PROTECTIVE EFFECTS OF VITAMINS C&E AGAINST SODIUM ARSENATE INDUCED EFFECTS ON MATERNAL WEIGHT IN ALBINO MICE

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ABSTRACT

Background: Exposure to arsenic through drinking water, food and occupational sources are common throughout the world. Women are more susceptible than men to the adverse effects of arsenic, as it interacts with estrogen hormones. This study was designed to evaluate the role of vitamin C&E in mitigating the toxic effects of sodium arsenate on maternal weight gain.

Materials and Methods: Thirty two Albino mice of BALB/c strain (twenty four females & eight males), 10 weeks old, weighing 30 – 35 gm were used; animals were divided into four groups having six female mice in each. Group A_1 served as control and was given a single I/P dose of weight related distilled water on 8^{th} day of gestation. Groups A_2, A_3 & A_4 animals received sodium arsenate (35 mg/kg) on 8^{th} gestational day and vitamin C & E were given by I/P injection,(9 mg/kg/day and 15 mg/kg/day) respectively, from 8^{th} day for rest of pregnancy period. The body weight of dams was recorded every day after the confirmation of pregnancy, till the time of sacrifice. The actual weight of dams was calculated as the difference between dam's total weight and that of total fetal weight.

Results: There was normal weight gain in dams of group A_1, whereas in group A_2 the maternal weight gain was reduced and the difference was statistically significant as compared to groups A_1, A_3 and A_4. It was therefore, concluded from the current results that vitamin C & E are useful in protecting sodium arsenate induced reduction of weight gain.

Key words: Sodium arsenate, maternotoxic, antioxidant.

INTRODUCTION

Sodium arsenate ($Na_2HAsO_4.\ 7H_2O$) is a colourless inorganic arsenical compound, highly soluble in water and among the most hazardous substance in the environment.[1,2] Various compounds of arsenic have been used widely as pesticide, insecticide and wood preservative.[3,4] Drinking water contaminated with arsenic is the main source of human exposure [3]. Chronic exposure to arsenic can lead to various health issues like diabetes mellitus, hypertension and asthma; cancers of skin, liver, kidney, bladder and prostate have also been reported.[5,6] Limited animal studies have shown arsenic compounds to be fetotoxic and teratogenic.[7] Ingested arsenic can cross the placental barrier resulting in its concentration of cord blood comparable to that in the maternal blood.[8] Epidemiological evidences indicate that exposure of pregnant women to elevated levels of arsenic in drinking water results in anemia which progresses as pregnancy advances.[9,10] Ingested arsenic adversely affects the maternal health as it interacts with steroid hormones and estrogen that makes women more susceptible to arsenic related toxic effects than men.[11] Women who have worked at the smelter or lived near it during the pregnancy have higher rates of spontaneous abortion, low birth weight and malformed children.[12,13]

Arsenic induced the production of free oxygen radicals that alter mitochondrial activity and genetic information.[14] Antioxidants aid in arsenic methylation which is excreted by kidneys. Ascorbic acid and Tocopherol (vitamin C and E) have specific role in mitigation of this heavy metal toxicity.[15]

Various cross sectional human studies carried out in Bangladesh, revealed that women of reproductive age, who were chronically exposed to high level of arsenic through drinking water, showed significant increase in premature, low birth weight babies and still birth, as compared to those who were not exposed.[13]

Talukder and Kabir (2001) reported that reduction of arsenic methylation resulted in maternal toxicity, prenatal mortality, low fetal weight, exencephaly and short tails.[16]

Similar observations were recorded by Lammon et al., (2003) that inhibition of arsenic methylation in mice, by pretreatment with periodate – oxidized adenosine (PAD) and administration of sodium arsenate 30 min after it; caused maternal toxicity, low fetal weight and prenatal mortality.[17] Stump et al., (1999) treated rats with a single Intraperitoneal (I.P) dose of sodium arsenate (35 mg/kg) on 9^{th} gesta-

tional day (GD), which decreased the maternal food consumption and reduced maternal weight gain.[18]

The maternal toxic effects of arsenic had been studied by various workers using human and animal models but prevention of toxicity on treatment with vitamin C and E had not been reported. The present study evaluated the protective role of Vitamin C and E as antioxidants for arsenic induced effects on maternal weight gain in albino mice.

