EXTENDED ABSTRACT

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Ameliorating role of antioxidant supplementation on sodium-arsenite induced adverse effects on developing rat cerebellum

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Abstract:

Arsenic (*As*) is being identified as an environmental contaminant of global concern. Consumption of ground water contaminated with inorganic arsenic (*iAs*) continues to be the major source of exposure to *iAs*. There are reports of functional deficits in later life following exposure to *iAs* during developmental period. The developing nervous system is especially vulnerable to environmental insults due to its higher rate of O_2 consumption and provision of weaker antioxidant (AOX) machinery. Oxidative stress has been reported as one of the major factors underlying *iAs* induced toxicity. Hence, it is of great importance to identify specific AOXs which could ameliorate the adverse effects induced by *iAs* exposure, especially in endemic areas.

The aim of the study was to determine the role of two AOXs i.e., alpha lipoic acid (ALA) and Curcumin (Cur) on *iAs* induced effects in developing rat cerebellum. The observations indicated deficits in locomotor function (lesser time spent on rota rod) and downregulation of the expression of proteins closely associated with synaptic functioning (Syp and PSD95) in the cerebellum of *iAs*-treated animals.

However, substantial recovery in these parameters was observed in antioxidant-supplemented groups, thereby suggestive of potential of ALA and Cur in amelioration of *iAs* induced developmental neurotoxicity.

List of abbreviations: AOX antioxidants, WB western blot, GSH reduced glutathione, MDA malondialdehyde, Syp synaptophysin, PSD95 Post synaptic density 95.

Introduction

Arsenic (*As*) is a well-known environmental contaminant with ubiquitous distribution in air, soil and water. Millions of people are exposed to *iAs* (inorganic arsenic) through consumption of contaminated drinking water. Consumption of rice irrigated with water contaminated with *iAs* forms a major component of infant diet and therefore is an important source of exposure to *iAs* in children (Davis et al., 2012). An association has been drawn between exposure to *iAs* during developmental period and the neurodegenerative changes with explicit functional deficits in later years of life (Vahter 2008). The role of *iAs* in inducing oxidative stress at cellular level has been largely discussed (Flora et al., 2005). Alpha Lipoic acid (ALA) and Curcumin (Cur) have been identified as multifunctional bioactive agents with remarkable antioxidant (AOX) activity in the nervous system. Their AOX activities are attributed to

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modulation of cellular redox system (Packer et al., 1995; Aggarwal et al., 2013). Keeping in mind the magnitude of *iAs* induced toxicity and the potential of ALA and Cur as antioxidants, the present work focused on evaluating the role of their supplementation on cerebellar oxidative stress in rats exposed to sodium arsenite ($NaAsO_2$) perinatally. We also determined the status of synapse associated proteins (Syp & PSD 95) (Sanyal et al., 2013) in rat cerebellum following exposure to *iAs* alone or in combination with AOXs.

Materials and Methods:

Five to six month old pregnant Wistar rats (18-19 days gestation) were housed under controlled laboratory conditions. The day of delivery of pups was considered as postnatal day zero (PND 0). The pups n=6/ subgroup/ technique) from various litters were randomly assigned to various groups based on the intraperitoneal (i.p.) administration of the test substance(s) such as control (I) (no treatment at all) and experimental (IIa, b: 1.5, 2.5 mg/kg bw $NaAsO_2$; IIIa, b: 1.5, 2.5 mg/kg bw $NaAsO_2$ along with 70 mg/kg bw ALA; IVa, b: 1.5, 2.5 mg/kg bw $NaAsO_2$ along with with 100 mg/kg bw Cur) (from PND 1 to PND 21).

Rota-rod test to assess the muscle coordination and postural balance was carried out (Shiotsuki et al., 2010) with three trials per day on three consecutive days (PND 20, 21, 22) designated as day 1, 2 and 3 respectively. The average time to fall off from the rod was determined from the recorded data and expressed as mean ± SD (Catre et al., 2012).
The animals were sacrificed on PND 22. The fresh cerebellar tissue was processed for WB analysis (Kaushal et al., 2014) and estimation of GSH & MDA levels (Ellman 1959, Ohkawa et al., 1979).

Results and discussion:

The lesser time spent on the rotating rod by the animals exposed to *iAs* alone was suggestive of impaired motor coordination. Significant improvement in these parameters was noted in the animals co-treated with ALA or Cur along with *iAs* (Fig. 1). These observations are suggestive of recovery in altered motor activity and behavioral deficits following supplementation of AOXs with *iAs*.

