# Chronic Arsenicosis Induced Oxidative Stress in Cattle: Role of Zn and Se

**Jeevan Ranjan Dash, Bakul Kumar Datta, Samar Sarkar, and Tapan Kumar Mandal**

## Introduction

High levels of arsenic in the ground water are a matter of concern, especially in the Indo-Bangladesh region where over a million people are reported to be suffering from arsenic poisoning. This kind of slow, low level, inevitable poisoning has caused serious concerns about the health of all living species in these areas (Majumder et al., 2012).

Arsenite ($As^{III}$) and arsenate ($As^{v}$) are the two major forms of the inorganic arsenic. While arsenite has a tendency to react readily with the sulphydryl groups of proteins and thus inhibit biochemical pathways, arsenate acts as a phosphate analogue and interferes with phosphorylation reactions (Valko et al., 2005). Most of the arsenate absorbed is reduced to arsenite in blood. The toxic effects manifested by both the molecules are very similar. However, the trivalent species (arsenite) is the biologically active form and the major source to arsenic toxicity represents a potential threat to the environment, human health and animal health due to their carcinogenic and other effects (Singh et al., 2004). Arsenic can result in acute and chronic toxicity. Zinc is one of the essential trace metals which have been studied for its protective value against arsenic. Selenium is known to promote the biliary excretion of exogenous selenium and selenite also augments the excretion of arsenic into bile (Flora et al., 1999; Gregus et al., 1998). Therefore in this experiment it has been tried to use zinc oxide-sodium selenite mixture as a

J.R. Dash • B.K. Datta • T.K. Mandal (✉)
Dept. of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, 37 K.B Sarani, Kolkata 37, India
e-mail: drtkm48@gmail.com

S. Sarkar
Dept. of Veterinary Medicine, Ethics and Jurisprudence, West Bengal University of Animal and Fishery Sciences, Kolkata 37, India



N.J. Raju et al. (eds.), *Management of Water, Energy and Bio-resources in the Era of Climate Change: Emerging Issues and Challenges*,
DOI 10.1007/978-3-319-05969-3_16

mitigation compound to reverse arsenicosis in cattle of some arsenic affected areas of West Bengal so as to curb the arsenic load in food chain which may have ultimate benefit to human being.

## Material and Methods

### *Design of Experiment*

Cattle from the six experimental villages namely Mitrapur, Nonaghata, Mandal Hat, Goetra, Ghetugachi and Dakhin Panchpota from Haringhata and Chakdah blocks of Nadia district, reported with high arsenic contamination, were covered under this study. The arsenicosis affected animals were selected as per the criteria depicted by Dash et al. (2013). Total 48 stressed cows affected with arsenicosis were selected for this experiment of which 22 animals were kept as untreated control and 26 animals were treated with Zn-Se mixture. The farmers were supplied with zinc oxide and sodium selenite mixture and advised to feed the selected animals at the dose rate of 10 mg/kg (Zinc oxide) and 0.1 mg/kg (sodium Selenite) orally once daily for six months and the samples were collected in monthly intervals for analysis.

### *Chemicals*

All chemicals of analytical grade were purchased from Rankem Pvt. Ltd., E-Merck (India), and Sigma Aldrich (USA). The kits used was from Cogent, India.

### *Enzymatic Assays*

5% RBC haemocylate was prepared in chilled phosphate buffer (pH 7.0). Superoxide dismutase (SOD), catalase (CAT) and malonaldehyde (MDA) were estimated by standard procedure of Misera and Fridivich (1972); Aebi (1974) and Nair and Turner (1984), respectively.

### *Hormone Analysis*

Plasma FSH (Follicle stimulating hormone), LH (Leutinising Hormone) and PrL (Prolactin) levels were analyzed using the AccuBind ELISA Microwells kit (Monobind Inc. USA).

### *Total Arsenic Analysis*

The milk, faeces and hair samples were digested with triacid mixture containing nitric acid, perchloric acid and sulphuric acid in the ratio of 10:4:1 according to the modified method of Datta et al. (2010). Total arsenic estimation was performed by atomic absorption spectrophotometer (AAS) with vapour generation accessories (VGA 77) (Varian AA 240, Russia).

