

## BIOCHEMICAL EFFECTS OF SODIUM FLUORIDE AND ARSENIC TRIOXIDE TOXICITY AND THEIR REVERSAL IN THE BRAIN OF MICE

NJ Chinoy,<sup>a</sup> SD Shah  
Ahmedabad, India

**SUMMARY:** Sodium fluoride (NaF) and arsenic trioxide (As<sub>2</sub>O<sub>3</sub>), singly or combined, at doses of 5 and 0.5 mg/kg body weight, respectively, were administered orally to mice for 30 days to investigate their biochemical effects on the brain (cerebral hemisphere). The effects of withdrawal of the treatment and ingestion of vitamin C, vitamin E, and calcium (as phosphate) were also investigated. During treatment, levels of dehydroascorbic acid and lipid peroxides increased, but the activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase, as well as the levels of glutathione, total ascorbic acid, and reduced ascorbic acid decreased. The data suggest that metabolic changes associated with the treatments could be the result of free radical toxicity rendering the brain more susceptible to injury. Withdrawal of the NaF + As<sub>2</sub>O<sub>3</sub> treatment resulted in incomplete recovery after 30 days. However, administration of the antidotes alone or in combination during the withdrawal period provided almost complete recovery, possibly due to their antioxidant properties and/or synergistic action.

Keywords: Anti-oxidative enzymes; Arsenic trioxide; Brain biochemistry; Calcium; Free radicals; Sodium fluoride; Toxicity reversal; Vitamin C; Vitamin E.

### INTRODUCTION

Chronic intoxication from arsenic (arsenicosis) and fluoride (fluorosis) has been reported from China and India.<sup>1-5</sup> Fluoride injected subcutaneously in different doses (5, 10, 20, and 50 mg/kg body wt/day) for 100 days into male and female rabbits exerted an inhibitory effect in the brain on free fatty acids and caused a significant decline in RNA and proteins (soluble, basic, and total), as well as free amino acids. The protein depletion produced degenerative changes in Purkinje cells of the cerebellar cortex, loss of Nissl substance, and paralysis in treated animals.<sup>6-8</sup> Marked reduction in myelinated nerve fibers, external granular layer in cerebellum, and increased neuronal apoptosis have also been reported in humans, rats, and mice.<sup>9-12</sup> An effect on intelligence, altered behavior, poor motor coordination, loss of neuronal and cerebrovascular integrity, and changes in brain membrane lipids in humans, especially in children and animals drinking fluoridated water, have also been reported.<sup>13-20</sup> The learning abilities and memory of high-fluoride exposure groups were significantly lower than in control mice,<sup>21-23</sup> and the fluoride

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<sup>a</sup>For Correspondence: Reproductive Endocrinology and Toxicology Unit, Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380009, India.  
E-mail: zooldeptgu@satyam.net.in

induced neurotoxicity was greater in early stages of intoxication<sup>24</sup> as well as during early developmental stages in rats.<sup>25-26</sup>

Oxidative damage in the brain of rat offspring by a combined treatment with fluoride and arsenic has also been reported.<sup>27</sup> Overall, however, relatively little is known about their combined effects on the structure and metabolism of various soft tissues in animals and humans. The present study was therefore undertaken to investigate the effects of sodium fluoride and arsenic trioxide administered alone or in combination on the brain (cerebral hemisphere) of mice and, moreover, in the light of earlier work, to examine possible reversal of the induced effects by administration of vitamins C and E and calcium (as calcium phosphate).

### MATERIALS AND METHODS

Details of animal groups (each group containing 15–20 mice), experimental protocol, treatments, and doses are described in a previous paper.<sup>28</sup>

Biochemical studies of protein,<sup>29</sup> lipid peroxide,<sup>30</sup> superoxide dismutase (E.C.1.1.15.11),<sup>31</sup> catalase (E.C. 1.11.1.6),<sup>32</sup> glutathione,<sup>33</sup> glutathione peroxidase (E.C.1.11.1.9)<sup>34</sup> and ascorbic acid<sup>35</sup> were carried out on the cerebral hemisphere of control and all treated mice by the methods cited.

*Statistical analysis:* For each biochemical parameter a minimum of 5–6 replicates were assayed, and the data were statistically analysed by Student's t test and ANOVA.

