± 0.2[bd]	1023 ± 9[b]	100.5 ± 0.4[b]	7.11 ± 0.17[bd]	2.50 ± 0.22[b]
High dose					
Arsenic trioxide treated	19.3 ± 0.1[a]	852 ± 13[a]	93.1 ± 0.6[a]	2.43 ± 0.28[a]	0.73 ± 0.14[a]
Arsenic trioxide + vitamin E treated	23.0 ± 0.1[c]	941 ± 11[c]	96.6 ± 0.3[c]	3.32 ± 0.19[c]	1.69 ± 0.13[c]
30 d withdrawal	24.7 ± 0.1[c]	972 ± 8[c*]	98.6 ± 0.5[c]	4.13 ± 0.11[c]	1.92 ± 0.18[c]
30 d withdrawal + vitamin E treated	26.4 ± 0.2[cd]	1018 ± 10[cd]	100.4 ± 0.3[cd]	6.79 ± 0.25[cd]	2.36 ± 0.14[c]

Notes: Values are mean ± *SEM*; $N = 10$; [1] mg/100 mg fresh tissue weight; [2] μg/100 mg fresh tissue weight; [3] mg/ 100 mg fresh tissue weight; [a] $p < 0.05$ as compared with control; [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated; [d] $p < 0.05$ as compared with withdrawal of the treatment

Table 8 Effect of arsenic trioxide on enzymatic changes in the kidney of mice

Groups of animals	Parameters		
	Succinic dehydrogenase[1]	Alkaline phosphatase[2]	Acid phosphatase[2]
Vehicle control	5.80 ± 0.25	516.88 ± 13.86	195.67 ± 10.68
Vitamin E control	5.88 ± 0.30	514.80 ± 14.00	184.75 ± 8.88
Low dose			
Arsenic trioxide treated	4.25 ± 0.14[a]	303.80 ± 13.46[a*]	87.23 ± 11.38[a]
Arsenic trioxide + vitamin E treated	4.04 ± 0.10	377.30 ± 12.58[b]	119.81 ± 10.86[b]
30 d withdrawal	4.42 ± 0.15	398.16 ± 11.28[b]	128.27 ± 11.00[b]
30 d withdrawal + vitamin E treated	5.41 ± 0.31[bd]	458.28 ± 13.90[bd]	158.12 ± 9.92[b]
High dose			
Arsenic trioxide treated	3.25 ± 0.14[a]	243.18 ± 13.21[a*]	51.92 ± 10.50[a*]
Arsenic trioxide + vitamin E treated	3.00 ± 0.12	331.20 ± 12.85[c*]	89.01 ± 11.36[c*]
30 d withdrawal	3.81 ± 0.11[c]	372.77 ± 14.00[c*]	106.95 ± 9.66[c*]
30 d withdrawal + vitamin E treated	4.40 ± 0.19[cd]	446.18 ± 13.08[cd]	143.35 ± 8.76[cd]

Notes: Values are mean ± *SEM*; $N = 10$; [1] μg formazon formed/100 mg fresh tissue weight/15 min; [2] μmol of *p*-nitrophenol released/100 mg fresh tissue weight/30 min; [a] $p < 0.05$ as compared with control; [b] $p < 0.05$ as compared with low dose treated; [c] $p < 0.05$ as compared with high dose treated; [d] $p < 0.05$ as compared with withdrawal of the treatment

3 Discussion

Oral administration of arsenic trioxide[3 and 6 mg/(kg body weight · d)] for 30 d caused dullness, hair fall, appearance of pigmented spots on the skin and hyperkeratinosis. The effect was more pronounced in higher dose arsenic-treated mice than that of lower dose. These effects could be due to higher affinity of arsenic to bind bisulfhydryl group affecting keratin of the hair and skin. In addition, swelling of feet as well as, appearance of gangrenous feet in some animals in higher dose arsenic-treated group was also observed. Many investigators have reported similar clinical signs, in epidemiological, clinical and experimental animal studies(Chen, 1993; Dhar, 1997).

