

BENEFICIAL EFFECTS OF SOME ANTIDOTES IN FLUORIDE AND ARSENIC INDUCED TOXICITY IN KIDNEY OF MICE

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SUMMARY: Ingestion of sodium fluoride (NaF, 5 mg/kg/body weight) and/or arsenic trioxide (As₂O₃, 0.5 mg/kg body weight) for 30 days and possible beneficial effects of calcium phosphate and vitamins C and E supplementation for the next 30 days on some parameters in kidney of adult male mice (*Mus musculus*) were examined. The combined treatment caused a significant decline in total protein and creatinine and in the activities of acid and alkaline phosphatase (ACP and ALP), which are indicative of altered membrane permeability, disturbed cell functions, and probably tissue damage. These alterations are correlated with the histological changes in the kidney. Withdrawal of NaF + As₂O₃ treatment, caused insignificant recovery in most of the parameters studied. However, administration of antidotes alone or in combination during the withdrawal period resulted in almost complete recovery, which was more pronounced with the antidotes combined, probably due to their additive or synergistic action. Thus it is possible they could be used for the management of kidney toxicity induced by fluoride and arsenic.

Keywords: Acid and alkaline phosphatases; Arsenic and kidney; Calcium; Creatinine; Fluoride and kidney; Mice; Synergistic effects; Vitamin C; Vitamin E.

INTRODUCTION

The kidney is a site for potential fluoride toxicity, since it can be exposed to relatively high concentrations of fluoride, whose elimination depends mainly on kidney function, and acute renal failure would contribute to the accumulation of fluoride.¹ A close relationship exists between polyuria and changes in excretion of certain ions in fluorosis.² The kidney is damaged in adults exposed to fluoride for a long period, and therefore the excretion of fluoride is significantly diminished.³ The activity of α -glutathione S-transferase, a marker of tubular damage, particularly in the S3 segment of proximal tubule, and the activity of N-acetyl β -D-glucuronidase (NAG) were found to be increased by fluoride in rats,^{4,5} suggesting that the toxic effects on kidney were more in the proximal tubule than in the glomerular region. According to Cittanova *et al*,^{6,7} mitochondria and its Na-K ATPase is a target of fluoride toxicity in Henle's loop and the collecting duct cells of human kidney. Li *et al*⁸ reported that fluoride damaged the organelles involved in protein synthesis in kidneys of animals and humans affecting some renal enzymes. Extensive histological and ultrastructural changes have been observed in kidney of NaF-treated rabbits⁹ and mice.^{10,11} Administered at 50 mg/L in water, NaF induced renal apoptosis and reduction in the G (2) M period in cell cycle, which were overcome in rats given selenium and zinc.¹² There is also evidence that an elevated intake of fluoride causes nephrolithiasis in tribal populations.¹³

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Rapid elimination of arsenic in urine relieves the kidney of a high arsenic burden thereby possibly minimizing the potential adverse effects on the renal system.¹⁴ Bouletreau *et al*¹⁵ stated that direct chemical nephrotoxicity in acute intoxication causes tubular impairment in the proximal part with a mild alteration of the glomeruli. Studies by Hirata *et al*¹⁶ corroborated these observations. Acute renal failure and glomerular congestion were reported in humans given a single oral dose of As⁺³ of between 0.02-8mg/kg.¹⁴ Arsenic concentrations of 0.1–0.5 and ≥ 0.5 $\mu\text{g/L}$ were responsible for bladder and kidney cancer in Finland.¹⁷ Studies by Ginsberg¹⁸ showed that pentavalent arsenic (as arsenate, AsO₄³⁻) is actively transported by the kidney tubules in which it is reduced to the more toxic trivalent form, As⁺³. Reduction of inorganic arsenic by nascent hydrogen may also result in highly toxic arsine (AsH₃), whose uptake by erythrocytes causes hemolysis and oxidation, leading to arsenic acid, H₃AsO₄, which also damages the kidney.¹⁹

In the present investigation, studies were made of the effects of sodium fluoride and/or arsenic trioxide ingestion on the structure and physiology of the kidney of mice. In addition, in the light of recent work, the possible therapeutic effects of supplementation with vitamins C and E plus calcium (as calcium phosphate), alone or in combination, were also examined.

