

ADVERSE EFFECTS OF FLUORIDE AND/OR ARSENIC ON THE CEREBRAL HEMISPHERE OF MICE AND RECOVERY BY SOME ANTIDOTES

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SUMMARY: The effects of oral administration of sodium fluoride (NaF) and/or arsenic trioxide (As_2O_3) at 5 and 0.5 mg/kg body weight doses, respectively, for 30 days were studied on the physiology and histology of brain (cerebral hemisphere) of adult mice (*Mus musculus*). Recovery after 30 additional days by some antidotes (vitamins C and E and calcium phosphate) was also examined. The observed significant decline in levels of DNA and RNA and acetylcholinesterase activity in brain (cerebral hemisphere) of mice treated with NaF, As_2O_3 and NaF + As_2O_3 is related to its altered histology. The combined antidote treatment was conducive for recovery of this fluoride and arsenic induced toxicity in the brain. These results are viewed as having important implications for fluoride and arsenic endemic populations all over the globe.

Key words: Acetylcholinesterase; Arsenic trioxide and cerebral hemisphere; Brain histology; Calcium phosphate; Mice brain; Nucleic acids; Protein; Sodium fluoride and cerebral hemisphere, Vitamin C; Vitamin E.

INTRODUCTION

The widespread distribution of fluoride in nature is a direct source of adverse health effects in human populations.¹ Fluoride is also neurotoxic as shown in various studies, including those of Shashi in male and female rabbits,^{2,3} ultimately leading to paralysis. A study by Chlubek *et al*⁴ revealed marked shrinkage of the cerebellar external granular layer and Purkinje cells, plus perivascular myelin swelling, especially in the white matter of the brain in NaF-treated rats.

Ingestion of inorganic arsenic can also cause injury to the nervous system. Acute high dose exposures (1 mg As/kg body weight or above) often lead to encephalopathy, accompanied by headache, lethargy, mental confusion, seizures, hallucinations, and coma.⁵ Gurr *et al*⁶ reported that arsenic trioxide (As_2O_3), and As_2O_3 plus dithiothreitol, enhanced arsenic-induced apoptosis in NB4 cells. As(III) and As(V) inhibit protein synthesis and replace phosphate in the nucleotides during DNA synthesis.⁷ Although oxidative damage by a fluoride-arsenic combination in the brain of rat offspring and adult mice has been reported,⁸⁻¹⁰ relatively little is known about the effect of their co-treatment. Hence the present study was undertaken.

MATERIALS AND METHODS

Animals: Healthy, adult male mice (*Mus musculus*) of Swiss strain were divided into thirteen groups and treated with sodium fluoride (NaF), arsenic tri-

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oxide (As_2O_3), and their combination as well as with antidotes (vitamins C and E and calcium as calcium phosphate) using dose regimens as detailed in an earlier paper.¹⁰ At the end of each experiment, the animals were sacrificed by cervical dislocation. The brain (cerebral hemisphere) of all control and treated animals was dissected out carefully, blotted free of blood, and utilized to study some specific parameters.

Histology: The histology of cerebral hemisphere of control and all treated groups of animals was studied by using the standard haematoxylin-eosin (HE) method. The stained slides were used for histocytometric analyses using an ocular (scaled) eyepiece and a micrometer scale.

Biochemical parameters: Standard techniques were used to determine acetylcholinesterase (AChE)¹¹ and nucleic acids^{12,13} in the cerebral hemisphere of control and treated groups of mice.

Statistics: For each biochemical parameter a minimum of 5 or 6 replicates were assayed and the data were statistically analysed by Student's t test and ANOVA.

RESULTS

Histology of brain (cerebral hemisphere): The brain histology of control mice showed well developed neurons (Figures 1 and 2).