Arsenic treatment also caused significant dose-dependent reduction in body weight. Weight loss during chronic arsenic toxicosis has been reported (Reichl, 1990). Arsenic treatment also caused significant reduction in absolute weight of liver and kidney (Tables 3, 6) which could be due to altered biochemical changes reported in present investigation (Tables 3,7). Rise in relative liver weight in arsenic-treated groups(both low and high) suggest that weight loss of liver is less prominent than rest of the body. Our data corroborates with the finding of Bencko and Nemenckova (Nemenckova, 1971). However this is not observed in arsenic trioxide and vitamin E co-treated groups. Hence vitamin E may provide protection to the whole body except liver (with respect to the relative liver weight) against arsenic toxicity. Relative weights of liver of groups Ⅸ and Ⅹ are found to be significantly low in comparison to groups Ⅶ and Ⅷ respectively. This also indicates that vitamin E is worsening the situation by enhancing arsenic-induced loss of liver weight or by decreasing the body resistance (regarding maintenance of weight).

The glycogen content was significantly reduced in liver and kidney of arsenic-treated mice. Szinicz and Forth (Szinicz, 1988) have also reported reduction in glycogen concentration during arsenic poisoning. Marked carbohydrate depletion(glucose and glycogen mainly, also lactose, maltose etc.) was reported to be a major problem in acute arsenic

poisoning (Szinicz, 1988). The data of Reichl *et al*. (Reichl, 1990) suggested that the glucose supplement by external food intake plays a crucial role in treatment against arsenic poisoning.

Alkaline phosphatase activity was significantly decreased in kidney of arsenic-treated animals (Table 2). Alkaline phosphatase activity is mainly localized in the brush border of proximal convoluted tubules and mostly helps in reabsorption of glucose from the lumen by secondary active transport including SGLT 1, 2, GLUT 1, 2 and Na^+-K^+-ATPase (Ganong, 2001). The significant reduction in alkaline phosphatase activity in the kidney of arsenic-treated mice is indicative of decreased reabsorption of glucose from the lumen. This could also be due to reduced generation of ATP as SDH activity is significantly reduced in the kidney (Table 8) of arsenic-treated mice. Acid phosphatase activity was significantly decreased in the kidney of arsenic-treated groups of mice. This might be a result of alterations in the biosynthesis and/or activity of acid phosphatase. Significant increase in acid phosphatase activity could be due to increased synthesis of lysosomal enzymes as a response to increased cell degeneration and other pathological liver injuries (Hirata, 1990).

Arsenic treatment caused significant reduction in protein concentration. Arsenic is known to react with –SH groups of proteins and enzymes (Webb, 1966), while arsenate may interfere with phosphorylation reaction due to its chemical similarity with phosphate. Studies on the interaction of arsenic with subcellular components of tissues of rabbits exposed to arsenite have shown that the main sites of localization are the nuclear and soluble cytoplasmic fraction of tissues (Vahter, 1983). Thus the reduction in protein concentration might be due to alteration in enzymatic activity involved in protein biosynthesis.

Oral administrations of arsenic trioxide caused significant reduction in total, dehydro and reduced ascorbic acid contents in the liver and kidney of mice. Active site for the synthesis of ascorbate is the liver in mammals except in man and guinea pigs. Thus significantly decreased ascorbic acid content might be due to altered biosynthesis, transport and/or its utilization. During radical scavenging action, ascorbic acid is suggested to be transformed into dehydroascorbate. Reduced glutathione is required for the conversion of dehydroascorbate back to ascorbate. The fall in the level of the reduced glutathione (Vahter, 1980) affects the conversion of dehydroascorbate back to ascorbate and this probably explains the lower level of ascorbic acid in the arsenic-treated mice.

The present study also shows that 30 d withdrawal of the treatment caused significant amelioration in arsenic-induced toxicity in the mice (Table 2—8). The methylation of inorganic arsenic to dimethyl arsenic (DMA) is generally considered as a detoxification mechanism in mammals and has been shown that the excretion of arsenic in man is faster following exposure to DMA than to inorganic arsenic. In both mice and rabbits injected with [^{74}As] DMA, the excretion was almost complete within 24 h indicating that essentially no retention occurred in the tissues (Vahter, 1983).