### *Statistical Analysis*

The data were analyzed by paired 2-tailed 't'-test using SPSS 17.0 software. $P < 0.05$ were considered significant.

## Results

### *Stress Enzymes Status*

There was a significant increase ($p < 0.05$) in the SOD (Fig. 1) and CAT (Fig. 2) level from 30$^{th}$ day and MDA (Fig. 3) level decreased significantly ($p < 0.05$) from 90$^{th}$ day onwards in the group treated with Zn-Se mixture.

### *Hormonal Status*

FSH (Fig. 4a) and LH (Fig. 4b) levels were increased from 60$^{th}$ day onwards and Prl (Fig. 4c) was found to increase significantly ($p < 0.05$) from 30$^{th}$ day of initiation of the treatment.

## Discussion

Oxidative stress is an imbalance between free radical generation and the antioxidant defense system. Many reports evidenced a decrease in the levels of antioxidants after arsenic exposure (Son et al., 2001). Decreased antioxidant levels due to arsenic toxicity is reported both in human (Wu et al., 2006) and animals (Das et al., 2012) and this effect is due to generation of reactive oxygen species (ROS) (Shi et al., 2004) leading to cell damage and death.

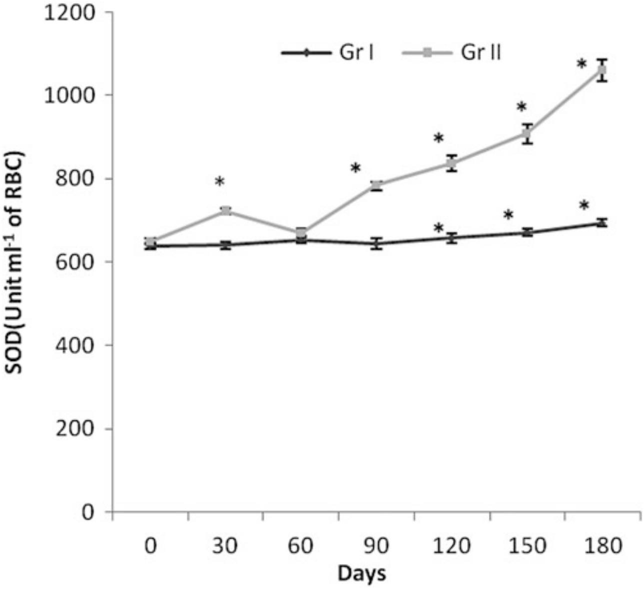


**Fig. 1** SOD (unit $ml^{-1}$ of RBC) status of cattle from experimental villages before and after oral administration of zinc and selenium combination for six months. Gr I: Untreated control group ($n = 22$), Gr II: Treated with zinc oxide and sodium selenite @ 10 and 0.1 mg/kg for 180 days ($n = 26$). * $p < 0.05$, compared to respective 0 day

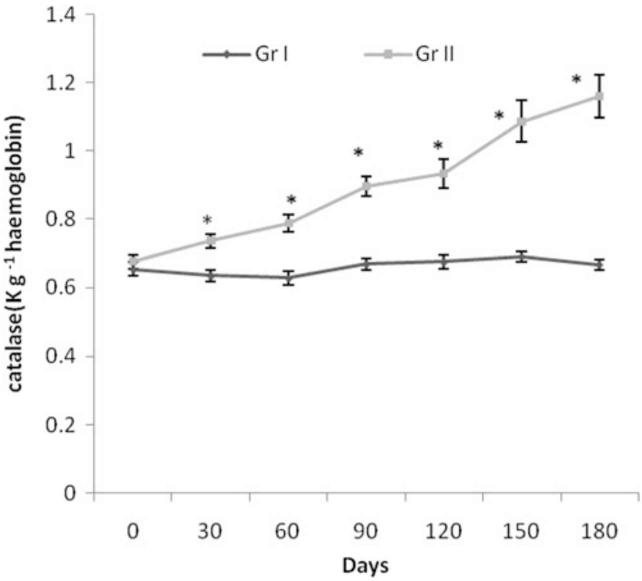


**Fig. 2** Catalase (K $g^{-1}$ haemoglobin) status of cattle from experimental villages before and after oral administration of zinc and selenium combination for six months. Gr I: Untreated control group ($n = 22$), Gr II: Treated with zinc oxide and sodium selenite @ 10 and 0.1 mg/kg for 180 days ($n = 26$). * $p < 0.05$, compared to respective 0 day