MATERIALS AND METHODS

Animals: Healthy, Swiss strain adult male mice (*Mus musculus*), weighing between 30 and 40 g, were obtained from the National Institute of Occupational Health (NIOH) and Cadila Health Care, Ahmedabad, Gujarat, India, under the Animal Maintenance and Registration No. 167/1999/CPCSEA from The Ministry of Social Justice and Empowerment, Government of India and Committee for the purpose of Control and Supervision of Experiments on Animals, Chennai, India. The mice were divided into thirteen groups and treatments given as per the details shown in the experimental protocol table.

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

Biochemical study: Standard techniques were used to determine the levels of protein²⁰ and creatinine²¹ and the activities of alkaline phosphatase (E.C.3.1.3.1.) and acid phosphatase (E.C.3.1.3.2.)²² in kidney of control and all treated groups of mice.

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

Experimental protocol

Group	Treatment and dose (15 to 20 mice in each group)	Duration (days)	Day of autopsy
I	Control + distilled water	-	*
II	Control + olive oil (0.2 mL/animal/day)	30	31 st
III	Control + ascorbic acid (AA) (15 mg/animal/day)	30	31 st
IV	Control + calcium phosphate (Ca) (25 mg/animal/day)	30	31 st
V	Control + vitamin E (vit. E) (2 mg/0.2mL olive oil/animal/ day)	30	31 st
VI	NaF - treated (5 mg/kg body weight)	30	31 st
VII	As ₂ O ₃ - treated (0.5 mg/kg body weight)	30	31 st
VIII	NaF + As ₂ O ₃ treated (5 mg + 0.5mg/kg body weight)	30	31 st
IX	Same as in Group VIII then withdrawal for further 30 days	30+30	61 st
X	Same as in Group VIII+AA for 30 days	30+30	61 st
XI	Same as in Group VIII+Ca for 30 days	30+30	61 st
XII	Same as in Group VIII+vit. E for 30 days	30+30	61 st
XIII	Same as in Group VIII+AA+Ca+vit. E (Same dose as in Groups III, IV, and V, respectively) for 30 days.	30+30	61 st

* Sacrificed along with treated mice.

RESULTS

Histology: The kidney of control mice consisted of several proximal and distal convoluted tubules and Bowman's capsule with glomeruli (Figures 1 and 2).

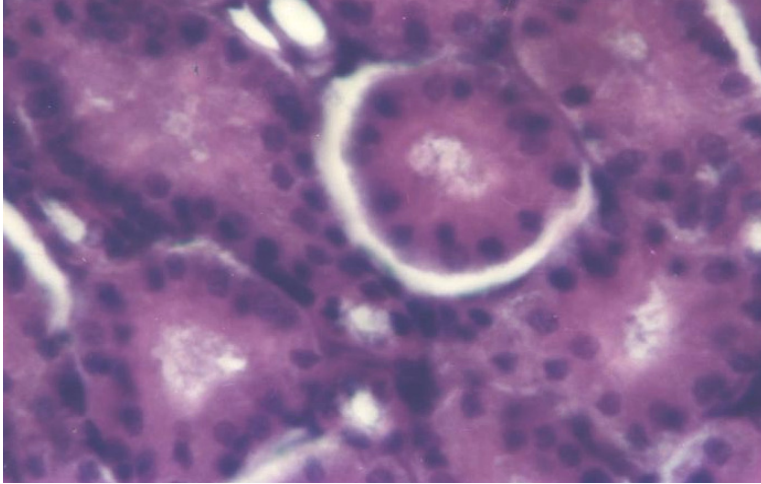


Figure 1. Transverse section of kidney of control (Group I) mice showing tubules and glomerulus. HE staining (X 780).

