Orginal Article

ANTIOXIDANTS PREVENTED THE FETAL RESORPTIONS INDUCED BY SODIUM ARSENATE IN ALBINO MICE

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ABSTRACT:

Background and Objectives: Epidemiological studies have revealed the increased prevalence of spontaneous abortion, stillbirth, and premature babies among women who were exposed to high levels of arsenic in consumable water during their reproductive years. The study explored the fetal toxicity in albino mice inoculated by sodium arsenate and its prevention by Vitamins C & E.

Material and Methods: Gravid albino mice of BALB/c strain twenty-four in number were randomly distributed into 4 groups containing 6 animals in each group. Control group 1 was injected with distilled water 0.1ml/kg/day I/P for 18 days. A single dose of sodium arsenate 35mg/kg was injected I/P on 8th gestational day to groups 2, 3 & 4. Vitamins C and E 9 mg/kg/day and 15 mg/kg/day respectively, were given by intraperitoneal injections to groups 3 and 4 starting from 8th gestational day and continued for the rest of the pregnancy period. The fetal resorption sites were counted both early & late, litter sizes were logged. Morphological malformations were examined grossly.

Results: An increased incidence of abortion, fetal resorptions, and a significant decrease in litter size were manifested in group 2. Groups 3 & 4 showed noticeable improvement in litter size and the number of fetal resorptions were reduced. There was a statistically significant difference in means among the groups ($p<0.000$).

Conclusions: The results exposed the antioxidant potential of ascorbic acid and alpha-tocopherol in inhibiting the arsenic borne fetal toxicity in mice.

Key Words: Fetal resorptions, Antioxidants, Alpha-tocopherol

INTRODUCTION:

Arsenic is among the harmful substances in the environment, its inorganic salts are highly toxic and water-soluble.[1] These salts have the potential to cause structural or functional defects in conceptuses, abortion, and infertility in humans and animals.[2,3] In many countries of the world humans are susceptible to arsenic in clean water above the approved level (10µg/lit), which is associated with the development of skin and cancers of various organs.[4,5] The population of South East Asia, the West Bengal India and Bangladesh are more vulnerable to arsenic contamination in drinking water where its concentration at certain places rises to > 100 µg/lit.[6,7] In Pakistan the concentration of arsenic in water sources is found to be much higher (32-1900µg/l) than the permissible level in 27 districts along the course of river Indus and northern Pakistan.[8,9] In Pakistan 47 million people are vulnerable to arsenic through the contaminated groundwater wells which is above the WHO permissible level (10µg/l).[10]

Epidemiological studies carried out in Bangladesh, Nigeria, Romania, and Hungary had suggested associations between a high concentration of arsenic in consumable water and spontaneous abortion, still and premature births.[11-14]

Human and animal data from various studies supported the association between the detrimental reproductive effects and drinking water soiled with arsenic.[15] Anisur Rahman 2010, conducted a cohort study in Bangladesh and reported that prenatal arsenic exposure resulted in a decrease in size at birth.[16]

Hans ZJ 2011, demonstrated that prenatal arsenate exposure in chick embryos resulted

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in neural tube defects owing to arsenic-induced oxidative stress.[17] Robinson JF 2011 revealed the specific gene response in mouse embryos to different doses of arsenic and cadmium during the process of neurulation.[18] Various studies have documented the loss of human pregnancies with consumption of groundwater soiled with arsenic.[19]

In another study male Wistar rats were exposed to arsenic compounds in different concentrations in drinking water for 56 days, resulting in a decrease in reproductive functions and fertility.[20] A case-control study conducted in Egypt showed a positive correlation of fetal growth retardation and high concentrations of heavy metals including arsenic in blood and urine samples of 60 women.[21] Arsenic compounds in different concentrations were fed to rats for 6 weeks prenatally and during the gestation, fetal resorptions, abortions, decrease in fetal weight and cardiac malformations were reported.[22]

Arsenic induced these effects due to chromosomal damage and enhances mutagenesis by interfering with the DNA repair due to the production of free radicals.[23] Arsenate is a chemical analogue to phosphate; it disengages oxidative phosphorylation by replacing for phosphate in ATP synthesis.[24]

Antioxidants can prevent the damaging effects of free radicals by inhibiting oxidation reactions.[25] Heavy metals exert their toxic effects by generating free radicals which could be scavenged by antioxidants.[26]

Tsang V et al., 2012, evaluated the effects of gestational inorganic arsenic and high doses of folate on DNA methylation in mice. They reported adverse effects on DNA methylation.[27]

McDougal et al., 2017, established in zebrafish embryos that deficiency of vitamin E resulted in fetal resorptions, mortality & malformations.[28] Flora G et al., 2015, proposed therapeutic measures for chronic arsenic poisoning by the combination of different chelating agents.[29]

The chelating agents are itself teratogenic and couldn't be used effectively during pregnancy to prevent arsenic toxicity; therefore the research was aimed to investigate the antioxidant potential of Vitamins C & E in averting the damaging outcomes of free radicals induced by arsenic and subsequently prevent the fetal toxicity.

