

## Arsenic induced free radical toxicity in brain of mice

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The present study was designed to investigate the *in vivo* effects of oral administration of arsenic trioxide ( $As_2O_3$ ; 0.5 and 1 mg/kg body weight/day for 45 days) on cerebral hemispheres and cerebellum in male mice, *Mus musculus*. Arsenic reduced the concentration of glutathione (GSH) in cerebral hemisphere and cerebellum at both the dose levels; while increased lipid peroxidation (LPO) in cerebral hemisphere and cerebellum regions. Further, the activities of antioxidant enzymes viz., superoxide dismutase and catalase also declined in these two regions with dose indicating oxidative stress. This effect is caused by the action of reactive oxygen species (ROS) induced by arsenic exposure.

**Keywords:** Arsenic, Free Radical Toxicity, Oxidative Stress, Brain

The main sources of human exposure to arsenic are through inhalation of arsenic dust particles and ingestion of drinking water<sup>1</sup>. Arsenic and its inorganic compounds have long been known to be neurotoxic. Common symptoms of acute inorganic arsenic poisoning are nausea, anorexia, vomiting, epigastric and abdominal pain and diarrhoea. Dermatitis, muscle cramps, cardiac abnormalities and hepatotoxicity, bone marrow suppression, haematologic abnormalities and vascular lesions have also been reported<sup>2-7</sup>. Acute arsenic exposure is also known to cause neurologic symptoms such as hyperpyrexia, convulsions, tremor, coma etc<sup>8</sup>. Jha *et al*<sup>9</sup> have reported hyperkeratosis together with peripheral neuropathy, weakness and sensory motor flaccid quadriplegia in acute and chronic arsenic toxicity. Central nervous system deficits (hearing loss, eye damage, abnormal EEGs, mental retardation, epilepsy) occurred in infants who had been fed arsenic contaminated milk for 1-2 months<sup>10</sup>. Chronic exposure to arsenic dust in smelter workers at a concentration  $\leq 0.5$  mg/m<sup>3</sup> caused a decrease in peripheral nerve conduction velocities, encephalopathy, polyneuropathy, tremor and axonal degeneration<sup>8,11-14</sup>. Mohamed *et al*<sup>15</sup> in a study on arsenic exposed patients in Bangladesh reported neurologic disorders that ranged from sleep disorder and memory impairment to paralysis. Recent study<sup>16</sup> on cell cultures showed that trivalent arsenic

enhanced oxidative stress in a variety of mammalian cells and this association may be closely associated with the development of arsenic-related neural diseases. Arsenic induced neurotoxicity is manifested as a peripheral neuropathy involving both sensory and motor nerves resulting in numbness and parasthesia, diminished sensation of touch, pain, heat and cold and muscle weakness<sup>17</sup>.

Reactive oxygen species pathway could be a mechanism of oxidative stress<sup>18</sup> and action of arsenic carcinogenesis in man<sup>19</sup>. Arsenic is also known to exert its toxicity through the generation of reactive oxygen species (ROS), which include  $H_2O_2$  and other chemical forms known as free radicals such as superoxide ( $\bullet O_2^-$ ), hydroxyl ( $OH\bullet$ ) and peroxy ( $ROO\bullet$ ) radicals<sup>20</sup>.

Cerebral hemisphere of brain is related to most important functions viz., thought, voluntary movement, language, reasoning and perception, while cerebellum is concerned with movement, balance and posture. Moreover, since not much information is there on brain, long-term (chronic) effect of trivalent arsenic have been investigated on cerebral hemispheres and cerebellum of the adult mouse brain.

### Materials and Methods

Healthy, adult male albino mice (*Mus musculus*) of Swiss strain, weighing between 35-40 g, were obtained from Alembic Pharmaceuticals, Vadodara, following institutional ethical guidelines. The animals were housed in an air-conditioned animal house at  $26^\circ \pm 2^\circ C$  and exposed to 10-12 hr of daylight and were

maintained on a standard chow and water was given *ad libitum*. The animals were divided into 3 groups. As<sub>2</sub>O<sub>3</sub> (HiMedia Laboratories Limited, Mumbai, India) was suitably diluted with water. Group I animals served as control animals. Group II and Group III animals were orally administered with As<sub>2</sub>O<sub>3</sub> for 45 days (chronic exposure) at the doses of 0.5 and 1 mg/kg body wt respectively based on earlier standardization. The selected doses in the present study are 1/80<sup>th</sup> of LD<sub>50</sub> for low dose and 1/40<sup>th</sup> of LD<sub>50</sub> for high dose.

At the end of each treatment, animals were weighed and sacrificed by cervical dislocation. The cerebral hemispheres and cerebellum regions were dissected out carefully, blotted free of blood and weighed to the nearest milligram and utilized for study.

Lipid peroxidation (LPO) was determined by the method of Ohkawa *et al*<sup>21</sup>. The activities of catalase (E.C.1.11.1.6) and superoxide dismutase (SOD) (E.C.1.15.1.1) were analysed by the modified method of Luck<sup>22</sup>, and spectrophotometric method of Kakkar *et al*.<sup>23</sup> respectively and glutathione (GSH) was estimated by the method of Grünert and Philips<sup>24</sup>. For all biochemical estimations, a minimum of 8-10 replicates were done for each parameter and tissue. Data were statistically analysed using Student's t-test. Percent values were also calculated. The results are presented in Table 1.