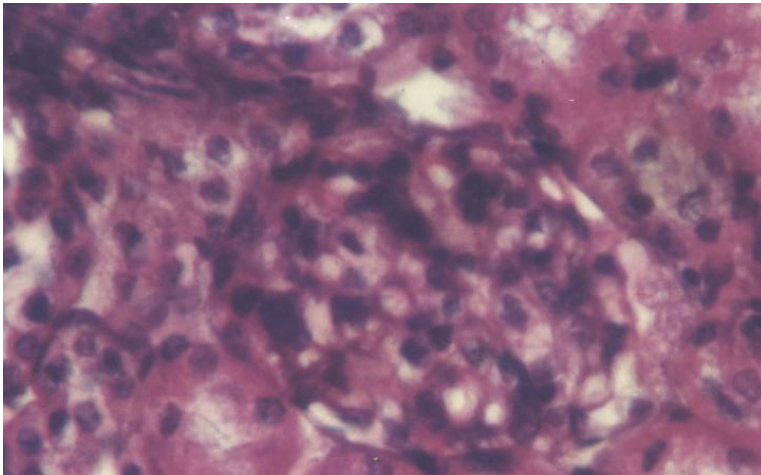


Figure 2. Transverse section of kidney of control (Group I) mice showing glomerulus. HE staining (X 870).

The sodium fluoride treatment for 30 days caused vacuolization as compared to the controls (Figure 3).

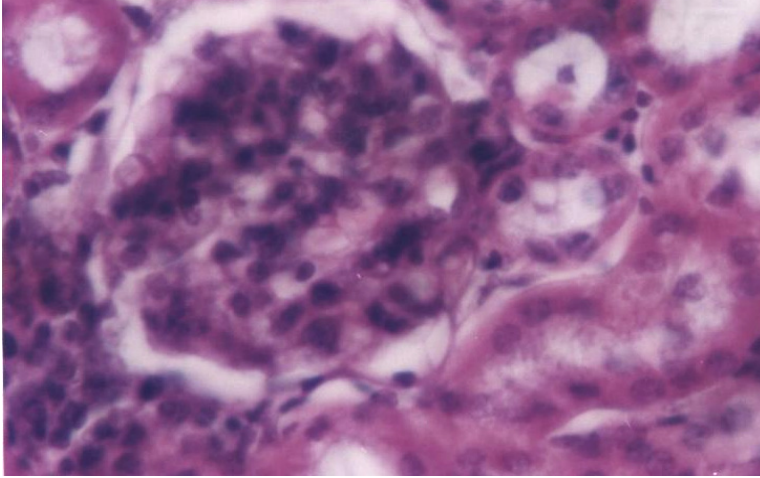


Figure 3. Transverse section of kidney of NaF treated mice. HE staining (X 720).

After the arsenic trioxide alone treatment for 30 days, the tubules were not compactly arranged and vacuolization was observed. The epithelium in the proximal and distal tubules was deeply eosinophilic and necrotic. The nuclei were strongly pyknotic, and the tubular lumen was obliterated. Numerous hemorrhagic regions were prominent (Figure 4).

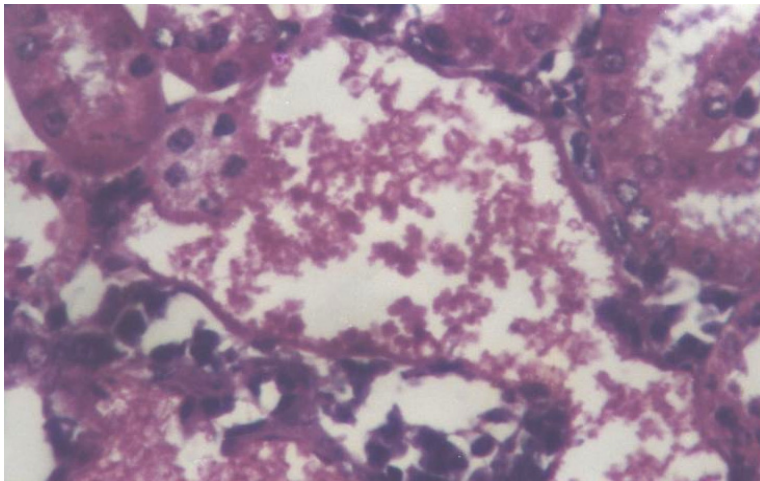


Figure 4. Transverse section of kidney of As₂O₃ treated mice. HE staining (X 920).

The combined treatment with NaF + As₂O₃ resulted in more severe histological changes in the kidney than in the individual treatments. Extensive necrosis of the tubules, shrunken glomeruli with pyknotic nuclei, infiltration of leukocytes especially near the glomeruli, and hemorrhagic patches were more prominent throughout the kidney (Figure 5).

