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EFFECTS OF SELENIUM, IRON AND ZINC SUPPLEMENTATION ON ARSENIC CONCENTRATION IN TISSUES WITH GROSS PATHOLOGY IN LONG EVANS RATS

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ABSTRACT

The study was carried out to know the effects of selenium, iron and zinc supplementation on gross pathology and tissue arsenic concentration in Long Evans rats during the period from August to October 2003. A total of 25, one-month-old male rats were randomly divided into 5 equal groups as A, B, C, D and E. Rats of group A were kept as control without giving any treatment. Rats of groups B, C and D were given normal rat pellet (25 g / rat / day) and in addition to arsenic trioxide (As_2O_3) @ 400 mg / litre, they were treated with sodium selenite (NaSe) @ 1 mg / litre, ferrous sulfate ($FeSO_4$) @ 150 mg / litre and zinc sulfate ($ZnSO_4$) @ 50 mg / litre respectively while rats of group E were treated with only arsenic trioxide (As_2O_3) @ 400 mg / litre drinking water daily for 42 days. All the rats of groups B, C and D showed slight congestion in the liver, spleen, heart and kidney and haemorrhage in stomach and intestine but the rats of group E showed heavy congestion in the liver, spleen, heart and kidney and rose-red inflammation in the stomach and severe haemorrhagic enteritis. Among the treated groups, the rats of groups B (0.0126-0.27 ppm), C (0.03-0.32 ppm) and D (0.04-0.288 ppm) which were supplemented with sodium selenite, ferrous sulfate and zinc sulfate respectively along with arsenic trioxide showed reduced arsenic concentrations in different tissues while the rats of group E treated only with arsenic trioxide showed higher concentrations of arsenic in tissues (0.05-0.4 ppm). The concentration of arsenic in the liver, kidney, spleen, heart, stomach, intestine, muscle and dermis of the treated rats were 0.27-0.40 ppm, 0.06-0.22 ppm, 0.052-0.20 ppm, 0.0126-0.102 ppm, 0.054-0.16 ppm, 0.064-0.176 ppm, 0.028-0.07 ppm and 0.03-0.05 ppm respectively. It may be concluded that supplementation of selenium, iron and zinc reduces the arsenic concentration in tissues like liver, kidney, spleen, heart, intestine, stomach, muscle and dermis of rats as well as lower the tissue damage caused by arsenic. The present results also indicate that arsenic is deposited mainly in liver followed by kidney, spleen, dermis, heart and muscle.

Key words: Selenium, iron, zinc, gross pathology, tissues, arsenic concentration, Long Evans rats

INTRODUCTION

Arsenic is one of the most environmental hazardous elements. Arsenic poisoning is a momentous issue for public health as well as for livestock. Arsenic poisoning causes acute erosion in omasum and abomasum, haemorrhagic enteritis, and epicardial and subendocardial haemorrhages in heifers (Deckert *et al.,* 1983). The thyroid tissue of rats pretreated with arsenic alone exhibits obvious toxic changes, whereas only minor or no changes are found in the tissues when pretreated with selenium (Glattre *et al.,* 1995). Arsenic concentration in liver, kidney and rumen contents of cattle varied (Riviere *et al.,* 1981). Despite several reports on arsenic issue, information with respect to effects of selenium, iron and zinc supplementation on arsenic concentration in tissues with gross pathology in Long Evans rats is meagre. The present study was, therefore, undertaken to evaluate the effects of selenium, iron and zinc supplementation on arsenic concentration in tissues with gross pathology in Long Evans rats.

MATERIALS AND METHODS

The present research was carried out in the Experimental Pharmacology and Toxicology Laboratory, Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh during the period of August to October 2003. A total of 25 one-month-old male Long Evans rats purchased from ICDDR, B, Mohakhali, Dhaka were used in this experiment. All the rats maintained on good housing conditions and were provided with normal feed and water. After acclimatization they were randomly divided into 5 equal groups as A, B, C, D and E, each consisting of 5 rats. Rats of group A was kept as control i.e., they were given normal feed and water. Rats of group B were given arsenic trioxide @ 400 mg / litre plus sodium selenite @ 1 mg / litre. Rats of group C were administered with arsenic trioxide @ 400 mg / litre plus ferrous sulfate @ 150 mg / litre. Rats of group D were fed with arsenic trioxide @ 400 mg / litre plus zinc sulfate @ 50 mg / litre. Group E was given only arsenic trioxide @ 400 mg / litre. All the chemicals i.e., arsenic trioxide, sodium selenite, ferrous sulfate and zinc sulfate were fed to the different groups of rats with drinking water daily for 42 days.

At the end of the experiment (i.e., day 42) the rats were anaesthetized in a desiccator by chloroform and thereafter post-mortem examinations were performed. The liver, kidney, heart, spleen, stomach, intestine, muscle and parts of dermis were excised and blotted dry. The stomach and intestine were always rinsed out with water to remove the contents including food residues. Tissue homogenates of the liver, kidney, spleen, heart, stomach, intestine, muscle and dermis of rats of each group were made with the aid of mortar and pestle by adding normal saline @ 5 ml / g of tissue. These tissue homogenates were subjected to detect arsenic by Reinsch test (Simmons and Gentzkow, 1956) followed by estimation of arsenic by 'Merck Arsen test' kit (Merck, Germany).

RESULTS AND DISCUSSION

All the rats of groups B, C and D which were treated with arsenic trioxide along with sodium selenite, ferrous sulfate and zinc sulfate respectively showed slight congestion in the liver, kidney, spleen and heart and the stomach and intestine showed slight hemorrhage. Rats of group E treated only with arsenic trioxide showed heavy congestion in the liver, kidney, heart and spleen, rose-red inflammation in the stomach mucosa and severe haemorrhagic enteritis. On the other hand, the visceral organs of rats of group A (Control) were apparently normal. Similar findings were reported by Deckert *et al.* (1983).

