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Effectiveness of zinc in modulating perinatal effects of arsenic on the teratological effects in mice offspring

Mohammad Ahmad[a], Mohammad A. M. Wadaan[b], Muhammad Farooq[b], Maha H. Daghestani[c] and Ahmed S. Sami[d]

[a] Department of Medical Surgical Nursing, College of Nursing.
[b] Department of Zoology, College of Science.
[c] Department of Zoology, Center for Scientific and Medical Female Colleges.
[d] Department of Animal Production, College of Food and Agricultural Sciences; King Saud University, Riyadh, Saudi Arabia.

ABSTRACT

Exposure to arsenic via drinking water is considered as a worldwide problem. Studies have shown that arsenic exposure during pregnancy affects embryogenesis and offspring development in rats and mice. Zinc as a micronutrient regulates many physiological functions, including an antioxidative role under various toxic conditions. However, studies on the perinatal protective effect of zinc on offspring need further attention. The present study was designed to evaluate the potential protective role of zinc in mitigating the adverse effects in the offspring of arsenic exposure during pregnancy. The arsenic (40mg/kg body weight) and zinc (4% w/v) doses formed the only drinking fluid source for the experimental groups of dams during the perinatal period of the experiment. The early development of sensory motor coordination reflexes together with morphological development in the male pups was measured during the weaning period. In adolescence, the offspring were tested for their motor behavior. The enzyme γ-glutamyl transferase (γ-GT) and the oxidative stress indices like reduced glutathione (GSH) and lipid peroxidation (TBARS) were also estimated in the serum of the young adult male mice. Perinatal arsenic exposure caused depletion in body weight gain, delay in morphological development and retardation in the development of all sensory motor reflexes of the pups. In young adults, significant decrease in motor behavior with significant decrease in GSH level in the serum was observed. On the other hand, γ-GT and TBARS were significantly increased in the serum due to arsenic treatment. However, animals exposed to arsenic in the presence of zinc showed a remarkable ameliorating effect of zinc on all observed teratological and biochemical arsenic toxicity in male offspring. It was observed that zinc has an antioxidative role in the perinatal toxicity of arsenic. It is concluded from the present study that zinc consumed during the perinatal period of pregnancy can ameliorate the possible toxicities of arsenic exposure in the offspring by acting as an ameliorative supplement.

Key words: Arsenic, zinc, perinatal, mice offspring, sensory motor reflexes, behavior, oxidative stress.

INTRODUCTION

Chronic arsenic (AS) poisoning caused by exposure via drinking water has been reported in many countries of the world (Frisbie *et al.,* 2002; Tian *et al.*, 2001) and is considered as a widespread and worldwide problem (Jin *et al.*, 2004; Mishra and Flora, 2008). Chronic exposure to AS may lead to a variety of symptoms including skin pigmentation, numbness, hypertension, diabetes, cardiovascular disease, anemia, neurological disorders and liver and kidney diseases (Szymanska-Chabowska *et al.*, 2002), whereas, acute AS exposure may cause nausea, vomiting, diarrhea, weakness, loss of appetite, shaking, cough, headache and neuropathy. AS is a known human carcinogen and causes various forms of malignancy and tumors of the skin, urinary bladder, liver, kidney and lung (Smith *et al.*, 1992; Chen *et al.*, 1992; Cui *et al.*, 2006; Suzuki *et al.*, 2008; Liu and Walkes, 2008). The health effects of toxic levels of AS are multidimensional in both human and animal populations (Nandi *et al.*, 2006). Liver and kidneys are considered as the primary targets for its toxico-pathological manifestations, and there are reports of biochemical alterations indicative of hepatic and renal system involvement in AS toxicity in animals (Biswas *et al.*, 1998; Santra *et al.,* 1999). After ingestion, dissolved AS compounds are readily absorbed through the gastrointestinal tract and distributed in the blood to the liver, kidney, spleen, lung and many other organs; it affects nearly all organ systems of the body (Guha Majumdar, 2005). Epidemiological and animal studies have suggested potent mutagenic and carcinogenic abilities of AS (Brown and Kitchin, 1996; Chen and Shi, 2002), toxic and teratogenic effects in chick embryogenesis (Gilani and Alibhai, 1990). Experimental studies have also shown that AS exposure during pregnancy affects embryogenesis and offspring development in mice (Ma *et al.,* 1994), rats (Zhang *et al.*, 1997; 1999) and rodents (Wang *et al.*, 2006).