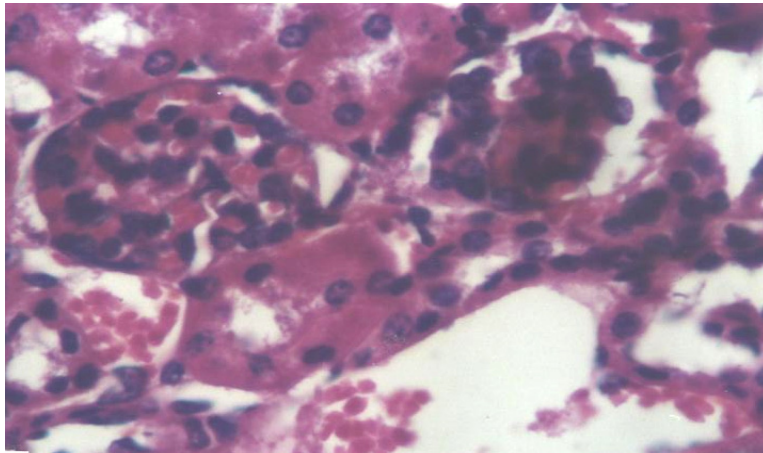


Figure 5. Transverse section of kidney of NaF + As₂O₃ treated mice. HE staining (X 660).

The withdrawal of treatment for 30 days resulted in very little recovery in the kidney histology. However, treatment with antidotes, either individually or in combination (Groups X–XIII), resulted in marked recovery as compared to treatments in Groups VI–VIII (Figures 6 and 7).

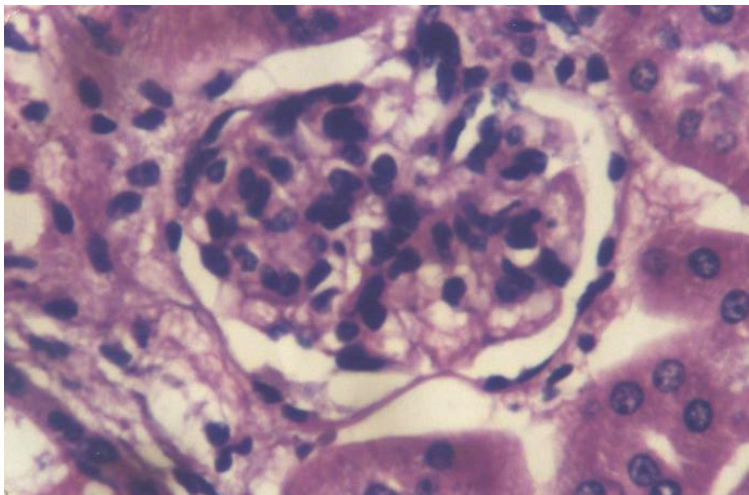


Figure 6. Transverse section of kidney of Group X (withdrawal + ascorbic acid treated) mice. HE staining (X 690).

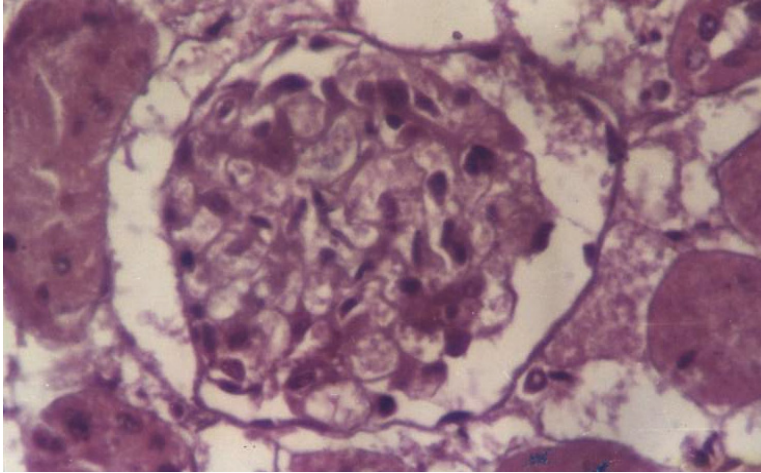


Figure 7. Transverse section of kidney of Group XIII (withdrawal + All antidotes) mice. HE staining (X 860).