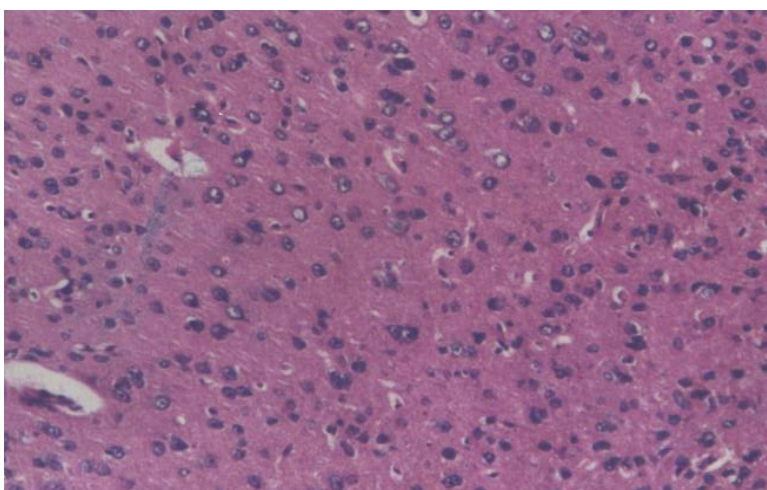


Figure 1. Transverse section of cerebral hemisphere of control (Group I) mice. HE staining (X 150).

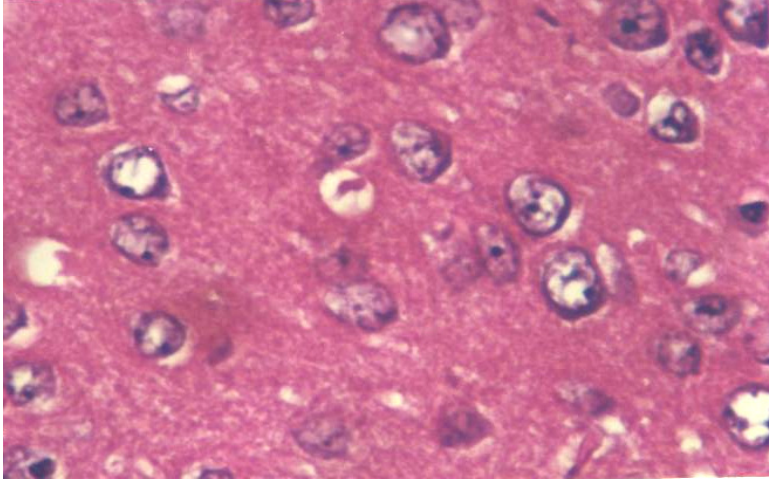


Figure 2. Magnified view of Figure 1. HE staining (X 600).

The brain of 30-day NaF-treated mice revealed vacuolization and pyknosis of nuclei (Figure 3).

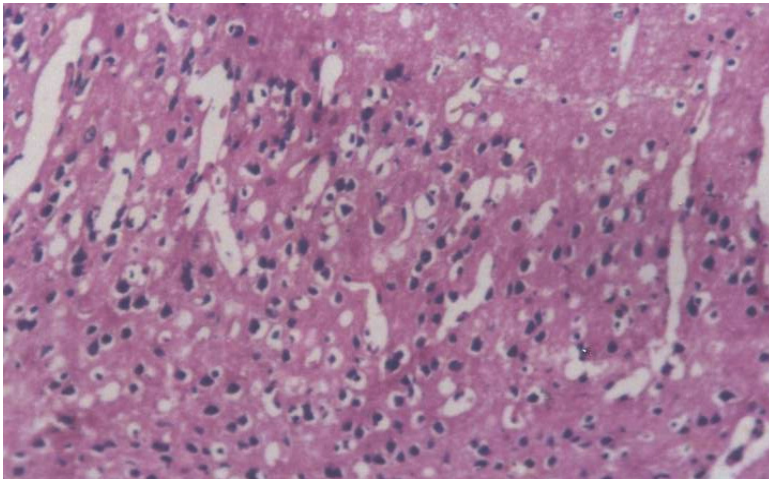


Figure 3. Transverse section of cerebral hemisphere of NaF treated mice. HE staining (X 120).

The treatment with arsenic trioxide (As_2O_3) caused necrosis of tissue and vacuolization. The effect was greater than by NaF treatment (Figures 4 and 5).