More recently, the enhanced production of reactive oxygen species has been implicated to contribute to cell injury associated with AS exposure (Flora, 1999; Ramos *et al.,* 1995). Indirect evidence of a role of oxidative stress in AS toxicity includes therapeutic and prophylactic efficacy of exogenously administered nutritional antioxidants such as ascorbic acid, a-tocopherol, methionine, thiamine and cysteine in acute and chronic AS toxicity in laboratory rats (Nandi *et al.,* 2005; Flora, 1999; Ramanathan *et al.,* 2002). Several mechanisms have been put forth to explain the mechanism of AS toxicity.

Zinc is a micronutrient as well as a trace element that is essential for a broad range of biological activities, and is nontoxic with the exception of a very high dose (Berthholf, 1988). Zinc is a fundamental element of more than 200 metalloenzymes and affects the activity and stability of many of them. Several studies have demonstrated the protective

Corresponding author: Dr. Mohammad Ahmad. College of Nursing Department of Medical Surgical Nursing. King Saud University. P.O.Box 642, Riyadh – 11421, Saudi Arabia. E-mail: mbadshah@ksu.edu.sa. Phone : +966-505195887. Fax : +966-11-4693770

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antioxidative role of zinc under various toxic conditions (Rostan *et al.*, 2002; Sidhu *et al.*, 2004a and b; Goel *et al.*, 2005; Bhalla *et al.*, 2007; Malhotra and Dhawan, 2008). Zinc is present in the brain at high concentrations and regulates many physiological functions (Singh *et al.*, 1995; Goel *et al.*, 2005). It is an essential catalytic or structural element of many proteins (Choi and Koh, 1998). Concentrated in synaptic vesicles, zinc is released during neuronal activity. Extracellular zinc modulates the activity of ion channels such as the NMDA receptors (Paoletti *et al.*, 1997) and store-operated Ca2+ channels (Jan *et al.*, 1999). Furthermore, the relevance of zinc in cognitive development and CNS homeostasis is well known (Takeda, 2001). In addition, zinc is also necessary to mobilize defenses against reactive oxygen species (ROS) and H_2O_2-induced apoptosis (Chung *et al.*, 2005; Chung *et al.*, 2006).

Therefore, it is important to explore the possibility of the protective potential of zinc in mitigating the adverse effects in the offspring of AS exposure during pregnancy, which would be a step forward in the management of patients who undergo AS poisoning. Furthermore, the present study may open new prospects for zinc to act as a nutritional and protective supplement for women during pregnancy to protect their offspring from the possible toxic effects of AS exposure during the perinatal period, which may ultimately prove beneficial for the health of the offspring by ameliorating adverse effects of AS. The present study has been designed to evaluate the effects of zinc on the perinatal toxicity of AS on the morphology and development of reflexes in mouse pups and on motor behavior and oxidative stress in the serum of male adolescent offspring.

MATERIALS AND METHODS

Experimental animals

Male and female Swiss-Webster strain mice (8-10 weeks old) were obtained from the animal husbandry of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were housed in opaque plastic cages (three females and one male in each cage) under hygienic conditions in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. All animals were maintained under reversed lighting conditions with white lights on from 22.30 to 10.30 hours local time. The ambient temperature was regulated between 18 and 22 °C. After pregnancy (appearance of a vaginal plug was considered as day one of pregnancy), the males were removed from the cages and the females were subjected to experimental treatments. Food (laboratory animal diet, F-648, Grain Silos and Flour Mills Organization, Riyadh) and water were available *ad libitum* unless otherwise indicated. Only a trace quantity of zinc was present in this rat food according to the manufacturer. All procedures were carried out in accordance with the ethical guidelines for care and use of laboratory animals, and all protocols were approved by the local Ethics and Care of Experimental Animals Committee.

Pregnant female animals were divided into four groups with ten animals in each. Group I consisted of untreated mice and served as controls. Group II was treated with zinc in drinking water. Group III consisted of mice treated with AS in drinking water and Group IV consisted of mice co-administered with a mixture of AS and zinc.

