

EFFECTS OF ARSENIC ALONE AND IN COMBINATION WITH SELENIUM, IRON AND ZINC ON CLINICAL SIGNS, BODY WEIGHT AND HAEMATOBIOCHEMICAL PARAMETERS IN LONG EVANS RATS

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ABSTRACT

The effects of selenium, iron and zinc supplementation along with arsenic on clinical signs, body weight and haematobiochemical parameters were studied in 25 mature male long Evans rats (*Rattus norvegicus*) of one month old during the period from August to October 2003. These 25 rats were randomly divided into five equal groups (A, B, C, D and E) of which rats of group A received normal feed and water and rats of group B were given arsenic trioxide (As_2O_3) @ 400 mg / liter plus sodium selenite (NaSe) @ 1 mg / liter; rats of group C were given As_2O_3 @ 400 mg / liter plus ferrous sulfate ($FeSO_4$) @ 150 mg / liter; rats of group D were given As_2O_3 @ 400 mg / liter plus zinc sulfate ($ZnSO_4$) @ 50 mg / liter and rats of group E were given only As_2O_3 @ 400 mg / liter drinking water daily for 42 days. All the rats of groups B, C and D showed very mild toxic signs, i.e., excitement, restlessness, anorexia, ruffled hair coat, salivation and mild skin lesion on tail region. Rats of group E (only As_2O_3) showed various toxic signs like excitement, restlessness, anorexia, ruffled hair coat, salivation, muscle tremor, ataxia, incoordination and posterior paralysis. All the toxic signs observed in group E were more severe and skin lesions were found in all parts of the body especially on tail region. Rats of group E showed highly significant ($p < 0.01$) decrease in body weight, total erythrocyte count, Hb content and packed cell volume (PCV). On the other hand highly significant ($p < 0.01$) increase of ESR values were recorded in group E. Biochemical examination revealed that the activities of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were significantly ($p < 0.01$) elevated in all the treated groups than that of control. But in the rats which were treated only with arsenic trioxide, the elevation of SGPT and SGOT were much higher than other treated groups. It may be concluded that the combined administration of NaSe, $FeSO_4$ or $ZnSO_4$ along with As_2O_3 were found to be less toxic than administration of As_2O_3 alone because selenium, iron, and zinc have protective effects against some toxic effects of arsenic.

Key words: Arsenic, selenium, iron, zinc, haematological parameters, rats

INTRODUCTION

Arsenic poisoning is one of the most important recent problems in Bangladesh. It is important for public health as well as for livestock. The ground water arsenic poisoning in Bangladesh was first confirmed in 1993 in Chapai Nawabgonj District (Smith *et al.*, 2001). Almost 57 million people in Bangladesh (around 40% populations) are at risk for cancer and other diseases because of arsenic contaminated tube-well water (Mohammad, 2002). Ground water in 59 out of 64 districts of Bangladesh is contaminated by deadly arsenic (Shafiuddin and Karim, 2002). Arsenic is ubiquitous in nature, i.e., present in all natural resources like soils, rocks, water, organisms, plants, atmosphere, etc. Most of the arsenic related environmental problems are the result of mobilization under natural conditions. However, man has an important additional impact through different activities such as combustion of fossil fuel, the use of arsenical pesticides, herbicides, crop desiccants and the use of arsenic as an additive to livestock feed, particularly for poultry. The use of arsenical compound for wood preservation is still common. The impact on the environment of the use of arsenical compounds will remain for some years. Many scientists from different countries are working on the arsenic problem in Bangladesh but no one has come up with an explanation based on convincing evidence that pinpoints the source of arsenic that is poisoning the people of Bangladesh. So, considering all possible factors, this pioneering work has been undertaken to determine the effects of feeding arsenic trioxide alone and in combination with selenium, iron and zinc in rats by observing toxic signs, body weight and haematobiochemical parameters.

MATERIALS AND METHODS

The experiment was carried out in the Experimental Pharmacology and Toxicology Laboratory, Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh during the period from August to October 2003. A total of 25 one-month-old male Long Evans rats were used in this experiment. All the rats were kept under close observation in order to acclimatize to the new environment for a period of one week. 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, each consisting of five rats and were marked as group A, B, C, D and E.