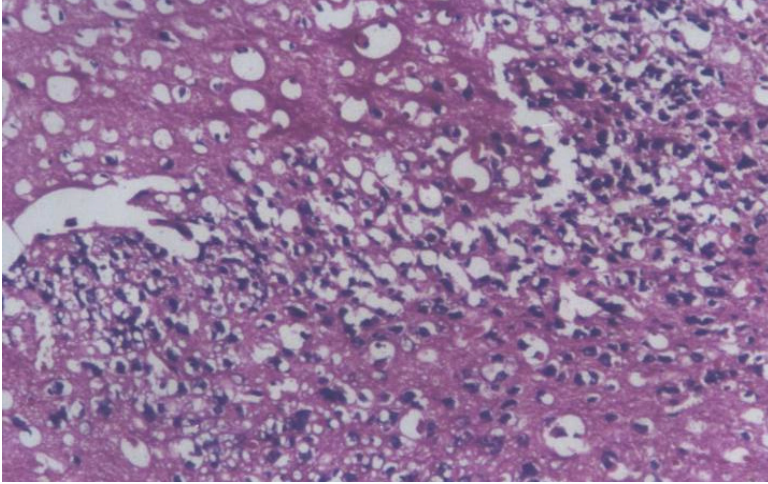


Figure 4. Transverse section of cerebral hemisphere of As_2O_3 treated mice. HE staining (X 200).

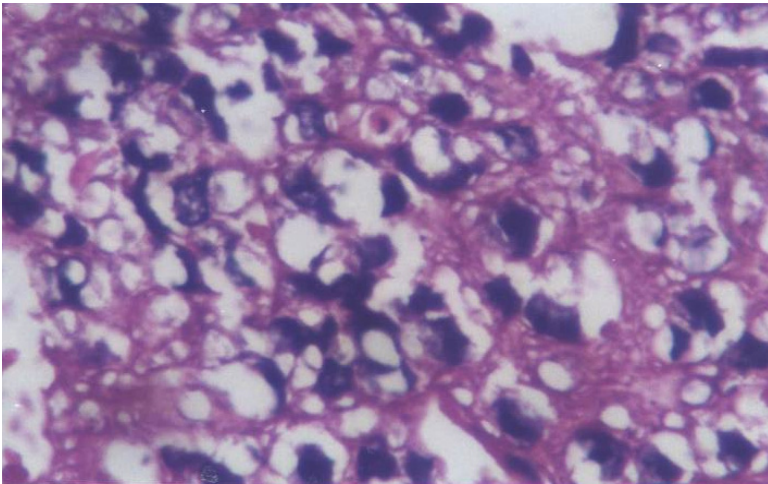


Figure 5. Magnified view of Figure 4. HE staining (X 500).

The combined treatment with NaF + As₂O₃ revealed, besides the vacuolated regions, some patches of lymphocyte infiltration (Figures 6 and 7).

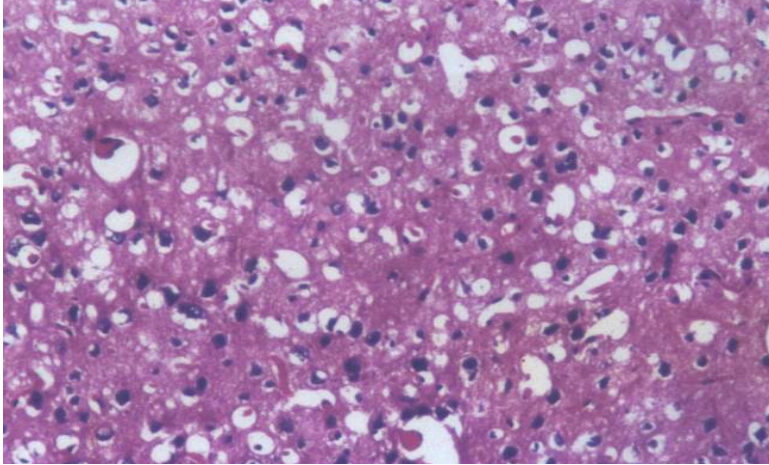


Figure 6. Transverse section of cerebral hemisphere of NaF + As₂O₃ treated mice. HE staining (X 160).