Arsenic and Zinc administration

AS (sodium arsenate; analytical grade, Riedel de Haen, Germany) was dissolved in tap water in such a way that it resulted in a dose of 40mg/kg body weight of the animal per day. After calculating the average volume of water consumed by the animals in one day, and by knowing the average body weight of the animals, AS was dissolved in tap water (0.04 mg/ml) that resulted in a dose of 40 mg/kg body weight per day. Zinc was administered in the form of zinc sulfate (analytical grade, Riedel de Haen, Germany) in drinking water at a dose of 4% w/v, which was equivalent to 40 mg/kg body weight per day. These AS and zinc doses formed the only drinking fluid source for the experimental groups of dams during the perinatal period of the experiment. Our pilot studies used two (20 and 40 mg/kg) doses of AS and zinc; these two doses are within the ranges of doses reported in published literature. It was observed in our pilot studies that the higher dose (40 mg/kg) inflicted maximum effects in the animals in comparison to the lower dose of 20 mg/kg (data not shown). Thus only the highest effective doses of zinc and AS were selected for the present study. Furthermore, our pilot studies did not show any reaction or precipitation upon mixing the zinc and AS salts together in a single bottle. Thus for the mixture of zinc and AS we used zinc and AS dissolved in the same bottle for the administration as the drinking fluid. Treatment of mothers started from day one of pregnancy and was continued until postnatal day 15 (PD15) after birth of the offspring and thereafter the mothers were switched to plain tap water. All pregnant mice were housed individually. The drinking fluid containing AS and/or zinc doses was changed to fresh preparations every third day. The control group received plain tap water only.

Behavioral observations

On the day of birth (postnatal day 0, PD0) the pups were culled to only eight per dam and were left with their mothers until PD22. During this weaning period, three pups of each litter were color marked at random (without consideration of gender) and were subjected to various behavioral tests (described below) under dim light (ca 8 lux). In all, 21 pups belonging to seven litters from each treatment category were considered. For statistical analysis, the mean of all three color marked pups per litter was considered as a single score. Thus seven replicates from each treatment category were considered in these observations. All observations were recorded on PD1 and repeated every other day until PD21 in the same three color-marked pups of each litter. These observations were used to measure the early development of sensory motor coordination reflexes together with morphological development in the pups.

Body weight:

The pups were weighed every other day from PD1 until PD21.

Righting reflex:

The time taken by a pup placed on its back to turn over and place all four paws on the substrate was recorded. An upper limit of 2 min was set for this test.

Cliff avoidance activity:

Pups were placed on the edge of a table top with the forepaws and face over the edge. The time taken by the pups to back away and turn from the "cliff" was recorded. Again an upper limit of 2 min was chosen. A latency of 2 min was attributed when the animal fell from the "cliff".

Rotating reflex:

The surface used to measure the rotating reflex was the same as that used for the righting reflex, except that it was inclined at an angle of 30°. The pups were placed on this surface with their heads pointed downwards. The time elapsed until the pup rotated its body through 180° geonegatively and faced its head upwards was recorded as the rotating time. The upper limit of this test was also set at 2 min.

Eye opening and hair appearance:

The days at which the body hair fuzz appeared and the eyes opened were also recorded.

Motor Tests of young adult males:

The offspring were weaned on PD21, after which the males were separated and kept in groups of two or three. Subsequently, 10 males from each treated group (including representatives from each of the 7 litters) were subjected to motor activity tests. The young adult males were placed in an experimental wooden arena measuring 80 X 80 X 30 cm and the floor was divided into 64 squares of equal size. Various behavioral elements were observed as described by Ajarem (1987 and Ajarem and Ahmad (1991). Elements of locomotor activity included the number of squares crossed, wall rears, rears and washes, as well as the duration of locomotion and immobility. These visual observations in the arena lasted for 300 sec for each animal.

Biochemical study

At the age of PD21, one male pup was chosen from each litter, apart from the three color-marked pups that were used for the behavioral tests. Thus seven offspring from each experimental group were sacrificed by decapitation and their blood was collected using EDTA as the anticoagulant. The blood samples were centrifuged at 1000g for 10 min and the clear non-hemolyzed supernatant (plasma or serum) was used for various biochemical estimations.

γ-glutamyl transferase (γ-GT):

The enzyme γ-glutamyl transferase was assayed by the method described in Kit No.GT1065 of Randox, U.K., based on the principle of Szasz (1969). The specific activity was expressed as Units per liter (U/l).

Reduced glutathione (GSH):

Reduced glutathione (GSH) content was estimated according to the method of Ellman (1959). Briefly, 0.1 ml of 25% trichloroacetic acid was added to 0.5 ml of plasma. After protein precipitation by trichloroacetic acid, the samples were centrifuged to obtain the supernatant. Then 0.1 ml of the supernatant was incubated with 2.0 ml of freshly prepared (0.6 mmol/l) 5, 5 - dithiobis (2-nitrobenzoicacid). The optical density of the yellow complex was measured at 412 nm against a reference lacking plasma. For each set of assays, a standard curve was obtained for reduced glutathione (GSH) and used to calculate GSH content in serum from samples.