MATERIAL AND METHODS:

The albino mice of BALB/c strain (twenty-four females and eight males), were kept in the animal husbandry of the University of Health Sciences, Lahore under a controlled environment (temperature 22 ±1˚C and humidity 40%-60%) with a 12-hour light and dark cycle. The animals were 10 weeks old, weighed 30-35gm, were nurtured on customary pellet rodent diet and distilled water ad libitum. After the acclimation period of seven days, female mice were mated overnight with male mice of the same strain. Gestational day (GD) one was designated to the day when the copulatory plug was identified. The mice with positive copulatory plugs were randomly allocated into four groups with six faunas in each group. Cage cards were used to indicate the number of the mouse and its group. The control group 1was given weight-related distilled water by intraperitoneal injection, for 18 days. Mice of group 2 were injected with sodium arsenate 35 mg/kg by a single I/P injection on the 8th day of gestation; sodium arsenate was dissolved in distilled water before injecting. Animals of groups 3 and 4 received sodium arsenate 35 mg/kg on 8th GD by I/P injection and Vitamins C and E 9 mg/kg/day and 15 mg/kg/day respectively, from 8th day for the remainder of the gestation period. The dose was adjusted individually according to the weight of each dam.

The animals were dissected on the 18th day of conception. The uterine horns were exposed which appeared beaded by the fetuses. Uterine horns were incised in midline and examined for the number of live and dead fetuses. Gross morphological examination for malformations of all fetuses

was carried out under a Wolfe stereo dissecting microscope, ER- 59 – 1828, and the following parameters were looked for: i. Exencephaly ii. Cleft palate. iii. Abdominal hernia. iv. Polydactyl & Opened eyes. The total numbers of litters in each group were recorded and their means were calculated. The uterine horns were also examined for the early and late fetal resorptions, the number of fetal resorptions/dams were documented, and a mean of the total number of fetal resorptions was calculated. The uterine horns which lacked the mark of implantation were exposed and were put in 10% ammonium sulfide solution for revealing early implantation sites.

The software (SPSS) version 18.0 was made use of to analyze the data. For the numerical variables mean and standard deviations were calculated. ANOVA was used to evaluate the mean difference among the groups. Post-hoc Tukey was tested to assess the difference of means between the groups. The p value of ≤ 0.05 was contemplated as statistically significant.

RESULTS:

In the control group 1 there was no occurrence of abortions, stillbirths, fetal resorptions, or maternal mortality. The uterine horns were opened up on day 18 of pregnancy which exhibited the normally growing fetuses (Fig 1, A).

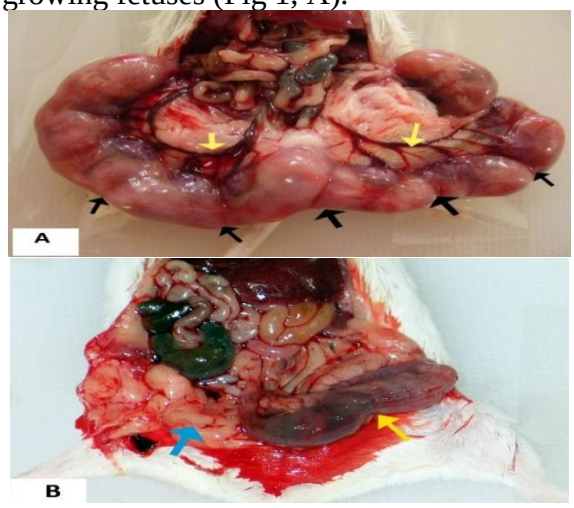

Fig 1: Photographs of mice on day 18 of pregnancy. A) Shows the dissected mouse of control group 1, displaying the uterine horns blood circulation through the uterine blood vessels (yellow arrows) on the mesometrial side of the uterus. Fetuses have seen growing normally through the wall of the uterus giving it a beaded appearance (black arrows). B) Dissected mouse treated with sodium arsenate (group 2), the right horn of uterus displays bleeding from the aborted fetuses (blue arrow). The left uterine horn shows a few of the remaining fetuses (yellow arrow).