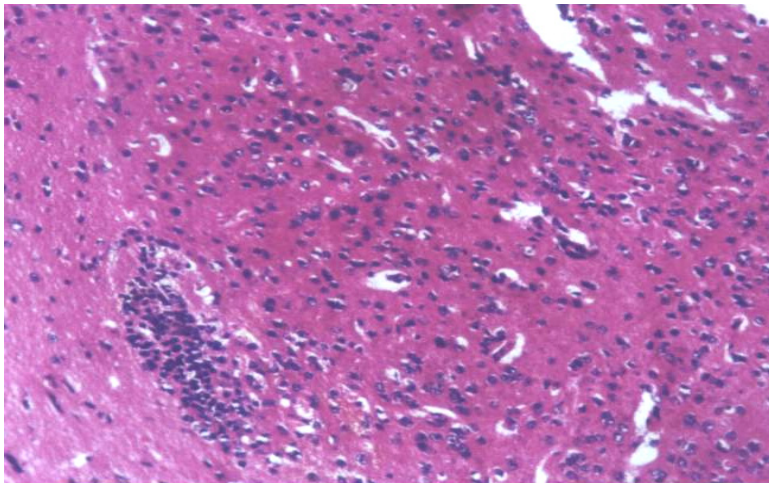


Figure 7. Transverse section of cerebral hemisphere of NaF + As₂O₃ treated mice. HE staining (X 150).

The withdrawal of NaF + As₂O₃ treatment resulted in some recovery, but vacuolization still persisted (Figure 8).

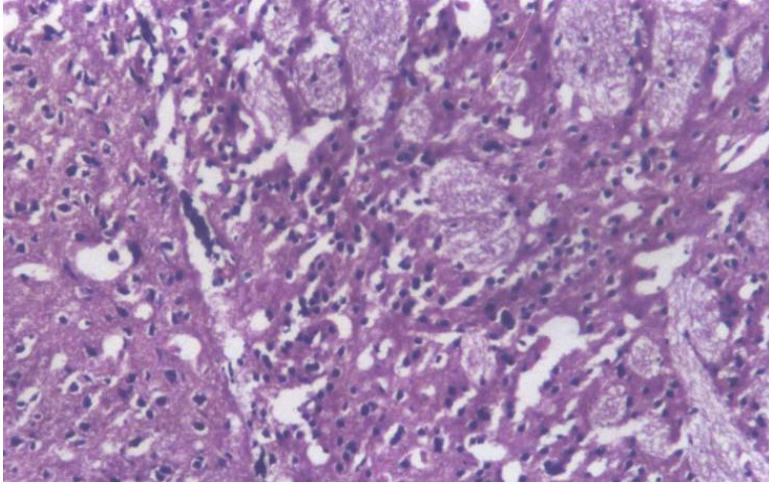


Figure 8. Transverse section of cerebral hemisphere of mice after withdrawal of NaF + As₂O₃ treatment. HE staining (X 200).

In the antidote treated mice (Groups X to XIII), much better recovery was observed (Figures 9 and 10).

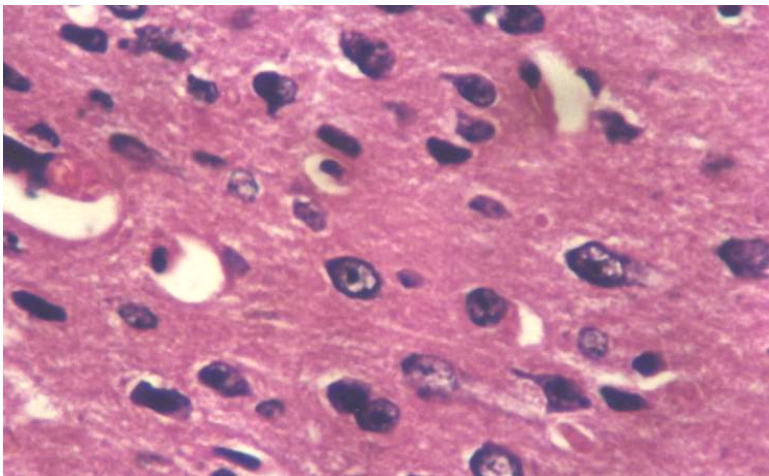


Figure 9. Transverse section of cerebral hemisphere of mice of Group XII (Withdrawal + vitamin E treatment). HE staining (X 600).