A significant dose dependent decrease was observed in the cerebellar GSH levels in group IIa (40.30%) and IIb (46.93%). Co-administration of ALA with *iAs* resulted in increase in GSH levels by 17.09% and 20.51% (IIIa and IIIb) respectively, while co-treatment with Cur (IVa and IVb) resulted in 24.4% and 28.8% increase in GSH levels as compared to *iAs* alone treated groups (IIa and IIb). MDA levels showed a significant dose dependent increase (36.79% and 47.19%) in groups IIa & IIb as compared to the controls. However, simultaneous administration of ALA with *iAs* (IIIa & IIIb) resulted in 16.05% and 20.42%

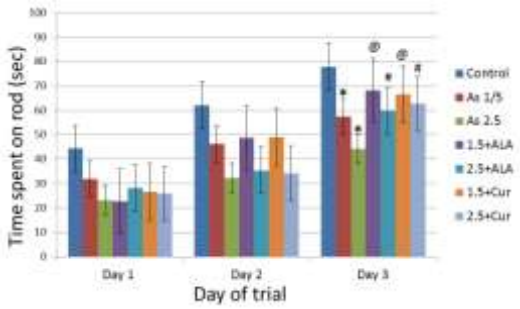

Figure 1. Graphic representation of the time spent (sec) on the rotating rod of Rota-rod by the control and the experimental animals.

Significant at $p<0.05$ values * compared to I, @ and # compared to IIa & IIb respectively
decrease in MDA levels whereas co- administration of Cur with *iAs* reduced cerebellar MDA levels by 15.55% (IVa) & 17.91% (IVb) as compared to groups IIa & IIb. WB analysis of synaptogenic proteins (Syp & PSD95) showed downregulation in *iAs* alone treated groups whereas co-administration of ALA or Cur along with *iAs* resulted in significant upregulation of protein expression (Fig.3).

The metabolically active nervous tissue is critically dependent on aerobic metabolism and, its richness in PUFA and iron, makes it highly vulnerable to ROS mediated oxidative damage. Hence, the neuronal dysfunction, induced by oxidative damage, could be associated with affliction of synaptic plasticity and neurogenesis.

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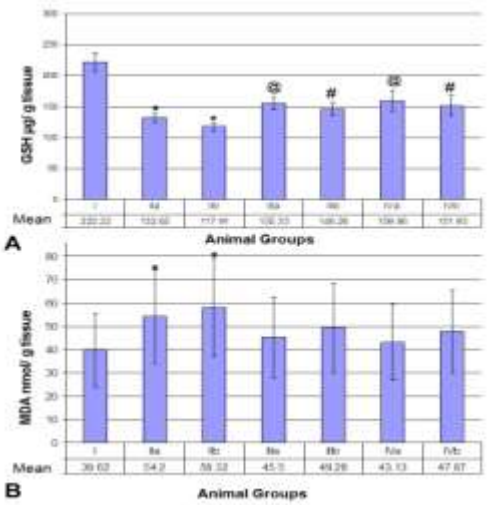

Figure 2. Bar diagrams showing levels of GSH (A) and MDA (B) in cerebellum of control (I) and experimental (IIa, b; IIIa, b; IVa, b) groups (n=6/group).
Values are Mean±SD. Significant at p<0.05 levels * compared to I, @ & # compared to IIa & IIb respectively

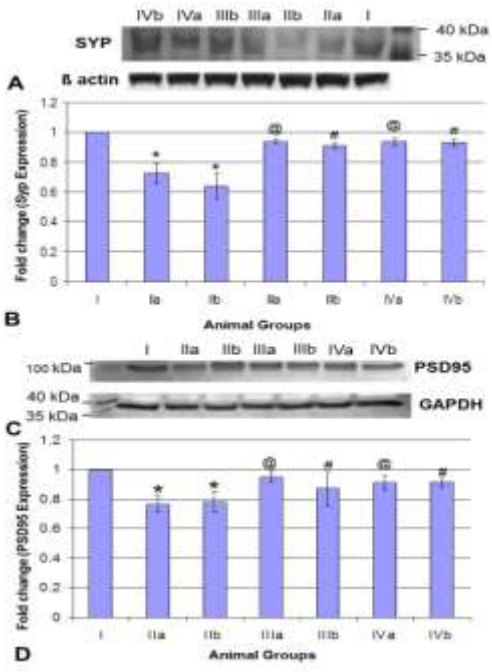

Figure 3. Immunoblots of Syp and PSD95 (A&C) and bar diagram showing fold change in Syp and PSD95 expression (B&D) in the cerebellum of control & experimental animals. Values are Mean±SD. Significant at p<0.05 levels * compared to I, @ & # compared to IIa & IIb respectively.

Conclusions:

Though, it is difficult to track the precise mechanism underlying the ameliorating role of ALA and Cur against *iAs* induced developmental neurotoxicity yet, the observations of the present study, suggest their multipronged approach in providing neuroprotective efficacy. Therefore it is possible that supplementation of antioxidants (ALA or Cur) with *iAs* ameliorates *iAs* induced toxicity by their ability to modulate redox potential and to up-regulate the proteins (Syp & PSD95) which play critical role in neuronal maturation during developmental period.

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