Lipid peroxidation (TBARS):

Lipid peroxidation (TBARS) was assayed using the method of Ohkawa *et al.* (1979). Briefly, a mixture of 8% solvent sodium dodecyl sulfate (0.2 ml), 0.9 % TBA (thiobarbituricacid; 0.2ml), and 20% acetic acid (1.5 ml) was prepared, to which 0.2 ml of plasma was added and the volume was made up to 4 ml by adding distilled water. After boiling for 1 hour the mixture was cooled, and 5 ml of solution of n-butanol + pyridine (vol/vol 15:1) was added and then centrifuged at 1000 g for 15 minutes, Absorbance in the supernatant was measured at 532 nm using a spectrophotometer.

Protein:

Protein concentrations were measured by the method of Lowry *et al.* (1951). Briefly, the samples were diluted with 100 mmol/l phosphate buffer (pH7.5) to a volume of 0.5 ml. The reactions were diluted with 0.5 ml 1.0 N sodium hydroxide followed by the addition of 5.0 ml reagent mixture (containing 48 ml 2% sodium carbonate, 1 ml 1% copper sulfate and 1.0 ml 2% sodium potassium tartarate). After 10 minutes of incubation at room temperature, the color was developed by the addition of 1.0 N Folin's reagent, and absorbance was measured using a spectrophotometer at 750 nm.

Statistical analysis:

The data were compared between the experimental groups by the analysis of variance (ANOVA) using a minitab computer program, and were subsequently analyzed by Student's t-test (Yamane , 1973), except the data of the motor test that was analyzed using the Mann-Whitney U test (Sokal and Rohlfe, 1981).

RESULTS

The body weight of the AS treated pups lagged behind their controls from the day of their birth (PD1) and remained so throughout almost all their weaning period until PD21. A significant decline in the body weight of the treated pups was observed on PD7, PD14 and PD21 (Fig. 1). Zinc alone had no effect on the body weight compared to the control. In contrast, zinc in combination with AS had a significant ($p<0.05$) protective effect on body weight compared to the arsenic group (Fig. 1).

Morphological development such as eye opening and body hair appearance were also affected by AS treatment (Fig.2). Both of these morphological parameters in the AS treated pups were significantly ($p<0.05$) delayed compared to the controls. Zinc alone had no effect whereas zinc in combination with AS had a significant ($p<0.05$) reduction in the delay of eye opening

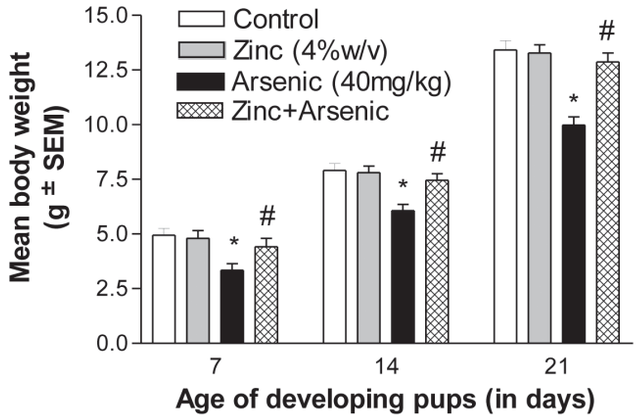

Figure 1. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the body weight gain of the developing pups on post natal day7 (PD7), PD14 and PD21 (weaning period). Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.
* represents statistically significant ($p< 0.05$) depletion as compared to the control and # represents significant ($p< 0.05$) amelioration in body weight decline compared to the arsenic alone treated group by Newman Keul's student's t test after one-way ANOVA.

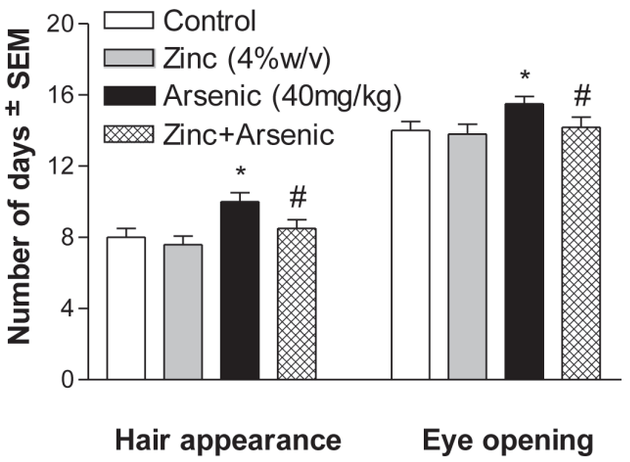

Figure 2. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the body hair fuzz appearance and opening of the eyes in the postnatal developing pups. Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.
* represents statistically significant ($p<0.05$) delay as compared to the control and # represents significant ($p< 0.05$) amelioration in body hair fuzz appearance and opening of the eyes compared to the arsenic alone treated groups by Newman Keul's student's t test after one way ANOVA.