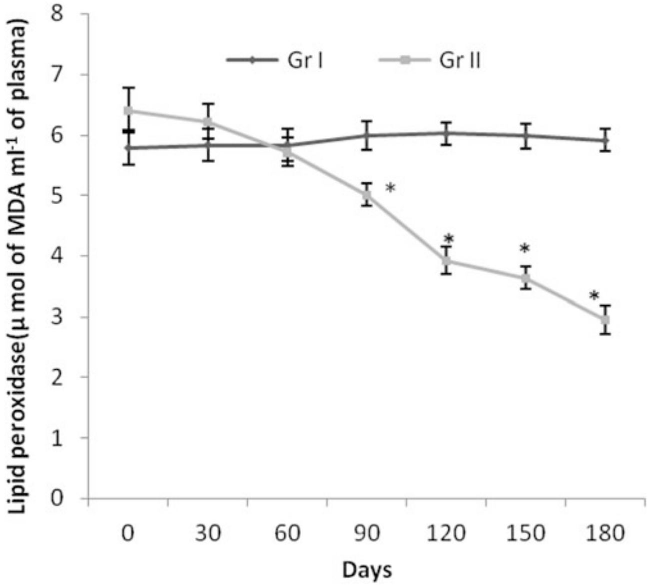


**Fig. 3** Lipid peroxidase (μmol of MDA $ml^{-1}$ of plasma) status of cattle from experimental villages before and after oral administration of zinc and selenium combination for six months. Gr I: Untreated control group ($n = 22$), Gr II: Treated with zinc oxide and sodium selenite @ 10 and 0.1 mg/kg for 180 days ($n = 26$). * $p < 0.05$, compared to respective 0 day

Lipid peroxidation is one of the important consequence of oxidative stress (Kumaraguruparan et al., 2002). Lipids, the most susceptible for peroxidative damage due to low energy necessary for the initiation of the process as well as the presence of unsaturated bonds (Balasinska, 2004). In the present study MDA production was decreased by 53.82% by the end of $180^{th}$ day of treatment period compared to the $0^{th}$ day in the group treated with the Zn-Se mixture, may be due to enhancement of cells natural protective system and increase in GSH production (Liu et al., 2003).

SOD (Superoxide dismutase) catalyzes the dismutation of superoxide anions and prevents the subsequent formation of hydroxyl radicals (Imlay and Linn, 1988). CAT (Catalase) catalyzes the removal of $H_2O_2$ formed during the reaction catalyzed by SOD (Kono and Fridovich, 1982). In this study, plasma SOD and CAT level was gradually increased from $30^{th}$ day in the treated group than the control group and recorded a hike of 63.58% and 71.59% respectively at the end of $180^{th}$ day of treatment which suggested that decreased accumulation of superoxide anion radical might be responsible for decreased lipid peroxidation following treatment with Zn-Se. This effect may be due to pronounced elimination of arsenic by Zn supplementation. This finding is in support of our previous findings that total arsenic concentration in milk decrease non-significantly though significant effect was observed from $150^{th}$ day, faecal arsenic increase significantly ($p < 0.05$) from