## Results and Discussion

A significant elevation was noted in lipid peroxidation after arsenic exposure at both the dosages in mice. Peroxidation of membrane lipids by free radicals results in loss of membrane integrity and function. Based on the observation that accumulation of LPO products in brain of patients with Alzheimer's disease, Romero *et al*<sup>25</sup>, tried to elucidate the role of oxidative stress and cellular antioxidants in  $\beta$ -amyloid induced apoptotic cell death of rat embryo neurons. The data of the present study gets support from a recent study conducted on humans exposed to low and high levels of arsenic indicating that mean serum levels of lipid peroxidation levels were significantly higher among high-exposed compared with low exposed group<sup>20</sup>. In contrast a significant depletion in glutathione (GSH) was observed following arsenic exposure. Arsenic ions are known to react with sulfhydryl groups<sup>26</sup> in cells<sup>27,28</sup> and proteins and enzymes<sup>14,28</sup>. Both methylation and reduction of arsenate to arsenite are saturable processes that depend on the availability of GSH. Arsenite has a high affinity for thiol, especially dithiol groups of proteins, a factor probably related to toxic effects of arsenic<sup>29</sup>. Thus, GSH offers first level of protection and its reduced level is correlated to an enhanced expression of toxicity<sup>30</sup>.

Superoxide dismutase (SOD), the antioxidant catalysing the dismutation of superoxide ( $\bullet\text{O}_2^-$ ) radical

Table 1—Reactive oxygen species parameters in cerebral hemisphere (CH) and cerebellum (C) of arsenic exposed mice [Values are mean  $\pm$  SE of 10 animals in each group. Figures in parenthesis are % increase (+) or decrease (-) over control]

Parameter		Control	0.5 mg/kg	1 mg/kg
Glutathione (GSH) <sup>a</sup>	CH	60.54 $\pm$ 1.67	39.65 $\pm$ 0.29 (+65)	35.81 $\pm$ 0.41 (+53)
	C	72.29 $\pm$ 4.63	38.34 $\pm$ 0.55 (+59)	32.83 $\pm$ 0.94 (+45)
Lipid peroxidation (LPO) <sup>b</sup>	CH	0.25 $\pm$ 0.01	0.42 $\pm$ 0.02 (+168)	0.74 $\pm$ 0.02 (+296)
	C	0.28 $\pm$ 0.01	0.47 $\pm$ 0.02 (+168)	0.62 $\pm$ 0.04 (+221)
Superoxide dismutase (SOD) <sup>c</sup>	CH	12.90 $\pm$ 0.16	8.55 $\pm$ 0.37 (-66)	4.70 $\pm$ 0.11 (-36)
	C	11.45 $\pm$ 0.64	7.94 $\pm$ 0.47 (-69)	4.83 $\pm$ 0.26 (-42)
Catalase <sup>d</sup>	CH	54.04 $\pm$ 2.32	36.28 $\pm$ 2.28 (-67)	29.32 $\pm$ 1.37 (-54)
	C	52.22 $\pm$ 2.98	37.65 $\pm$ 2.38 (-72)	22.68 $\pm$ 3.24 (-43)

<sup>a</sup> =  $\mu\text{g}/100$  mg fresh tissue weight; <sup>b</sup> =  $\times 10^4$  nanomoles of MDA/mg tissue weight/60 min

<sup>c</sup> = unit SOD/mg protein, <sup>d</sup> =  $\mu\text{moles H}_2\text{O}_2$  consumed/mg protein/minute

All experimental values are significant at  $P < 0.001$ .

into hydrogen peroxide, showed a decline in its activity after the exposure. This hydrogen peroxide is further decomposed by catalase enzyme whose activity also registered a significant fall (Table 1) following arsenic exposure in both regions of brain. In relation to the present study, there are results which support the participation of hydroxyl radicals in arsenic-induced disturbances in the central nervous system<sup>18</sup>. Because of high ATP demand, brain consumes O<sub>2</sub> rapidly and is thus susceptible to interference with mitochondrial function, which in turn leads to increased •O<sub>2</sub><sup>-</sup> (superoxide radical) formation<sup>31</sup>, leading to tissue oxidative damage. Recent studies have also indicated that arsenic could generate reactive oxygen species viz., dimethyl arsenic peroxy radical, superoxide anion, hydroxyl radicals causing cellular toxicity and/or carcinogenicity<sup>19</sup>. Mohamed *et al*<sup>15</sup>, also demonstrated neurotoxicity in patients exposed to arsenic in support of our data. Results of study conducted on transgenic mice also demonstrated role of superoxide dismutase, catalase and cellular glutathione peroxidase (GSHPx-1) protection against tissue injury<sup>32</sup>.

The present findings thus revealed that arsenic at both dosage levels brought about a reduction in antioxidant enzymes followed by an increase in lipid peroxidation levels causing neurotoxicity by generation of free radicals in brain tissue. Further studies are underway to combat neurotoxic effects exerted by arsenic exposure.

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