TABLE 1

Effect of perinatal oral exposure to zinc[a] and arsenic[b] independently and in combination in drinking water on the motor activity of male adolescent offspring (post natal day 22)

Treatment Groups	Median number (with ranges) of acts and postures					
	Number of squares crossed	Wall rears	Rears	Wash	Movement duration (sec)	Immobility duration (sec)
Control	371 (314 – 409)	33 (28 – 41)	15 (11– 22)	6 (3 – 10)	226.5 (192.5 – 288.5)	73.5 (12 – 108)
Zinc (4%w/v)	338 (298 – 387)	30 (25 – 40)	11 (9 – 19)	5 (4 – 9)	238 (184.5 – 290)	62 (10 – 115.5)
Arsenic (40mg/kg)	128** (87 – 193)	9** (4 – 15)	5** (0 – 9)	7 (4 – 11)	92.5*** (78.5 – 122.5)	208*** (177.5 – 221.5)
Zinc + Arsenic	256## (183 – 317)	24## (19 – 35)	10## (8 – 18)	6 (2 – 9)	196## (149.5 – 263.5)	104.5## (36.5 – 150.5)

** and *** significantly different ($P < 0.01$ and $P < 0.001$ respectively) from the control by Mann-Whitney U test . ## significantly different (*P<0.01)* from the arsenic alone treated offspring by the Mann-Whitney U test. [a and b] Fresh zinc and arsenic doses formed the sole available drinking fluid to the dams from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.

and body hair appearance compared to the AS alone group (Fig. 2).

Perinatal exposure of mice to AS had a significant effect on the early development of all sensory motor reflexes in the pups studied here. Pups born to treated mothers were lethargic and sluggish from the very first day (PD1). During the first two weeks of postnatal development AS had significant ($p<0.05$) suppressive effect on the righting reflex and cliff avoidance activity, as shown in Fig. 3 and 4, respectively, whereas the rotating reflex (Fig.5) was significantly ($p<0.05$) suppressed only during the first week of postnatal development. Exposure to zinc alone had no significant effect on these reflexes and resembled the control animals. However, zinc in combination with AS reduced the suppressive effects significantly ($p<0.05$) compared to the AS alone group in all the developing sensory motor reflexes of the pups (Figs. 3, 4 and 5).

The locomotor activity test (Table 1) showed that perinatal AS exposure affected almost all the elements of acts and postures of the male offspring. The number of squares crossed, wall rears, rears and movement duration decreased significantly ($p<0.05$), whereas the duration of immobility increased significantly ($p<0.05$) in the AS treated animals compared to the controls. Exposure to zinc alone had no effect on any of the parameters. However, zinc in combination with AS increased significantly ($P<0.05$) all locomotor parameters compared to the offspring exposed to AS alone (Table 1) and was able to bring the affected behavioral activities within the normal untreated control range (Table 1).

The level of γ-glutamyltransferase (GGT) activity in the plasma of the male offspring was significantly increased

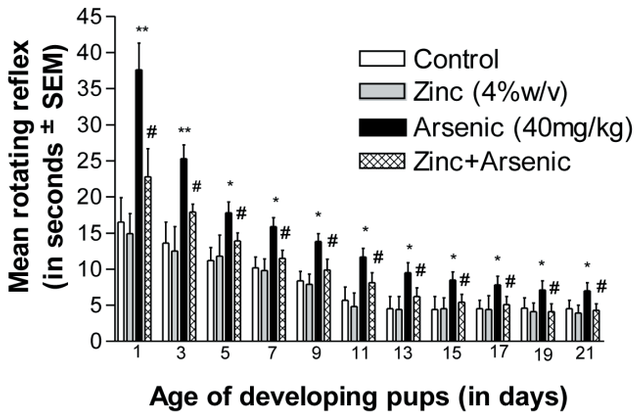

Figure 4. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the mean rotating reflex of the developing pups from post natal day1 (PD1) until PD21 (weaning period). Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.
* and ** represent statistically significant ($p<0.05$ and $p<0.01$ respectively) delay in righting reflex as compared to the control and # represents significant ($p<0.05$) amelioration in delay of rotating reflex development compared to the arsenic alone treated groups by Newman Keul's student's t test after one way ANOVA.