In sodium arsenate treated group 2 there were spontaneous abortions, therefore more animals were added to the group (n=10) to balance the number of the group. The animals started aborting on 17th and 18th gestational days. The mice were dissected which showed bleeding from the aborted fetuses and a few numbers of partially formed fetuses (Fig1, B). In groups 3 and 4, the sodium arsenate was administered in consort with Vitamins C and E respectively; no incidence of spontaneous abortions or maternal mortality was observed in these groups. The fetuses were well developed with all normal body parts. There was no incidence of stillbirth nor was there any evidence of exencephaly, cleft palate, abdominal hernia, polydactyl, or opened eyes (Fig 2, A&B).

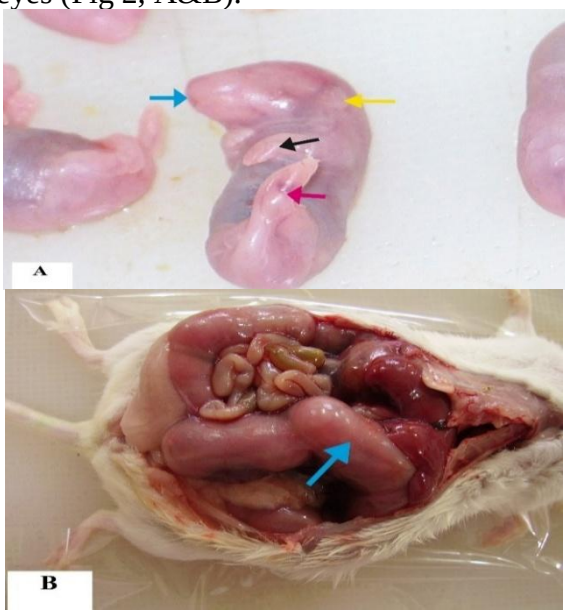

Fig 2. Photograph of mice fetuses from groups 3&4. A) Shows a fetus; with well-formed jaws (blue arrow), ear (yellow arrow) forelimbs (black arrow), turned up tail (red arrow). B) A dissected female

mouse, showing well-formed fetuses in uterine horns (blue arrow) occupying the lower abdomen.

In sodium arsenate treated group 2 early and late fetal resorptions were observed. The mean number of resorptions among various groups was statistically significant (Table 1, Fig 3 A& B).

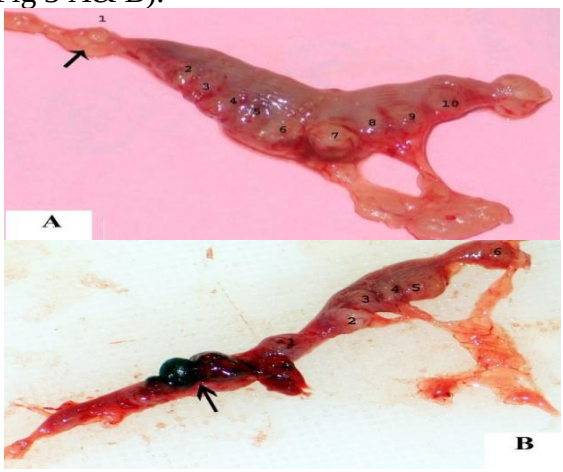

Fig. 3. Photograph of uterine horns of mice (Group 2). A) Showing resorptions at the implantation sites; the opened up uterine cavity showing site of early resorption turned into the yellow fat body (arrow), and the number indicating the sites of resorption. B) Uterine horn, dissected to show the late resorptions (arrow) and the number indicating sites of early resorptions.

Table 1: Comparison of fetal parameters among various groups.

Parameters	Control group 1 (n=6)	Sodium arsenate group 2 (n=10)	Sodium arsenate + Vit C group 3 (n=6)	Sodium arsenate + Vit E group 4 (n=6)	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Number of fetal resorptions.	0.00 ± 0.00	3.7 ± 2.6	0.33± 0.5	0.5± 0.8	p<0.000 *
Total number of fetuses.	9.5± 1.4	5.5± 1.8	10.2± 2.3	8.5± 2.2	p<0.000 *

The sight of resorption was made discernable by opening the uterine horns and placing it in a 10% ammonium sulphide solution. Groups 3&4 showed a minor number of resorptions. Post-hoc Tukey test applied for multiple comparisons among the groups showed a significant difference in mean of the total number of resorptions between the groups 1&2, 2&3, 2&4; the number of fetal resorptions was considerably higher in group 2where as it was reduced in groups 3&4; the difference of means of the total number of resorptions between groups 1& 3&4 was statistically insignificant (Table 2A).

Table 2A: Multiple comparisons of mean of the total number of resorptions among various groups according to the Tukey test.

Comparison among groups		**Mean Difference**	**Level of Significance**
Groups (α)	Group compared (β)	(α-β)	p-value
(1)	(2)	-3.7	0.001*
	(3)	-0.33	0.985
	(4)	-0.50	0.952
(2)	(3)	3.37	0.003*
	(4)	3.20	0.005*
(3)	(4)	-0.167	0.998

*The mean difference is statistically significant between groups 1&2, 2&3, 2&4. The mean difference is statistically insignificant between groups 1&3, 1&4, 3&4.