Among 5 groups, one group of rats (group A) was kept as control fed with normal feed and water while the remaining groups were received arsenic trioxide @ 400 mg / liter drinking water daily for 42 days. In addition, groups B, C and D were treated with sodium selenite @ 1 mg / liter, ferrous sulfate @ 150 mg / liter and zinc sulfate @ 50 mg / liter drinking water respectively daily for 42 days and observed carefully for appearance of toxic signs.

Body weight of the control and treated rats were taken before commencement of treatment and then weekly during 42 days of feeding. Blood samples were collected from the tail vein of all the groups in screw capped vial with and without anticoagulant at day 0 and day 42 for haematological and biochemical studies respectively. The haematological parameters were determined as per method cited by Lamberg and Rothstein (1977). The biochemical parameters were performed by UV method using IFCC (International Federation of Clinical Chemistry) used Humalyzer 2002 (Human type, Germany).

Data were analyzed statistically by Student's 't' test (Bailey, 1981).

RESULTS AND DISCUSSION

Rats of group A (control group) were quite normal during the whole experimental period. Rats of groups B (arsenic trioxide @ 400 mg / L plus sodium selenite @ 1 mg / L), C (arsenic trioxide @ 400 mg / L plus ferrous sulfate @ 150mg / L) and D (arsenic trioxide @ 400 mg / L plus zinc sulfate @ 50mg / L) showed very mild toxic signs, i.e., excitement, restlessness, anorexia, salivation and very mild skin lesion found on tail region. Rats of group E (only arsenic trioxide @ 400 mg / L) showed various toxic signs like excitement, restlessness, anorexia, salivation, muscle tremor, ruffled hair coat, ataxia, posterior paralysis, incoordination, reduced feed consumption. Many other authors reported clinical signs similar to the present findings (Harding *et al.*, 1968; Confer *et al.*, 1980; Riviere *et al.*, 1981; Balatskii, 1989 and Blackley *et al.*, 1990). The mean body weight of rats of group B, C and D were decreased gradually but not significant statistically (Table 1).

Table 1. Effect of arsenic trioxide alone and in combination with selenium, iron and zinc supplementation on mean body weight in rats (Mean \pm SE)

| Days | Group A (Control) (n = 5) | Group B (As ₂ O ₃ @ 400 mg / L + NaSe @ 1 mg / L) (n = 5) | Group C (As ₂ O ₃ @ 400 mg / L + FeSO ₄ @ 150 mg / L) (n = 5) | Group D (As ₂ O ₃ @ 400 mg / L +ZnSO ₄ @ 50 mg / L) (n = 5) | Group E (Only As ₂ O ₃ @ 400 mg / L) (n = 5) |
|------|---------------------------------|--|---|---|---|
| 0 | 178.94 \pm 7.50 | 184.66 \pm 5.37 | 179.56 \pm 9.76 | 176.22 \pm 11.33 | 183 \pm 4.85 |
| 7 | 184 \pm 7.45 | 180.12 \pm 5.44 | 177.2 \pm 9.92 | 174.8 \pm 11.10 | 179.38 \pm 4.76 |
| 14 | 188.8 \pm 7.22 | 178.28 \pm 5.15 | 175.68 \pm 8.88 | 172.08 \pm 10.22 | 173.04 \pm 4.9 |
| 21 | 192.4 \pm 7.4 | 175.94 \pm 5.17 | 172.26 \pm 8.18 | 170.2 \pm 10.12 | 167.78 \pm 5.21 |
| 28 | 196.50 \pm 8.81 | 173.12 \pm 4.35 | 170.08 \pm 8.24 | 169.18 \pm 9.84 | 163.2 \pm 5.05* |
| 35 | 200.8 \pm 8.86 | 170.32 \pm 4.59 | 168.10 \pm 7.47 | 167.76 \pm 9.91 | 158.82 \pm 5.20** |
| 42 | 202 \pm 9.12 | 168.24 \pm 8.68 | 165.5 \pm 6.81* | 165.7 \pm 9.62 | 152.6 \pm 5.10** |

n = Number of rats weighed, **Significant at p < 0.01, *Significant at p < 0.05.