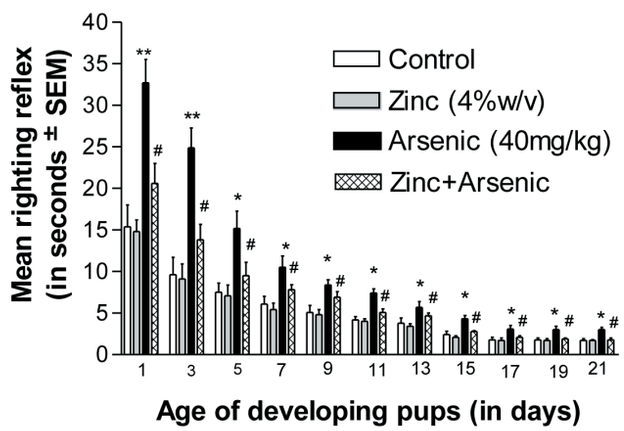

Figure 3. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the mean righting reflex of the developing pups from post natal day1 (PD1) until PD21 (weaning period). Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), eafter which the dams were switched to plain tap drinking water.
* and ** represent statistically significant ($p<0.05$ and $p<0.01$ respectively) delay in righting reflex as compared to the control and # represents significant ($p<0.05$) amelioration in delay of righting reflex development
compared to the arsenic alone treated groups by Newman Keul's student's t test after one way ANOVA.

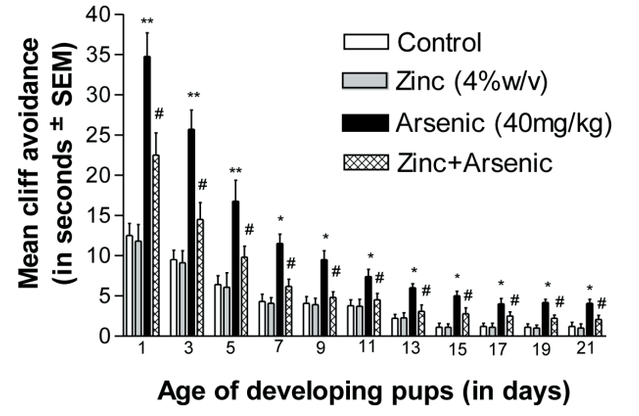

Figure 5. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the mean cliff avoidance reflex of the developing pups from post natal day1 (PD1) until PD21 (weaning period). Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.
* and ** represent statistically significant ($p<0.05$ and $p<0.01$ respectively) delay in avoidance of the cliff reflex as compared to the control and # represents significant ($p< 0.05$) amelioration in delay of the cliff avoidance reflex development compared to the arsenic alone treated groups by Newman Keul's student's t test after one way ANOVA.

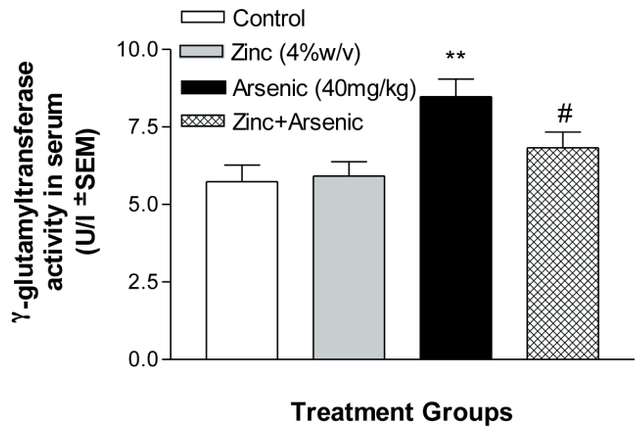

Figure 6. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the γ glutamyltransferase (GGT) activity in the serum of the male adolescent offspring. Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.
** represents statistically significant ($p<0.01$) increase in GGT activity as compared to the control and # represents significant ($p<0.05$) amelioration in GGT activity stimulation by bringing back the level of GGT to the level of The control group as compared to the arsenic alone treated group by Newman Keul's student's t test after one way ANOVA.

after perinatal exposure to AS (Fig. 6). Administration of zinc along with AS led to a significant decrease in the level of GGT compared to the AS alone treatment group and was able to bring the raised level of GGT to within the normal untreated control range (Fig. 6).