Arsenic also undergoes significant biliary excretion. Rats excrete 24%—7% of injected arsenite in bile within 2 h (Gregus, 1985). Glutathione plays a role in the biliary excretion of arsenic (Gyurasics, 1991). Primarily the kidneys excrete inorganic arsenic. The process is dependent on the rate and extent of methylation (Flora, 1997). Rates of elimination vary with dose, route of exposure, animal species and forms of arsenic. Other factors such as diet probably influence arsenic metabolism as well (Flora, 1997). The rate is generally lower in humans than in mice, dogs and monkeys. The half-life in humans and most experimental animals is fairly short; 40%—65% of a single dose is excreted within 2 d, another 30% in less than 10 d and the remaining 5% or less, in more than a month. Thus amelioration of arsenic toxicity on withdrawal of the treatment could be due to almost total excretion of arsenic from the body.

It was also observed that co-treatment with vitamin E as well as vitamin E administration during withdrawal period also significantly ameliorates arsenic-induced toxicities. Arsenic reacts with protein thiol group to form 1-3-dithiol-2-arsa-hexacycles. Most of monomethyrated pentavalent arsenic intermediate is reduced by GSH to a monomethylated trivalent arsenic compound, which is then methylated to a dimethylated pentavalent arsenic derivative. Methylarsenic acid [$MeAsO(OH)_2$] and dimethyl arsenic acid [$Me_2AsO(OH)$] are generated by hydrolysis of methylated heterocycles. These methyl arsenic compounds, much less toxic than arsenite, are excreted in urine (Hirata, 1990).

A positive correlation between arsenic concentration and lipid peroxidation levels has been reported in the liver and kidney of rats (Ramos, 1995). Yamanaka and Okada (Yamanaka, 1994) also reported induction of lung specific DNA damage by metabolically methylated arsenics via the production of free radicals. Vitamin E is a potent biological antioxidant and therefore provides protection to the body tissues and organs (Odin, 1997).

Vitamin E, an antioxidant, helps GSSG (Oxidized glutathione) to convert back into GSH (reduced glutathione) thus in turn help to convert mono and dehydro ascorbic acid to ascorbic acid. Thus vitamin E helps to stabilize the changes by increasing antioxidant capacity of the cell. However, significant changes in ascorbic acid contents were not observed in between group Ⅲ and Ⅴ as well as between group Ⅳ and Ⅵ. It indicates that the dose of vitamin E may be an important factor. Also vitamin E prevents conversion of free or protein bound sulfhydryls to disulfide (Gyurasics, 1991). Also vitamin E prevents all membrane lipids and

unsaturated fatty acids (UFAs) against oxidative degradation (Odin, 1997). Thus vitamin E possibly provides protection against arsenic intoxication.

References:

Beatty C H, Basinger G H, Dully C C *et al*., 1966. Comparison of red and white voluntary skeletal muscle of several species of primates[J]. J Histochem Cytochem, 149: 590—600.

Bencko V, Nemenckova H. 1971. Oxygen consumption by mouse liver homogenate during drinking water arsenic exposure. Part 1[J]. J Hyg Epidemiol Microbiol Immunol, 15: 104—110.

Bessey O A, Lowry O H, Brock M J, 1945. Method for the determination of alkaline phosphatase with five cubic millimeters of serum[J]. J Biol Chem, 159: 399.

Chen H, Pellett L H, Anderson H J *et al*., 1993. Protection by vitamin E, selenium and β-carotene against oxidative damage in rat liver slices and homogenate[J]. Free Radical Biol Med, 14: 473—482.

Chinoy N J, Sharma A, 1998. Amelioration of fluoride toxicity by vitamin E and vitamin D in reproductive functions of male mice[J]. Fluoride, 31: 203—216.

Cikrt M, Benko V, Tichy M *et al*., 1980. Biliary excretion of ^{74}As and its distribution in the golden hamster after treatment with ^{74}As(III) and ^{74}As(V)[J]. J Hyg Epidemiol Immuno, 9: 384—388.