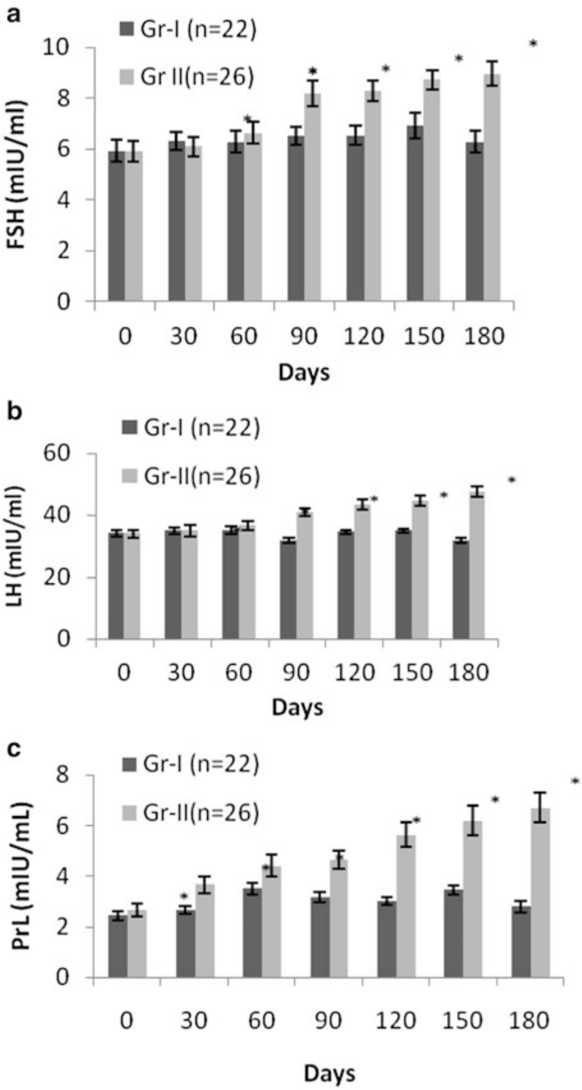


**Fig. 4** (**a**) FSH, (**b**) LH and (**c**) PrL levels in plasma of cattle from experimental villages before and after oral administration of zinc and selenium combination for six months. $* = p < 0.05$, $n =$ number of animals

$90^{th}$ day onwards and accumulation of total arsenic in hair decrease gradually but not significantly in cattle treated with Zn and Se mixture (Dash et al., 2013). It is also in corroboration with the findings of Modi et al. (2006) who have reported that iron or zinc either alone or in combination with monoisoamyl di mercapto succinic acid (DMSA) produce more pronounced elimination of arsenic in male mice. Selenium may be enhancing the protective effect against oxidative damage by augmenting the excretion of arsenic into bile (Flora et al., 1999) and it was in support of our previous finding that concentration of faecal arsenic increase gradually from $90^{th}$ day onwards in cattle treated with Zn-Se.

Dairy cows undergo massive metabolic adaptations during the onset of lactation, and it was postulated that some of these physiological events may negatively impact the health of the dairy cows (Sordillo et al., 2009). During this experimental period we observed a significant increase in the levels of FSH and LH from 60 days onwards and PrL level from 30 days onwards in the treated group (Figs. 4 a, b, c) which can be due to decreased oxidative stress by rapid elimination of arsenic from the body of the animals receiving Zn-Se treatment.

## Conclusion

From the above study it can be concluded that zinc oxide-sodium selenite mixture can potently reduce the oxidative stress induced by arsenicosis in cattle by enhancing the dismutation of superoxide radicals and reducing the lipid peroxidation by enhancing the excretion of arsenic from the body and thereby may enhance the physiological performance of the animal and can curb the entry of arsenic into the human food chain.

**Acknowledgement** We acknowledge West Bengal University of Animal and Fishery Sciences and National Agriculture Innovation Project (NAIP), ICAR, New Delhi in collaboration with World Bank for financial support.

## References

Aebi, H. (1974). Catalase: Methods of enzymatic analysis. Ed. Bergmeyer HV **2:** 674-677.

Balasinska, B. (2004). Evaluation of antioxidant status in living organisms. *Med Weter*, **60:** 579-583.

Das, T.K., Mani, V., Kaur, H., Kewalramani, N., De, S., Hossain, A., Banerjee, D. and Datta, B.K. (2012). Effect of vitamin E supplementation on arsenic induced oxidative stress in goats. *Bulletin of Environmental Contamination and Toxicology*, **89(1):** 61-66.

Dash, J.R., Datta, B.K., Sarkar, S. and Mandal, T.K. (2013). Chronic arsenicosis in cattle:Possible mitigation with zinc and selenium. *Ecotoxicology and Environmental Safety,* **92**:119-122.