TBARS level showed a significant increase ($p<0.05$) in the plasma of the male offspring exposed perinatally to AS (Fig. 7). However, perinatal administration of zinc along with AS led to a significant decrease ($p<0.05$) in the level of TBARS compared to the AS alone treatment group and was able to raise the level of TBARS to within normal untreated control range (Fig. 7). The level of GSH was significantly decreased ($p<0.05$) in the blood serum after perinatal AS exposure (Fig. 8) c whereas perinatal exposure to zinc alone was ineffective on GSH level, but exposure to zinc in combination with AS was able to increase significantly ($p<0.05$) the GSH level compared to the AS alone exposed offspring, and was effective in restoring the decreased level of GSH within the normal control range (Fig. 8).

DISCUSSION

The present results suggest that perinatal exposure to AS is toxic to the offspring and influences their postnatal developing morphology and sensory motor reflexes during the weaning period, and also their motor behavioral activities and the levels of GGT enzyme and TBARS and GSH activities (causing oxidative stress) in the serum of the adolescent offspring. However, animals exposed to AS in the presence of

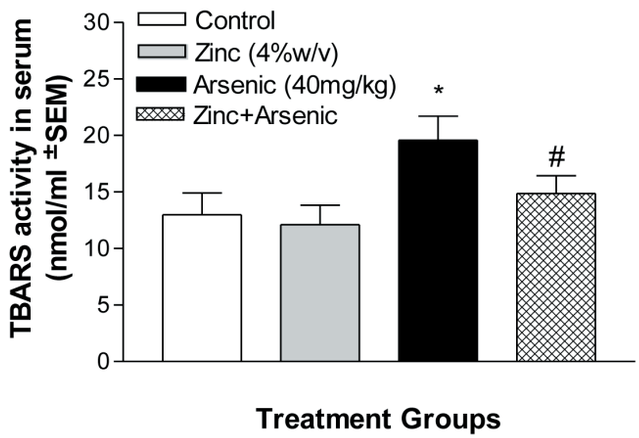

Figure 7. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the oxidative stress enzyme lipid peroxidation (TBARS) activity in the serum of the male adolescent offspring. Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), eafterwhich the dams were switched to plain tap drinking water.
* represents statistically significant ($p<0.05$) increase in TBARS activity compared to the control and # represents significant (p 0.05) amelioration in TBARS activity stimulation by bringing back the level of TBARS to the level of the control group compared to the arsenic alone treated group by Newman Keul's student's t test after one way ANOVA.

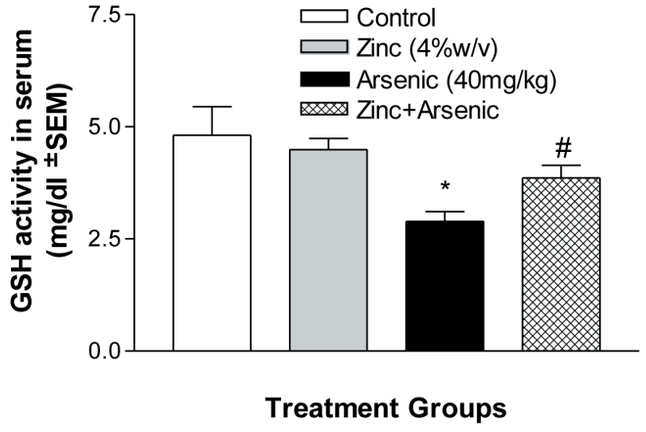

Figure 8. Effect of perinatal oral exposure to zinc (4%w/v) and arsenic (40mg/kg body weight), independently and in combination in drinking water on the oxidative stress enzyme reduced glutathione (GSH) activity in the serum of the male adolescent offspring. Fresh zinc and arsenic doses formed the only available drinking fluid to the dams 24h from day one of pregnancy until PD15 after birth of the offspring (perinatal exposure period), after which the dams were switched to plain tap drinking water.
* represents statistically significant ($p<0.05$) decrease in GSH activity compared to the control and # represents significant ($p<0.05$) amelioration in GSH activity depletion by restoring the level of GSH to the level of the control group compared to the arsenic alone treated group by Newman Keul's student's t test after one way ANOVA

zinc showed a remarkable ameliorating effect of zinc on the AS toxicity since these offspring did not exhibit significant behavioral or biochemical deficits compared to the groups exposed to AS alone. Furthermore, the ineffectiveness of zinc alone to inflict any behavioral or biochemical change suggests the non-toxicity of zinc itself in the animals.