Qualitative determination of arsenic in tissue samples was performed by Reinsch test. The result of Reinsch test are presented in Table 1.

Table 1. Qualitative determination of arsenic in tissues

Groups	Organs					
	Liver	Kidney	Heart	Spleen	Muscle	Dermis
Group A (Control)						
A_1	–	–	–	–	–	–
A_2	–	–	–	–	–	–
A_3	–	–	–	–	–	–
A_4	–	–	–	–	–	–
A_5	–	–	–	–	–	–
Group B (As_2O_3 + NaSe)						
B_1	+	+	–	–	–	–
B_2	–	–	–	–	–	–
B_3	+	–	–	+	–	–
B_4	–	+	–	–	–	–
B_5	+	–	–	–	–	+
Group C (As_2O_3 + $FeSO_4$)						
C_1	+	+	–	–	–	–
C_2	–	–	–	–	–	–
C_3	–	–	–	–	–	–
C_4	–	–	–	–	–	–
C_5	–	–	–	–	–	–
Group D (As_2O_3 + $ZnSO_4$)						
D_1	+	+	–	–	–	–
D_2	–	–	–	+	–	–
D_3	–	–	–	–	–	–
D_4	–	+	–	–	–	–
D_5	+	–	–	–	–	–
Group E (Only As_2O_3)						
E_1	+++	++	+	+	+	+
E_2	+++	++	+	+	+	+
E_3	+++	++	+	+	+	+
E_4	+++	++	+	+	+	+
E_5	+++	++	+	+	+	+

– = Indicates absence of color change of Cu wire, + = Indicates mild color change (blackish), ++ = Indicates moderate color change (blackish), +++ = Indicates severe color change (black) of Cu wire in Reinsch test.

Among the treated groups, few samples of the groups B, C and D which were treated with sodium selenite, ferrous sulfate and zinc sulfate along with arsenic trioxide were positive for arsenic by Reinsch test while all the samples from rats of group E which were treated only with arsenic trioxide were positive for arsenic with mild to marked change of colour of copper wire. In the present study the result of Reinsch test revealed that storage of arsenic was the highest in the liver followed by kidney, spleen, dermis, heart and muscle. Selley *et al.* (1974) reported that arsenic is stored mainly in liver, kidney and spleen which supports the result of the present study.

The semi-quantitative estimation of arsenic in the tissue samples was performed by "Merck Arsen test" kit (Merck, Germany). The semi-quantitative results obtained by "Merck Arsen test" kit are presented in Table 2. Among the treated groups, the rats of groups B (0.0126-0.27 ppm), C (0.03-0.32 ppm) and D (0.04-0.288 ppm) which were supplemented with sodium selenite, ferrous sulfate and zinc sulfate respectively along with arsenic trioxide showed reduced arsenic concentrations in different tissues while the rats of group E treated only with arsenic trioxide showed higher concentrations of arsenic in tissues (0.05-0.4 ppm). In the present study the semi-quantitative estimation revealed that the concentration of arsenic in the liver, kidney, spleen, heart, stomach, intestine, muscle and dermis of the treated rats were 0.27-0.40 ppm, 0.06-0.22 ppm, 0.052-0.20 ppm, 0.0126-0.102 ppm, 0.054-0.16 ppm, 0.064-0.176 ppm, 0.028-0.07 ppm and 0.03-0.05 ppm respectively. Similar results were also reported by many other workers (Krockza and Schah, 1973; Sahli, 1982; Pace *et al.*, 1997).

Table 2. Concentration of arsenic in different tissues of rats

Groups (n = 5)	Treatment	Arsenic concentration (ppm) (Mean ± SE)							
		Liver	Kidney	Spleen	Heart	Stomach	Intestine	Muscle	Dermis
A	Control	0	0	0	0	0	0	0	0
B	As_2O_3 @ 400 mg / L + NaSe @ 1 mg / L	0.27 ± 0.12	0.15 ± 0.04	0.076 ± 0.01	0.0126 ± 0.04	0.060 ± 0.01	0.106 ± 0.04	0.028 ± 0.01	0.03 ± 0
C	As_2O_3 @ 400 mg / L + $FeSO_4$ @ 150 mg / L	0.32 ± 0.04	0.093 ± 0.02	0.052 ± 0.01	0.08 ± 0.02	0.054 ± 0.01	0.064 ± 0.01	0.03 ± 0.01	0.04 ± 0
D	As_2O_3 @ 400 mg / L + $ZnSO_4$ @ 50 mg / L	0.288 ± 0.07	0.06 ± 0.2	0.096 ± 0.04	0.078 ± 0.03	0.054 ± 0.01	0.116 ± 0.06	0.04 ± 0.01	0.04 ± 0.03
E	Only As_2O_3 @ 400 mg / L	0.40 ± 0	0.22 ± 0	0.20 ± 0.3	0.102 ± 0.03	0.16 ± 0.02	0.176 ± 0.03	0.07 ± 0.03	0.05 ± 0

n = No. of rats per group.

It may be concluded that supplementation of selenium, iron and zinc reduces the arsenic concentration in tissues like liver, kidney, spleen, heart, intestine, stomach, muscle and dermis of rats as well as lower the tissue damage caused by arsenic. The present results also indicate that arsenic is deposited mainly in liver followed by kidney, spleen, dermis, heart and muscle.

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