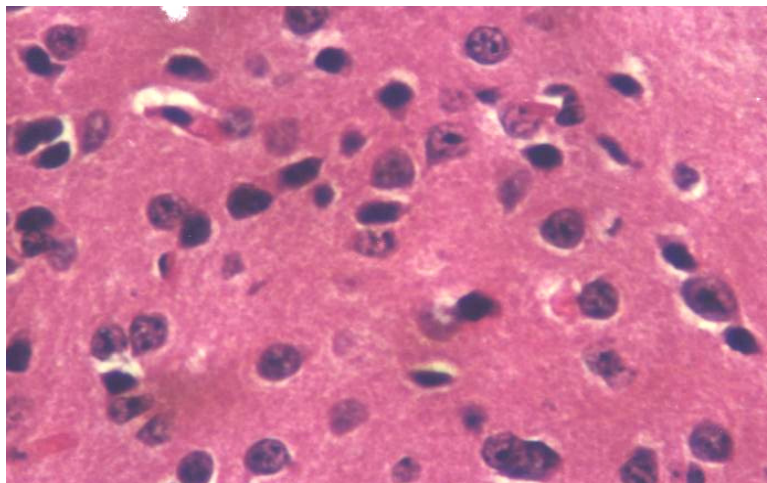


Figure 10. Transverse section of cerebral hemisphere of mice of Group XIII (withdrawal + all antidotes). HE staining (X 900).

Acetylcholinesterase: The activity of acetylcholinesterase in brain decreased significantly ($P<0.001$) after 30 days of NaF, As_2O_3 , or NaF + As_2O_3 treatments (Groups VI to VIII) as compared to control. Upon withdrawal of treatment (Group IX) there was significant recovery in enzyme activity ($P<0.01$), but in the antidote-treated Groups X to XIII, recovery was even more significant ($P<0.001$) as compared to Group VIII (Table 1).

DNA and RNA: The DNA and RNA levels decreased significantly ($P<0.001$) in the cerebral hemisphere of mice by NaF, As_2O_3 , and NaF + As_2O_3 treatments (Groups VI-VIII) as compared to control. In the Group VII (arsenic trioxide) treated mice, the maximum decline was observed. The nucleic acid levels in Group IX (withdrawal) showed significant recovery (DNA, $P<0.02$; RNA, $P<0.01$) after 30 days as compared to Group VIII. Significant recovery also occurred in the cerebral hemisphere of animals administered with antidotes individually (vitamin C, calcium, $P<0.001$; vitamin E for DNA only, $P<0.01$) and in combination ($P<0.001$) in comparison to Group VIII (Table 1).

Table 1. Acetylcholinesterase (AChE activity/mg protein), DNA and RNA levels (g/100 mg fresh tissue weight) in cerebral hemisphere of mice in Groups I-XIII^a