MATERIALS AND METHODS

Thirty two Albino mice of BALB/c strain were procured from National Institute of Health Islamabad (twenty four females and eight males), 10 weeks old and weighing 30 – 35 gm; they were kept under controlled environment (temperature 22 ± 2˚C and humidity 50% ± 5%), at the Experimental Research Laboratory of University of Health Sciences Lahore. Animals were acclimatized for 7 days, and were kept under12 hour cycle of light and darkness. The mice were fed on standard rodent diet and distilled water ad libitum. For the purpose of mating, three female mice were kept overnight with a single male; the appearance of vaginal plug, was regarded as a gestational day (GD) one.

A permanent picric acid mark of identification for group and mouse was placed on the body of pregnant females using cotton buds. Animals were randomly divided into four groups of six each, A_1, A_2, A_3 and A_4 respectively. The mice were placed in respecttive cages (n = 6); A_1 served as a control and the other three as experimental groups. Group A_1 received weight related distilled water by intraperitoneal (I /P) injection, for 18 days. Group A_2 animals were treated with sodium arsenate 35 mg/kg ($Na_2HasO_4.7H_2O$ – Fluka) by a single I/P injection on 8th day of gestation; sodium arsenate was dissolved in distilled water before injecting. Groups A_3 and A_4 animals received sodium arsenate 35mg/kg on 8th GD and Vitamin C and E by I/P injection, 9 mg/kg /day and 15 mg/kg /day respectively, from 8th day to the end of the pregnancy period.

The body weight of the animals was recorded in the beginning, and subsequently daily after the confirmation of pregnancy till the time they were sacrificed on 18th GD. The uterine horns were opened and fetuses were extracted and weighed. The weight of all fetuses from a single dam was recorded and its final actual weight was calculated by subtracting the total fetal weight.

The effects of sodium arsenate was calculated by comparing the mean of the weight gain of the dam of experimental group, compared to the mean of group weight gain of the control and those of treated with vitamins C and E respectively.

Statistics

The data was analysed by using software Statistical Package for Social Sciences (SPSS) version 13.0. Mean and standard deviations were calculated for the quantitative variables. ANOVA was applied to assess the significance of difference among the groups. The post – hoc Tukey test was applied to assess the difference of means between the groups. The p value of ≤ 0.05 was considered statistically significant.

RESULTS

In comparison with control group, there was a statistically significant difference in maternal weight gain of experimental group A_2 as compared to their initial weight. Their weight suffered a reduction following the injection of sodium arsenate; decrease in weight continued throughout the gestation period. Comparison of mean maternal weight at the time of sacrifice showed a statistically significant difference when compared among groups (Table 1).

There was a statistically significant difference in mean maternal weight at the time of sacrifice bet-

Table 1: *Comparison among various groups of mean weight of mice initial, final, fetal and actual weight gain of dams.*

Parameters	*A_1 Control Group (n=6)*	*A_2 Sodium arsenate (n=10)*	*A_3 Sodium arsenate + Vit C (n = 6)*	*A_4 Sodium arsenate + Vit E (n = 6)*	*p value*
	Mean ± S.D	*Mean ±S.D*	*Mean±S.D*	*Mean±S.D*	
Initial weight of animals in g	30.8 ±1.3	30.8 ± 1.3	31.5 ± 1.8	32.0 ± 1.7	p<0.40
Animal weight at the time of sacrifice in g	61.0 ± 3.3	40.6 ± 5.2	59.5 ± 3.2	56.2 ± 3.7	p<0.001*
Total fetal weight in g	16.3 ± 2.9	11.4 ± 3.8	17.6 ± 4.0	14.1 ± 4.6	p<0.02
Maternal weight gain in g	13.9 ± 3.0	1.6 ± 2.8	10.4 ± 4.0	10.1 ± 3.6	p<0.001*