The average number of litters was reduced in sodium arsenate treated group 2 as compared to groups 1, 3&4. The comparison of means of the total number of fetuses among the groups was statistically significant (Table 1). The number of fetuses/dams was also more in groups 1, 3, and 4 as compared to group 2. The Post-hoc Tukey test was applied for multiple comparisons among the groups; there was a significant difference in mean of the total number of fetuses between the groups 1&2, 2&3, 2&4. (Table 2B). The data are given in (Fig 4).

Table 2B: Tukey test showing multiple comparisons of mean of the total number of fetuses among various groups.

Comparison among groups		Mean Difference	Level of Significance
Groups (α)	**Group compared (β)**	**(α-β)**	**p-value**
(1)	(2)	4.0	0.003*
	(3)	-o.7	0.930
	(4)	1.0	0.803
(2)	(3)	-4.7	0.000*
	(4)	-3.0	0.028*
(3)	(4)	1.7	0.450

* The mean difference is statistically significant between groups 1&2, 2&3, 2&4. The mean difference is statistically insignificant between groups 1&3, 1&4, 3&4.

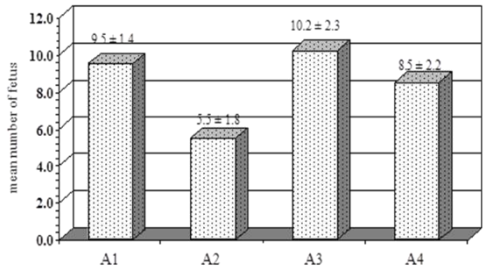

Fig 4. Bar chart showing the comparison of mean of the number of fetuses among various groups.

DISCUSSION:

In this study administration of intraperitoneal injection of sodium arsenate on 8th GD in group 2 resulted in a decreased number of litters, frequent fetal resorption, and spontaneous abortion. Spontaneous abortions and decreased fecundity in mice after arsenite toxicity which leads to the placental insufficiency attributing these effects have been reported.[19] The fetotoxic effects of sodium arsenate manifested as an increased rate of fetal resorption had been documented by Sampayo et al., 2017, Gandhi 2012 and Markowski 2011.[30-32] In our work the external malformations like craniofacial, skeletal, limb defects, abdominal hernia, polydactyly, and opened eyes were not observed as had been reported by Wlodarczyk 2014 & Javanmard 2011 .[33,34] This may be due to the high rate of resorptions and abortion. The sodium arsenate induced oxidative stress which causes DNA damage through the production of free radicals having possibly an effect on the developing embryo eliminating the abnormal conceptuses. Han Z et.al, 2011, revealed that the embryos with neural tube defects showed a significantly higher concentration of free radicals.[17]

In groups (3&4) sodium arsenate along with Vitamins C and E were injected respectively, there was a considerable increase in the mean of the number of litters as equated to group 2 and even greater than in the control group 1. There was no incidence of spontaneous abortion and the number of fetal resorptions decreased in groups 3&4. This suggested that due to their antioxidant properties the Vitamins C and E had prevented the fetotoxicity, avert the high level of free radicals in the body and block the DNA damage.[35] Davis 2010, discussed the significance of antioxidants to combat the hypoxic intrauterine environment and reported that surge of antioxidants like Vitamins C, E and A were found in the cord blood of term infants as compared to preterm infants.[36]

A fundamental balance between oxidant and antioxidant molecules is essential for a normal pregnancy to take place, interruption in this balance could contribute to defective embryo development.[37] In this study arsenic presumably broke down the balance between oxidant and antioxidant levels by producing free radicals and resulted in abortions and fetal resorptions, while the antioxidant potential of Vitamins C&E had inhibited this inequity of oxidant and antioxidant molecules and prevented the fetotoxicity.

CONCLUSION:

This study concludes that the spontaneous abortions & fetal resorptions induced due to free radical formation by arsenic, have been prevented by Vitamins C & E proving that the free radicals can be scavenged by these antioxidants & hence prevented the fetotoxicity. However, further studies to assess the effects of these vitamins are

requisite on human conceptuses in areas where women in their childbearing years are susceptible to arsenic through the polluted water supply.

ACKNOWLEDGMENTS:

The author acknowledges the support provided by the laboratory staff of the anatomy department of the University of Health Sciences, Lahore.

AUTHOR'S CONTRIBUTION:

FQ: Conduction of study, design, hypothesis formation, analysis & interpretation of data, drafting, revising critically & final submission of manuscript & responsible for correspondence as contributing author.
MT: Supervisor of the project. Integrity & technical aspects had been investigated by him.

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