### RESULTS

*Protein and lipid peroxide (LPO):* The treatments of Groups VI–VIII (NaF, As<sub>2</sub>O<sub>3</sub> and NaF + As<sub>2</sub>O<sub>3</sub>) significantly (P<0.001) decreased protein levels but increased (P<0.001) LPO in the brain (cerebral hemisphere) as compared to control Groups I–V. However, the recovery was more significant (P<0.001) in protein than in LPO (P<0.05) upon withdrawal of NaF + As<sub>2</sub>O<sub>3</sub> treatment for 30 days (Group IX) as compared to Group VIII. The recovery was also significant (P<0.001) in Groups X–XIII (Group XI LPO P<0.01), wherein antidotes were administered during the withdrawal period as compared to Group VIII (Table 1).

*Superoxide dismutase (SOD) and catalase:* A significant (P<0.001) decline in the SOD and catalase activities of brain (cerebral hemisphere) occurred after the treatments of Groups V–VIII as compared to control Groups I–V. In Group IX the recovery was not significant in both enzymes as compared to Group VIII. On the other hand, significant recovery (P<0.001; Group XII SOD, P<0.01) was obtained in Groups X–XIII as compared to Group VIII (Table 1).

**Table 1.** Protein (mg/100 mg fresh tissue wt), lipid peroxide (LPO) (nanomoles of MDA/mg tissue wt/60 min), superoxide dismutase (SOD) (units/mg protein), catalase (units/min/mg protein), in brain (cerebral hemisphere) of Groups I-XIII mice<sup>a</sup>

Group	Treatment	Protein	LPO	SOD	Catalase
I	Control, untreated	15.11 ± 0.13	32.48 ± 0.12	0.57 ± 0.01	61.48 ± 0.14
II	Control + olive oil	15.60 ± 0.07	32.82 ± 0.14	0.54 ± 0.01	61.62 ± 0.10
III	Control + ascorbic acid (AA)	15.50 ± 0.02	32.75 ± 0.03	0.58 ± 0.01	61.30 ± 0.17
IV	Control + calcium phosphate (Ca)	15.14 ± 0.05	32.94 ± 0.04	0.57 ± 0.01	61.69 ± 0.03
V	Control + vitamin E (Vit. E)	15.24 ± 0.01	32.99 ± 0.08	0.58 ± 0.01	61.26 ± 0.06
VI	NaF treatment	09.65 ± 0.12 <sup>§</sup>	42.82 ± 0.08 <sup>§</sup>	0.46 ± 0.01 <sup>§</sup>	54.79 ± 0.04 <sup>§</sup>
VII	As <sub>2</sub> O <sub>3</sub> treatment	08.36 ± 0.15 <sup>§</sup>	52.91 ± 0.43 <sup>§</sup>	0.26 ± 0.01 <sup>§</sup>	32.68 ± 0.45 <sup>§</sup>
VIII	NaF + As <sub>2</sub> O <sub>3</sub> treatment	09.15 ± 0.14 <sup>§</sup>	54.81 ± 0.50 <sup>§</sup>	0.38 ± 0.05 <sup>§</sup>	47.79 ± 0.38 <sup>§</sup>
IX	Withdrawal of Group VIII treatment	12.00 ± 0.14 <sup>†</sup>	53.40 ± 0.36 <sup>*</sup>	0.46 ± 0.01	49.28 ± 0.40
X	Withdrawal of Group VIII treatment + AA	14.09 ± 0.26 <sup>§</sup>	31.21 ± 0.31 <sup>§</sup>	0.56 ± 0.01 <sup>§</sup>	58.41 ± 0.79 <sup>§</sup>
XI	Withdrawal of Group VIII treatment + Ca	12.88 ± 0.30 <sup>§</sup>	33.36 ± 1.6 <sup>‡</sup>	0.54 ± 0.01 <sup>§</sup>	57.23 ± 0.35 <sup>§</sup>
XII	Withdrawal of Group VIII treatment + Vit. E	12.34 ± 0.20 <sup>§</sup>	34.36 ± 0.36 <sup>§</sup>	0.53 ± 0.01 <sup>‡</sup>	53.73 ± 0.78 <sup>§</sup>
XIII	Withdrawal of Group VIII treatment + AA, Ca & Vit. E	14.78 ± 0.11 <sup>§</sup>	32.85 ± 0.58 <sup>§</sup>	0.55 ± 0.01 <sup>§</sup>	58.37 ± 1.01 <sup>§</sup>

a = Data are expressed as mean ± SE. \* = P<0.05; † = P<0.02; ‡ = P<0.01;

§ = P<0.001; where nothing is shown = nonsignificant.

Comparison between: Group I and Group VI or VII or VIII individually; Group VIII and Group IX or X or XI or XII or XIII individually.