*p value of ≤ 0.05 is statistically significant

ween the groups A_1&A_2, A_2&A_3 and A_2&A_4 (Table 2). There was a marked increase in maternal weight at the time of sacrifice as compared with their initial weight in all groups; however weight gain in group A_2 at the end of experimental period was significantly lower when compared with that of the control group (Fig. 1).

When total fetal weight of group A_1 was compared with experimental group A_2 the weight in litter, seemed to have been reduced to a level which was statistically significant whereas; the total fetal weight in groups A_3&A_4 were comparable to that of group A_1. The difference of mean of total fetal weight was statistically significant when compared among groups (Table 1). Post-hoc Tukey test showed statistically significant difference in mean of total fetal weight when compared among groups A_1&A_2, A_2&A_3 (Table 3).

There was a statistically significant drug related decrease in mean maternal weight gain of group A_2 when compared among the groups A_1, A_3 and A_4; (Table 1, 4). The data are given in (Fig. 2).

The mean difference is statistically highly significant between groups A_1&A_2, A_2&A_3, A_2&A_4. The mean difference is statistically insignificant between groups A_1 and A_3, A_1&A_4, A_3&A_4.

The mean difference is statistically highly significant between groups A_1 & A_2, A_2 & A_3, A_2 & A_4.The mean difference is statistically insignificant between groups A_1&A_3, A_1&A_4, A_3&A_4.

The mean difference is statistically highly signi-

Table 2: *Multiple comparisons among groups of mean maternal weight at the time of sacrifice according to Tukey test.*

Comparison among Groups		*Mean Difference*	*Level of Significance*
Groups (α)	*Group Compared (β)*	*(α – β)*	*p-value*
(A_1)	(A_2)	20.40	0.001*
	(A_3)	1.50	0.923
	(A_4)	4.83	0.211
(A_2)	(A_3)	-18.90	0.001*
	(A_4)	-15.57	0.001*
(A_3)	(A_4)	3.33	0.518

*p value of ≤ 0.05 is statistically significant

Table 3: *Multiple comparisons of mean total fetal weight among various groups according to Tukey test.*

Comparison among Groups		*Mean Difference*	*Level of Significance*
Groups (α)	*Group Compared (β)*	*(α-β)*	*p-value*
(A_1)	(A_2)	4.6	0.001*
	(A_3)	-1.3	0.93
	(A_4)	2.2	0.76
(A_2)	(A_3)	-6.1	0.03
	(A_4)	-2.6	0.55
(A_3)	(A_4)	3.4	0.41

*p value of ≤ 0.05 is statistically significant.

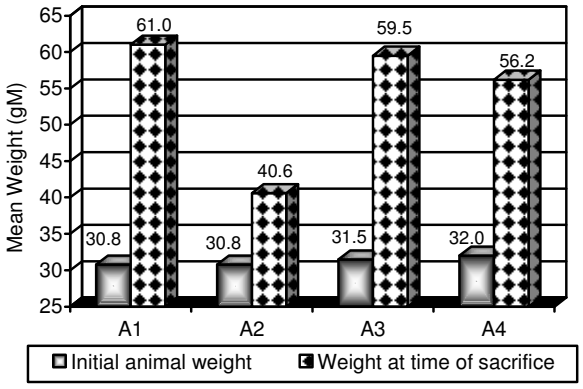

Fig. 1: *Comparison of Initial animal weight and weight at time of sacrifice among various groups.*