Cori C F, Cori G T, Green A, 1943. Crystalline muscle phosphorylase kinetics[J]. J Biol Chem, 15: 39—55.

Dhar R K, Biswas B G, Samanta G *et al*., 1997. Ground water arsenic calamity in Bangladesh[J]. Curr Sci, 73: 48—59.

Fiske C H, Subbarao Y, 1925. The colorimetric determination of phosphorus[J]. J Biol Chem, 66: 375.

Flora S J S, Pant S C, Malhotra P R *et al*., 1997. Biochemical and histopathological changes in arsenic intoxicated rats coexposed to ethanol[J]. Alcohol, 14: 563—568.

Ganong W F, 2001. Review of medical physiology. M]. 20th Edition.

Gregus C, Klaassen C D. 1985. Deposition of metals in rats: A comparative study of fecal, urinary and biliary excretion and tissue distributions of eighteen metals[J]. Toxicol Appl Phormacl, 85: 24—38.

Gyurasics A, Varga F, Gregus Z, 1991. Effects of arsenicals on biliary excretion of endogenous glutathione and xenobiotics with glutathione-dependent hepatobiliary transport[J]. Biochem Pharmacol, 4: 937—944.

Harrington J M, Middavgh J P, Morse D L *et al*., 1978. A survey of a population exposed to high concentrations of arsenic in well water in Fairbanks, Alaska[J]. Am J Epidemiol, 108: 377—385.

Hirata M, Tanaka A, Hisanaga A *et al*., 1990. Effect of glutathione depletion on the acute nephrotoxic potential of arsenite and on arsenic metabolism in hamster[J]. Toxicol Appl Pharmacol, 106: 469—481.

Lowry O H, Rosebrough N T, Farr A L *et al*., 1951. Protein measurement with the folin phenol reagent[J]. J Biol Chem, 193: 265—275.

Odin A P, 1997. Vitamins as antimutagens: Advantages and some possible mechanisms of antimutagenic action[J]. Mutat Res, 386: 39—67.

Ramos O, Carrizales L, Yanez L *et al*., 1995. Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels [J]. Environ Health Perspect, 103: 85—88.

Reichl F X, Szinicz L, Kreppel H *et al*., 1990. Effect of glucose in mice after acute experimental poisoning with arsenic trioxide(As_2O_3)[J]. Arch Toxicol, 64: 336—338.

Reichl F X, Kreppel H, Szinicz L *et al*., 1991. Effect of glucose treatment on carbohydrate content in various organs in mice after acute As_2O_3 poisoning [J]. Vet Human Toxicol, 33: 230—235.

Roe J H, Kuether C A, 1943. The determination of ascorbic acid in blood and urine through the 2-4-dinitrophenyl hydrazine derivation of dehydroascorbic acid[J]. J Biol Chem, 147: 399—407.

Seifter S, Dayton S, Novic B *et al*., 1950. Estimation of glycogen with anthrone reagent Arch[J]. Biochem, 25: 191—200.

Sigma Technical Bulletin No. 104. Sigma Chemical Comp. 3500[Z]. St. Louis 18. Mo. USA.

Szinicz L, Forth W, 1988. Effect of As_2O_3 on gluconeogenesis[J]. Arch Toxicol, 61: 444—449.

Tseng W P, 1999. Effect of dose-response relationships of skin cancer and blackfoot disease with arsenic[J]. Environ Health Perspect, 19: 109—120.

Vahter M, Marafante E, 1983. Intracellular interaction and metabolic fate of arsenite and arsenate in mice and rabbits[J]. Chem Biol Interaction, 47: 29—44.

Vahter M, Norin H, 1980. Metabolism of ^{74}As labelled trivalent and pentavalent inorganic arsenic in mice[J]. Environ Res, 21: 446—457.

Webb J L, 1966. Enzymes and metabolism inhibitors[M]. Vol. 3. New York: Academic Press.

Yamanaka K, Okada S, 1994. Induction of lung-specific DNA damage by metabolically methylated arsenics via the production of free radicals[J]. Environ Health Perspect, 102: 37—40.

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