*Glutathione (GSH) and glutathione peroxidase (GSH-Px):* The levels of glutathione and the activity of GSH-Px in brain (cerebral hemisphere) of NaF, As<sub>2</sub>O<sub>3</sub> or NaF + As<sub>2</sub>O<sub>3</sub> treated mice showed significant (P<0.001) decrease in comparison to all control Groups (I–V). Group IX (withdrawal

of treatment) showed insignificant recovery in both parameters after 30 days as compared to Group VIII. However, treatment of Groups X–XIII, resulted in significant recovery ( $P < 0.001$ ) in GSH and GSH-Px as compared to Group VIII (Table 2).

*Total ascorbic acid (TAA) and reduced ascorbic acid (RAA):* The TAA and RAA levels in brain showed a significant decline ( $P < 0.001$ ) after the treatments of Groups VI–VIII as compared to control (Groups I–V). Withdrawal of treatment (Group IX) resulted in insignificant recovery as compared to Group VIII in TAA and RAA. On administration of ascorbic acid, calcium phosphate, or vitamin E, alone or in combination (Groups X–XIII), resulted in very significant ( $P < 0.001$ ) recovery in both parameters in comparison to Group VIII (Table 2).

*Dehydroascorbic acid (DHA):* DHA levels in the brain increased significantly ( $P < 0.001$ ) in Groups VI–VIII as compared to control Groups I–V. The recovery was insignificant by withdrawal of treatment (Group IX) as compared to Group VIII. On administration of the three antidotes, alone or together (Groups X–XIII), the levels of DHA recovered significantly ( $P < 0.001$ ) as compared to Group VIII (Table 2).

#### DISCUSSION

Fluoride-induced free radical toxicity has been reported recently in the cerebral hemisphere of female and male mice.<sup>36,37</sup> Shao *et al*<sup>38</sup> found oxidative stress in the brain of rats with fluorosis wherein a decrease in polyunsaturated fatty acids occurred along with an increase in saturated fatty acids. Arsenic is also known to induce neuropathy in humans<sup>39</sup> and, when combined with fluoride, causes oxidative damage in the brain of offspring rats.<sup>27</sup> In the present study, levels of glutathione and activities of catalase, GSH-PX, and SOD were significantly decreased, whereas lipid peroxide levels were enhanced in the brain of adult rats by treatment with NaF, As<sub>2</sub>O<sub>3</sub>, or NaF + As<sub>2</sub>O<sub>3</sub>, in agreement with earlier reports.<sup>2-4,36,37,40,41</sup>

The treatments in Groups VI–VIII resulted in a significant decline in total ascorbic acid levels accompanied by an increase in the levels of dehydroascorbic acid (DHA) in the brain, suggesting the occurrence of stress leading to a rapid utilization of stored ascorbic acid or else non-conversion of DHA to reduced ascorbic acid due to a decrease in GSH. Alteration in ascorbic acid metabolism has also been reported in several organs of fluoride and arsenic treated rats and mice.<sup>4</sup>

Upon withdrawal of the combined treatment, some recovery occurred in most of the brain parameters, but in Groups X–XIII of animals treated with antidotes alone or in combination during the withdrawal period, there was almost complete recovery. These findings show that toxicity in the brain

could be overcome by certain antioxidant vitamins. The results also corroborate several earlier reports from our laboratory on reversal of fluoride, aluminium, and arsenic toxicity with vitamins C, D, and E in brain and other

**Table 2.** Glutathione (g/100 mg fresh tissue wt), glutathione peroxidase (GSH-Px) activity (nanomoles of NADPH oxidized/min/mg protein), total, dehydro and reduced ascorbic acid (TAA, DHA and RAA) (mg/g fresh tissue wt) in brain (cerebral hemisphere) of Groups I-XIII mice<sup>a</sup>