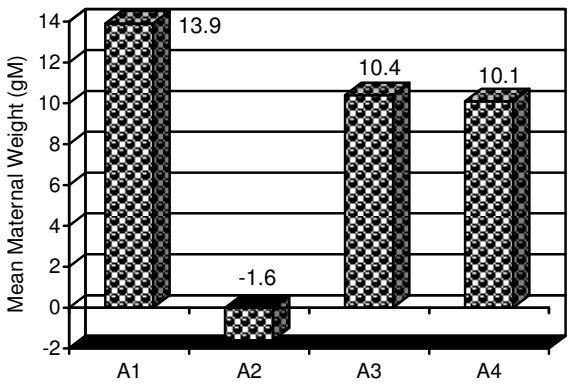

Fig. 2: *Comparison of mean maternal weight gain among various groups.*

ficant between groups A_1&A_2, A_2& A_3, A_2&A_4. The mean difference is statistically insignificant between groups A_1&A_3, A_1&A_4, A_3&A_4.

Table 4: *Multiple comparisons of mean maternal weight gain among various groups according to Tukey test.*

Comparison among Groups		*Mean Difference*	*Level of Significance*
Groups (α)	*Group Compared (β)*	*(α-β)*	*p-value*
(A_1)	(A_2)	9.70	0.001*
	(A_3)	2.20	0.668
	(A_4)	3.65	0.257
(A_2)	(A_3)	-7.49	0.001*
	(A_4)	-6.04	0.009*
(A_3)	(A_4)	1.45	0.875

*p value of ≤ 0.05 is statistically significant.

DISCUSSION

The sodium arsenate exposed group A_2 showed toxic effects on dams manifested as a decrease in weight gain following the injection of sodium arsenate, which continued throughout the gestation; similar effect with sodium arsenate had been reported by (Stump, 1999) in which the author observed that single I/P dose of sodium arsenate on 9th day of gestation resulted in maternal morbidity and reduction in actual weight gain.[18]

Holson et al., (1999) administered arsenic oxide in female rats by inhalation throughout the gestation period, and observed reduction in food consumption and also in weight gain.[20]

The Vitamin C supplementation in group A_3 and Vitamin E in group A_4 resulted in considerable maternal weight gain as compared to sodium arsenate group A_2. It suggested that both Vitamin C and E showed preventive effects on maternal weight loss due to sodium arsenate; these effects are attributed to the antagonistic effect of Vitamin C and E on arsenic absorption, and due to their antioxidant properties to interact with the free oxygen radicals and protect the cells from reactive oxygen species; antioxidant properties of Vitamin C had been studied by various researchers. Moller, (2004) observed in mononuclear blood cells that oxidative DNA damage was reduced by vitamin C supplemented with vitamin E.[21] Naidu, (2003) documented that vitamin C scavenges the free oxygen radicals and thus reduces the damage to macromolecules like lipids, DNA and proteins.[22]

The mean of the total fetal weight of arsenic exposed group A_2 was decreased to 11.45 ± 3.8, as compared to control group A_1 (16.27±2.9). Mason, (1988) reported that fetal weight was reduced after the arsenic exposure.[19] Huai Guan, et.al (2012) in a cross-sectional study showed that maternal blood arsenic concentration was negatively associated with fetal birth weight.[23] Similar effects of arsenic were described among newborns in Shanghai by Xu L, et al (2011).[24] However, Lammon (2003) reported that mean of the fetal weight remained unaffected after arsenic exposure.[17]

In sodium arsenate plus Vitamin C and E treated groups (A_3 and A_4) respectively, mean of the fetal weight increased considerably (17.6 ± 4.0 &14.08 ± 3.7) as compared to mean of the fetal weight in group A_2 (11.45 ± 3.8); suggesting that vitamin C and E directly reacted with ROS and lessened the toxic manifestations of heavy metals.[25]

In *conclusion* the present study showed that sodium arsenate exposure reduces the weight of mice (final, fetal and actual) by generating an oxidative stress. Vitamins C & E found to be an antioxidant for preventing the weight loss, which is manifested as a fair degree weight gain in Vitamin C&E treated groups.

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