Histocytometry of kidney tubules and glomeruli: The data revealed that the tubular diameter was not altered significantly throughout the treatments of Groups VI–VIII as compared to control but the height of tubular epithelium was significantly reduced in Group VI–VIII (Group VI $P < 0.001$; Groups VII and VIII $P < 0.01$) as compared to the controls (Table 1). Withdrawal of the NaF + As₂O₃ treatment (Group IX) for 30 days caused insignificant recovery as compared to Group VIII. However, administration of the antidotes alone or in combination (Groups X–XIII) brought about a significant recovery (Groups X, XII, XIII $P < 0.01$; Group XI $P < 0.02$) in comparison to Group VIII (Table 1). The glomerular diameter was decreased significantly ($P < 0.001$) in mice of Groups VI–VIII as compared to Group I. By the antidote treatments alone or in combination (Groups X–XIII), a highly significant ($P < 0.001$) recovery occurred as compared to Group VIII (Table 1).

Protein and creatinine levels: The levels of protein and creatinine decreased significantly ($P < 0.001$) in kidney of mice by treatment with NaF, As₂O₃, and NaF + As₂O₃ (Groups VI–VIII) as compared to the controls. In Group VII (As₂O₃ treated) the maximum decline was observed. Group IX (withdrawal) showed significant recovery ($P < 0.01$) in protein but was insignificant in creatinine recovery after 30 days as compared to Group VIII. A highly significant recovery ($P < 0.001$) occurred in both parameters of kidney of animals administered ascorbic acid (vitamin C), calcium phosphate, and vitamin E, either individually or in combination (Groups X–XIII), as compared to Group VIII (Table 1).

Alkaline and acid phosphatases: A significant decrease ($P < 0.001$) in alkaline and acid phosphatase activities in kidney in Groups VI–VIII (NaF, As₂O₃, and NaF + As₂O₃ treated) occurred as compared to control.

Table 1. Histocytometric measurements (μm) of kidney tubular epithelial cell height (ECH), glomerular diameter (GD), protein (mg/100 mg fresh tissue wt), creatinine ($\mu\text{g}/100$ mg fresh tissue wt), alkaline and acid phosphatase activities (ALP and ACP) (μmoles of p-nitrophenol released/mg protein) in kidney of mice in Groups I-XIII^a

Group	Treatment	ECH	GD	Protein	Creatinine	ALP	ACP
I	Control + distilled water	26.77 \pm 2.20	68.89 \pm 3.85	28.22 \pm 0.27	123.31 \pm 0.21	0.19 \pm 0.002	0.32 \pm 0.005
II	Control + olive oil	-	-	28.47 \pm 0.08	123.00 \pm 0.23	0.19 \pm 0.002	0.32 \pm 0.003
III	Control + ascorbic acid (AA)	-	-	28.54 \pm 0.08	122.71 \pm 0.15	0.19 \pm 0.002	0.32 \pm 0.004
IV	Control + calcium phosphate (Ca)	-	-	28.56 \pm 0.05	123.34 \pm 0.19	0.19 \pm 0.002	0.32 \pm 0.003
V	Control + vitamin E (Vit. E)	-	-	28.51 \pm 0.07	122.81 \pm 0.21	0.19 \pm 0.002	0.32 \pm 0.03
VI	NaF	13.63 \pm 3.20 [§]	45.81 \pm 3.19 [§]	21.14 \pm 0.29 [§]	92.99 \pm 0.23 [§]	0.16 \pm 0.002 [§]	0.29 \pm 0.003 [§]
VII	As ₂ O ₃	17.37 \pm 3.00 [‡]	39.04 \pm 4.38 [§]	12.42 \pm 0.15 [§]	62.11 \pm 0.32 [§]	0.12 \pm 0.002 [§]	0.23 \pm 0.007 [§]
VIII	NaF + As ₂ O ₃	15.89 \pm 2.30 [‡]	52.46 \pm 3.20 [§]	16.81 \pm 0.16 [§]	84.62 \pm 0.68 [§]	0.15 \pm 0.002 [§]	0.27 \pm 0.005 [§]
IX	Withdrawal of Group VIII treatment	18.18 \pm 2.80	52.64 \pm 4.48	19.23 \pm 0.17 [‡]	86.62 \pm 1.9	0.15 \pm 0.002	0.28 \pm 0.05
X	Withdrawal of Group VIII treatment + AA	24.30 \pm 2.60 [‡]	62.80 \pm 2.60 [§]	24.95 \pm 0.35 [§]	120.13 \pm 1.8 [§]	0.19 \pm 0.001 [§]	0.32 \pm 0.01 [§]
XI	Withdrawal of Group VIII treatment + Ca	19.70 \pm 2.70 [†]	59.20 \pm 3.69 [§]	25.40 \pm 0.55 [§]	112.84 \pm 0.77 [§]	0.18 \pm 0.002 [§]	0.29 \pm 0.009 [§]
XII	Withdrawal of Group VIII treatment + Vit. E	23.70 \pm 2.80 [‡]	61.88 \pm 2.56 [§]	26.99 \pm 0.03 [§]	119.86 \pm 0.37 [§]	0.18 \pm 0.001 [§]	0.31 \pm 0.01 [†]
XIII	Withdrawal of Group VIII treatment + AA, Ca & Vit. E	25.17 \pm 2.30 [‡]	67.90 \pm 2.22 [§]	27.81 \pm 0.14 [§]	122.04 \pm 0.72 [§]	0.19 \pm 0.002 [§]	0.32 \pm 0.008 [§]