Body weight, body hair appearance and opening of the eyes can serve as useful indicators of morphological developments in animals. The postnatal depletion in body weight and the delay in the appearance of the body hair and opening of the eyes in the AS-exposed pups may be indicative of lasting AS - induced postnatal effects in the pups. It has been shown experimentally that perinatal exposure to AS affects embryonic and offspring development in mice (Ma *et al.*, 1994), rats (Zhang *et al.*, 1997; 1999) and rodents (Wang *et al.*, 2006).

Perinatal AS exposure suppressed the preweaning reflexes in the developing mouse pups. Such decreases in early nerve reflex and neurobehavioral development of the rodent offspring due to AS exposure have been reported earlier also (Ma *et al.*, 1994; Zhang *et al.*, 1999). This suggests a direct intervention of AS in the developing pups *in utero.* At the same time, it is also likely that the pups received AS via their mother's milk during lactation. It is now well documented that significant quantities of compounds that are given to the mothers during late pregnancy may be transmitted to the offspring *in utero* and/or via mother's milk during lactation (Ajarem and Ahmad, 1991, 1998; Draski *et al.*, 1989; Mereu *et al.*, 1987). Furthermore, the suppressing effects of AS on the motor behavior of the offspring at the early adolescent stage suggest a deleterious effect and significant hypo activity brought about by perinatal AS exposure. Exposure to zinc alone caused no alteration in any of the observed behavioral parameters and resembled the control offspring, whereas exposure to zinc in combination with AS had an ameliorating effect on all observed behavioral parameters by reducing the intensity of the deleterious effects of AS alone and by bringing behavior back within the control range. Thus it indicates that zinc has a protective effect on the morphological, motor sensory reflexes and motor behavioral activities by modulating the adverse effects on the offspring of perinatal AS exposure.

The biochemical results in the present study suggest that GGT and the oxidative stress parameters of TBARS and GSH may serve as reliable biomarkers for detecting teratological and/or behavioral effects of AS toxicity in experimental models. These biomarkers are significantly altered in the serum of the offspring when their mothers are exposed to AS during the perinatal period. It is pertinent to note here that Kaltreider *et al*. (2001) reported that extremely low levels of AS exposure can cause cell damage or toxicity and alter hormonal functions in the glucocorticoid system which is centrally involved in the control of growth, glucose regulation and protein metabolism (Vahter *et al.*, 2002). Thus perinatal exposure to AS during pregnancy may result in the oxidative stress which ultimately may have resulted in the altered behavioral expressions. Zinc alone in the present study did not affect any of the biomarkers, however, zinc in combination with AS was significantly effective in ameliorating the deleterious effects of AS alone on GGT, TBARS and GSH. This shows that zinc administration results in a reversal in indicators of oxidative stress and may have a role in reducing the toxicity of AS. Interestingly, Fascineli *et al*. (2002) reported that zinc did not improve AS teratogenesis in their *in vivo* and *in vitro* studies. This might be due to the differences in the salt form of AS used in their studies. However, further studies including other oxidative stress parameters are required to ensure the antioxidative role of zinc in ameliorating the toxicity of AS. Several studies have demonstrated the antioxidative role of zinc under various toxic conditions (Rostan *et al.*, 2004). The protective potential of zinc has been evaluated under other toxic conditions also (Sidhu *et al.*, 2004 a, b; Goel *et al.*, 2005; Bhalla *et al.*, 2007; Malhotra and Dhawan, 2008). Extracellular zinc modulates the activity of ion channels of various receptors (Paoletti *et al.*, 1997; Jan *et al.*, 1999; Choi *et al.*, 2001). Furthermore, the relevance of zinc in CNS homeostasis is well known (Takeda, 2001). According to the present study, zinc consumed during the perinatal period of pregnancy can ameliorate the possible toxicity of AS exposure in the offspring.

CONCLUSION

The present study, together with the supporting literature, clearly suggest that AS toxicity in pregnant females may have significant ill health effects on the embryonic development of their offspring, from the toxicological, behavioral and biochemical points of view. Perinatal exposure to AS is toxic to the offspring and influences their postnatal developing morphology and sensory motor reflexes during the weaning period, and also their motor behavioral activities and the levels of GGT and oxidative stress indices such as TBARS and GSH activity in the serum of the adolescent offspring. Animals exposed to AS in presence of zinc show a remarkable ameliorating effect of zinc on the AS toxicity since such offspring did not exhibit significant behavioral or biochemical deficits compared to the group exposed to AS alone. Furthermore, the ineffectiveness of zinc alone to inflict any behavioral or biochemical effect clearly suggests the ameliorating effect of zinc on the behavioral and biochemical toxicity of AS, and it can be a useful supplement for protecting against possible AS toxicity during pregnancy.

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