Group	Treatment	Glutathione	GSH-Px	TAA	DHA	RAA
I	Control,	46.64 ±	12.13 ±	4.85 ±	1.13 ±	3.59 ±
	untreated	0.06	0.03	0.17	0.02	0.06
II	Control	46.22 ±	11.87 ±	4.84 ±	1.15 ±	3.69 ±
	+ olive oil	0.01	0.03	0.01	0.01	0.01
III	Control + ascorbic acid (AA)	45.95 ±	11.85 ±	4.83 ±	1.17 ±	3.67 ±
		0.02	0.04	0.01	0.01	0.01
IV	Control + calcium phosphate (Ca)	45.65 ±	11.76 ±	4.80 ±	1.17 ±	3.64 ±
		0.02	0.16	0.01	0.01	0.01
V	Control + vitamin E (Vit. E)	45.79 ±	12.01 ±	4.84 ±	1.15 ±	3.68 ±
		0.01	0.03	0.01	0.004	0.01
VI	NaF treatment	34.47 ±	9.82 ±	3.76 ±	1.28 ±	2.35 ±
		0.01 <sup>§</sup>	0.09 <sup>§</sup>	0.46 <sup>§</sup>	0.21 <sup>§</sup>	0.07 <sup>§</sup>
VII	As <sub>2</sub> O <sub>3</sub> treatment	18.12 ±	6.60 ±	2.19 ±	1.43 ±	0.74 ±
		0.33 <sup>§</sup>	0.38 <sup>§</sup>	0.03 <sup>§</sup>	0.020 <sup>§</sup>	0.05 <sup>§</sup>
VIII	NaF + As <sub>2</sub> O <sub>3</sub> treatment	23.70 ±	8.65 ±	2.72 ±	1.60 ±	1.01 ±
		0.34 <sup>§</sup>	0.15 <sup>§</sup>	0.16 <sup>§</sup>	0.06 <sup>§</sup>	0.05 <sup>§</sup>
IX	Withdrawal of Group VIII treatment	25.79 ±	8.89 ±	3.05 ±	1.56 ±	2.11 ±
		0.25	0.11	0.11	0.04	0.06
X	Withdrawal of Group VIII treatment + AA	43.98 ±	11.62 ±	4.67 ±	1.16 ±	3.28 ±
		0.91 <sup>§</sup>	0.12 <sup>§</sup>	0.23 <sup>§</sup>	0.07 <sup>§</sup>	0.16 <sup>§</sup>
XI	Withdrawal of Group VIII treatment + Ca	40.67 ±	11.38 ±	3.88 ±	1.24 ±	2.76 ±
		0.77 <sup>§</sup>	0.20 <sup>§</sup>	0.11 <sup>§</sup>	0.05 <sup>§</sup>	0.15 <sup>§</sup>
XII	Withdrawal of Group VIII treatment + Vit. E	41.10 ±	11.08 ±	4.57 ±	1.21 ±	3.27 ±
		0.54 <sup>§</sup>	0.19 <sup>§</sup>	0.08 <sup>§</sup>	0.02 <sup>§</sup>	0.08 <sup>§</sup>
XIII	Withdrawal of Group VIII treatment + AA, Ca & Vit. E	43.82 ±	11.86 ±	4.68 ±	1.15 ±	3.53 ±
		0.46 <sup>§</sup>	0.15 <sup>§</sup>	0.12 <sup>§</sup>	0.01 <sup>§</sup>	0.12 <sup>§</sup>

a = Data are expressed as mean ± SE. \* = P<0.05; † = P<0.02; ‡ = P<0.01;

§ = P<0.001; where nothing is shown = nonsignificant.

Comparison between: Group I and Group VI or VII or VIII individually; Group VIII and Group IX or X or XI or XII or XIII individually.

tissues of rats and mice,<sup>4,36,37,42</sup> as well as the work of others who have reported recovery by feeding antioxidants like SOD, glutathione,  $\beta$ -carotene, and some herbal extracts as well as by synthetic catalytic scavengers of reactive oxygen species.<sup>43-46</sup>

The mechanism of the ameliorative role of vitamins C and E and calcium phosphate in mitigating fluoride and aluminium toxicity in mice, rats, and rabbits has been presented earlier.<sup>28,36,37,42</sup> It is likely that both vitamins C and E act synergistically to activate several enzymes,<sup>36,37,42</sup> since  $\alpha$ -tocopherol (vitamin E) interacts with vitamin C, which enhances its radical-scavenging activity.<sup>47</sup> Vitamin E is known to protect polyunsaturated fatty acids from oxidation *in vivo* and to prevent the oxidation of free or protein-bound sulfhydryls to disulphides.<sup>48</sup> It is further known that vitamin E deficiency causes central nervous system disturbances,<sup>47</sup> while reduced glutathione and other sulfhydryl groups protect the cell membrane against free radical damage.<sup>3</sup> Calcium activates several enzymes, whereas both calcium and ascorbic acid are known to inhibit phosphodiesterase and to enhance cAMP levels.<sup>4,28</sup>

The present study thus demonstrates that toxic effects of NaF + As<sub>2</sub>O<sub>3</sub> in the brain of mice are, by and large, transient and reversible, and that dietary factors like vitamins C and E as well as calcium are useful for the amelioration of these toxic effects.

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