Group	Treatment	AChE	DNA	RNA
I	Control + distilled water	10.44 ± 0.09	575.65 ± 1.7	220.77 ± 1.05
II	Control + olive oil	10.64 ± 0.03	579.32 ± 1.4	221.31 ± 1.04
III	Control + ascorbic acid (AA)	10.55 ± 0.09	577.93 ± 1.6	221.96 ± 1.15
IV	Control + calcium phosphate (Ca)	11.08 ± 0.03	574.55 ± 1.6	219.36 ± 1.77
V	Control + vitamin E (Vit. E)	10.53 ± 0.07	576.71 ± 1.5	221.93 ± 2.04
VI	NaF	7.50 ± 0.06 [§]	375.34 ± 1.3 [§]	120.14 ± 1.80 [§]
VII	As ₂ O ₃	4.91 ± 0.09 [§]	210.91 ± 1.0 [§]	75.27 ± 1.37 [§]
VIII	NaF + As ₂ O ₃	5.00 ± 0.03 [§]	235.58 ± 1.6 [§]	95.31 ± 1.0 [§]
IX	Withdrawal of Group VIII treatment	6.78 ± 0.12 [‡]	242.25 ± 2.0 [†]	103.16 ± 1.7 [‡]
X	Withdrawal of Group VIII treatment + AA	9.55 ± 0.14 [§]	539.64 ± 1.3 [§]	200.0 ± 1.72 [§]
XI	Withdrawal of Group VIII treatment + Ca	8.66 ± 0.11 [§]	518.17 ± 1.4 [§]	191.53 ± 2.03 [§]
XII	Withdrawal of Group VIII treatment + Vit. E	8.62 ± 0.14 [§]	244.30 ± 1.6 [‡]	190.25 ± 2.32 [§]
XIII	Withdrawal of Group VIII treatment + AA, Ca & Vit. E	9.80 ± 0.20 [§]	535.96 ± 0.3 [§]	207.32 ± 2.27 [§]

^aData are expressed as mean ± S.E. * P<0.05; † P<0.02; ‡ P<0.01; § P<0.001; where no sign = not significant.

Comparisons between: Group I to Groups VI or VII or VIII individually;
Group VIII to Groups IX or X or XI or XII or XIII individually

Table 1a. ANOVA of various parameters

Parameter	Source of variation	SS	df	MSS	F-cal	F-tab
AChE	Between Groups	416.0629	12	34.67191	146.726	1.835815
	Within Groups	276.4754	117	0.23630		
DNA	Between Groups	154198.1	12	128498.4	91.02641	1.911928
	Within Groups	87522.97	62	1411.661		
RNA	Between Groups	191517.2	12	15959.7	583.068	1.96475
	Within Groups	1286.494	47	27.3722		

SS=Sum of squares; df=degree of freedom; MSS=Mean sum of squares;
F-cal=Fisher calculated; F-tab=Fisher tabulated.

DISCUSSION

The histology of the cerebral hemisphere was altered by NaF and/or As₂O₃ treatment for 30 days, wherein the effect by As₂O₃ was greater than by NaF treatment. This result is in agreement with data of others.^{4,14} Acute high dose exposure to arsenic can lead to encephalopathy, impaired memory, and emotional instability.⁵ Exposure to fluoride in drinking water has been reported by several workers to decrease intelligence, learning, and memory in children.¹⁵⁻¹⁷ The degenerative changes in the brain and apoptosis^{18,19} might also contribute to the results found here in fluoride-treated mice.

The reduced brain acetylcholinesterase (AChE) enzyme activity observed in the present study corroborates data of others in rats exposed for three months to arsenic trioxide²⁰ and in the brain of NaF-treated mice and rats as compared to controls.^{21,22} According to Parveen and Kumar,²³ AChE seems to be the most sensitive parameter for monitoring intoxication due to toxic compounds and drugs to mammals. The inhibition of AChE might alter synaptic transmission of impulses.

Arsenic can affect DNA repair and its methylation, and it can increase radical formation.²⁴ The decrease in RNA content of rabbit brain observed during acute and chronic fluoride intoxication seems to be due to fluoride induced inhibition of protein synthesis.³ The DNA and RNA levels in the cerebral hemisphere were significantly lower in NaF- and/or As₂O₃-treated mice in the present study, which could affect brain function.

The ingestion of the antidotes vitamins C and E as well as calcium phosphate, either individually or in combination, during the 30-day withdrawal period, resulted in significant recovery, probably due to the antioxidant properties of vitamins C and E^{10,25,26} and modulation of fluoride-induced toxicity in rats by calcium.²² Therefore, calcium and vitamins C and E have an important role in alleviating fluoride and/or arsenic induced toxic effects in the brain of mice. Thus, it appears likely that these dietary supplements could be beneficial for populations in endemic areas against fluorosis or arsenosis.

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