In group E decrease in body weight was highly significant (p < 0.01) at 35 and 42 days of treatment. A similar result on body weight was observed by many other workers (Byron *et al.*, 1967; Suren, 1977; Confer *et al.*, 1980). Total erythrocyte count (TEC) and haemoglobin content (Hb) were slightly reduced and packed cell volume (PCV) significantly (p < 0.05) reduced in groups B, C and D but highly significant (p < 0.01) reduction of TEC, Hb content and PCV were observed in group E (Table 2). In contrast to TEC, Hb content and PCV, ESR value was slightly increased in groups B, C and D but significantly (p < 0.01) increased in group E. Variable haematological values have been reported by Klassen (1991). It may be assumed that toxic effects induced by arsenic trioxide on bone marrow may be responsible for erythrocytopenia and leukocytosis observed in this study.

Effects of arsenic in rats

Table 2. Effect of arsenic trioxide alone and in combination with selenium, iron and zinc supplementation on certain haematobiochemical parameters in rats

| Parameters | Group A (Control) (n = 5) | | Group B (As ₂ O ₃ @ 400 mg/L +NaSe @ 1 mg/L) (n = 5) | | Group C (As ₂ O ₃ @ 400 mg/L +FeSO ₄ @ 150 mg/L) (n = 5) | | Group D (As ₂ O ₃ @ 400 mg/L +ZnSO ₄ @50 mg/L) (n = 5) | | Group E (Only As ₂ O ₃ @ 400 mg/L) (n = 5) | |
|-------------------------------------|---------------------------------|-----------------|---|---------------------|--|---------------------|--|---------------------|---|--------------------|
| | Pre- dosing | Post- dosing | Pre- dosing | Post- dosing | Pre- dosing | Post- dosing | Pre- dosing | Post- dosing | Pre- dosing | Post- dosing |
| TEC (10 ⁶ /cmm) | 7.936 ± 0.91 | 7.959 ± 0.13 | 7.935 ± 0.07 | 7.554 ± 0.22 | 7.938 ± 0.16 | 7.755 ± 0.05 | 7.936 ± 0.14 | 7.724 ± 0.18 | 7.937 ± 0.18 | ** 4.668 ± 0.14 |
| Hb (g%) | 8.00 ± 0.22 | 8.25 ± 0.17 | 8.06 ± 0.06 | 7.70 ± 0.98 | 8.12 ± 0.11 | 7.80 ± 0.12 | 8.20 ± 0.12 | 7.68 ± 0.25 | 8.00 ± 0.22 | ** 6.10 ± 0.18 |
| PCV (%) | 35.00 ± 1.00 | 35.20 ± 1.12 | 35.25 ± 0.94 | * 30.85 ± 0.95 | 35.45 ± 0.46 | * 32.60 ± 0.75 | 35.00 ± 0.94 | * 30.85 ± 0.95 | 35.01 ± 0.50 | ** 26.80 ± 0.37 |
| ESR (mm in 1 st hour) | 0.5 ± 0.16 | 0.55 ± 0.09 | 0.5 ± 0.16 | * 0.85 ± 0.03 | 0.5 ± 0.16 | 0.72 ± 0.22 | 0.5 ± 0.16 | 0.73 ± 0.09 | 0.5 ± 0.16 | ** 4.2 ± 0.37 |
| SGPT (U / L) | 160 ± 0.14 | 163 ± 0.16 | 161 ± 0.95 | ** 180.50 ± 0.25 | 162 ± 0.97 | ** 190.62 ± 0.14 | 162 ± 0.96 | ** 181.78 ± 0.22 | 163.35 ± 0.84 | ** 220 ± 0.12 |
| SGOT (U / L) | 165 ± 0.12 | 165 ± 0.25 | 163 ± 0.94 | ** 183 ± 0.16 | 164 ± 0.99 | ** 195 ± 0.14 | 165 ± 0.94 | ** 178 ± 0.22 | 166 ± 0.97 | ** 230 ± 0.12 |

Pre-dosing at day 0 of commencement of experiment, Post-dosing at 42 days of treatment, n = Number of blood samples, ** Significant at p < 0.01, * Significant at p < 0.05.

Biochemical examination revealed that the activities of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were significantly (p < 0.01) elevated in all the treated groups than that of control. But in the rats which were treated only with arsenic trioxide, the elevation of SGPT and SGOT were much higher than other treated groups. The present findings could not be compared with the findings of other workers due to lack of available literature on biochemical parameters. It may be concluded that arsenic is highly toxic both for man and laboratory animals and some cheap chemicals like selenium, iron and zinc supplementation may be helpful in reducing arsenic toxicity.

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