Datta, B.K., Mishra, A., Singh, A., Sar, T.K., Sarkar, S., Bhatacharya, A., Chakraborty, A.K. and Mandal, T.K. (2010). Chronic arsenicosis in cattle with special reference to its metabolism in arsenic endemic village of Nadia district, West Bengal, India. *Science of the Total Environment*, **409:** 284-288.

Flora, S.J.S., Kannan, G.M. and Kumar, P. (1999). Selenium effects on gallium arsenide induced biochemical and immunotoxicological changes in rats. *Chemico-Biological Interactions*, **122:** 1-13.

Gregus, A., Gyurasics, A. and Koszorus, L. (1998). Interactions between selenium and group Va-metalloids (arsenic, antimony and bismuth) in the biliary excretion. *Environmental Toxicology and Pharmacology*, **5:** 89-99.

Imlay, J.A. and Linn, S. (1988). DNA damage and oxygen radical toxicity. *Science*, **240:** 1302-1309.

Kono, Y. and Fridovich, I. (1982). Superoxide radicals inhibit catalase. *Journal of Biological Chemistry*, **257:** 5751-5754.

Kumaraguruparan, R., Subapriya, R., Kabalimoorthy, J. and Nagini, S. (2002). Antioxidant profile in the circulation of patients with fibroadenoma and adenocarcinoma of the breast. *Clinical Biochemistry*, **35:** 275-279.

Liu, L., Trimarchi, J.R., Navarro, P., Blasco, M.A. and Keefe, D.L. (2003). Oxidative stress contributes to arsenic induced telomere attrition, chromosomal instability and apoptosis. *Journal of Biological Chemistry*, **278:** 31998-32004.

Majumder, A., Biswas, T. and Kole, S.C. (2012). Geobacillus Stearothermophilus – An Arsenic Oxidizing Bacterium Isolated from Arsenic Polluted Field of West Bengal, India. National Conference on Biotechnology and the Environment, NIT, Durgapur.

Misera, H.P. and Fridivich, I. (1972). The role of superoxide dismutase ion in the auto-oxidation of epinephrine and a single assay for super-oxide dismutase. *Journal of Chemotherapy*, **247:** 3170-3185.

Modi, M., Kaul, R.K., Kannan, G.M. and Flora, S.J.S. (2006). Preventive Effects of Zinc and N-acetylcysteine Administration against Arsenic Intoxication in Rats. *Journal of Trace Elements in Medicine and Biology*, **20:** 179-204.

Nair, V. and Turner, G.A. (1984). The thiobarbituric acid test for lipid-peroxidation, structure of the adduct with malonaldehyde. *Lipids*, **19:** 804.

Shi, H., Shi, X. and Liu, K.J. (2004). Oxidative mechanism of arsenic toxicity and carcinogenesis. *Molecular and Cellular Biochemistry*, **255:** 67-78.

Singh, T.S. and Pant, K.K. (2004). Equilibrium, kinetics and thermodynamic studies for adsorption of $As^{(III)}$ on activated alumina. *Separation and Purification Technology*, **36:** 139-147.

Son, M.H., Kang, K.W., Lee, C.H. and Kim, S.G. (2001). Potentiation of arsenic-induced cytotoxicity by sulfur amino acid deprivation (SAAD) through activation of ERK1/2, p38 kinase and JNK1: The distinct role of JNK1 in SAAD-potentiated mercury toxicity. *Toxicology Letters*, **121:** 45-55.

Sordillo, L.M. and Aitken, S.L. (2009). Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary Immunology and Immunopathology*, **128:** 104-109.

Valko, M., Morris, H. and Cronin, M.T. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, **12:** 1161-1208.

Wu, M.M., Chiou, H.Y., Hsueh, Y.M., Hong, C.T., Su, C.L. and Chang, S.F. (2006). Effect of plasma homocysteine level and urinary monomethylarsonic acid on the risk of arsenic-associated carotid atherosclerosis. *Toxicology and Applied Pharmacology*, **216:** 168-175.