^aData are expressed as mean \pm 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
Tubular epithelial cell height (ECH)	Between Groups	820.5221	8	891.2578	13.99786	1.7895
	Within Groups	333.1758	81	91.6753		66
Glomerular diameter (GD)	Between Groups	944.9964	12	128498.4	91.0264	1.9119
	Within Groups	59.7210	62	1411.661		28
Protein	Between Groups	2456705.5853	12	273.9469	463.2018	1.8358
	Within Groups	1782140.4415	117	0.59142		15
Creatinine	Between Groups	12007.7884	12	41.11303	408.4141	1.8358
	Within Groups	3294.0267	117	0.100665		15
Alkaline phosphatase	Between Groups	10646.2417	12	5.803921	98.2028	1.8358
	Within Groups	908.2987	117	5.91014		15
Acid phosphatase	Between Groups	596.4405	12	5.803921	80.98202	1.8358
	Within Groups	378.4864	117	2.91014	8	15

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

The decrease in activity in As₂O₃-treated mice was greater than in Groups VI or VIII. The alkaline phosphatase activity of animals fed vitamins C and E and calcium phosphate, alone and in combination, recovered significantly (P<0.001) (Table 1). Recovery in acid phosphatase activity was also highly significant (P<0.001) by vitamin C and the combination of all three antidotes (Groups X and XIII) than with the individual treatments with calcium (P<0.05) and vitamin E (P<0.02), respectively (Table 1).

DISCUSSION

Adverse effects of combined arsenic and fluoride treatment on kidney in rats have been reported recently,²³ and they are known to alter protein levels in various tissues of treated mice independently or in combination.^{10,11,24} The results of the present study corroborate these findings.

Fluoride concentration in serum or tissues depends mainly on kidney function. A relationship between the dose of fluoride and renal tissue injury has been reported.¹ The levels of creatinine in serum of workers exposed to airborne fluoride at an aluminium plant in China were significantly decreased.²⁵ Similar results were obtained in the present study which indicated that, along with the kidney, the muscle function would also be affected, since creatinine is a metabolite of phosphocreatine, which is an important energy source for muscle.

Acid phosphatase, a lysosomal enzyme, is one among the highly impaired enzymes in the early stage of acute toxic sodium fluoride nephropathy.²⁶ The activity of alkaline phosphatase was also reduced in plasma of subjects taking fluoride tablets²⁷ and in the kidney of mice and humans exposed to fluoride in drinking water^{8,28} as in the present study with NaF and As₂O₃ treatments. It is suggested that changes in the kidney could be related to histological and ultra-structural alterations as also reported by others.^{9,29,30}

The withdrawal of treatment brought about only partial recovery in levels of protein and creatinine. However, administration of ascorbic acid (vitamin C) manifested significant recovery in all parameters. Kennedy³¹ found that the severity of damage by fluoride to humans is increased where the dietary intake of protein, calcium, and vitamin C are low. The ingestion of vitamin C and E and calcium phosphate also showed significant recovery in all parameters which were affected by NaF + As₂O₃ treatment, probably due to their additive or synergistic action. These results are significant since kidney malfunction induced by the toxicants could be, by and large, overcome by dietary factors.

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