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BENEFICIAL EFFECTS OF SOME VITAMINS AND CALCIUM ON FLUORIDE AND ALUMINIUM TOXICITY ON GASTROCNEMIUS MUSCLE AND LIVER OF MALE MICE

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SUMMARY: Treatment of male mice (*Mus musculus*) with sodium fluoride (NaF, 10 mg/kg body weight), alone or in combination with aluminium chloride ($AlCl_3$, 200 mg/kg body weight), was investigated for its effects on gastrocnemius muscle and liver. Recovery after one-month withdrawal of treatment and responses to some antidotes, *viz* calcium, ascorbic acid, and vitamin E administered alone or in combination were also studied. NaF alone or in combination with $AlCl_3$ caused a significant decrease in protein levels and activity of succinate dehydrogenase (SDH) in liver and gastrocnemius muscle, thereby indicating altered protein and oxidative metabolisms in these tissues. Cholinesterase activity declined significantly with all the treatments in both tissues, probably affecting the synaptic transmission due to altered acetylcholine release or metabolism and altering muscle contraction.

All three treatments caused changes in liver function as shown by a significant increase in serum transminases, accumulation of glycogen and inhibition of phosphorylase activity thereby indicating that carbohydrate metabolism was affected. Similar changes occurred in muscle tissue. Gastrocnemius muscle and liver were therefore affected by sodium fluoride, aluminium chloride and in combination.

Recovery was not significant on withdrawal of NaF + $AlCl_3$ treatment. However, all three antidotes brought about significant recovery in the organs studied. Individually, ascorbic acid was the most beneficial in bringing about pronounced recovery. Thus, intoxication induced by sodium fluoride and aluminium chloride is transient and reversible.

Keywords: Aluminium chloride, Ascorbic acid antidote, Calcium antidote, Cholinesterase activity, Fluoride toxicity, Gastrocnemius muscle, Glycogen accumulation, Liver, Male mice, Oxidative metabolisms, Protein alteration, Phosphorylase inhibition, Serum transaminases, Sodium fluoride, Toxicity reversal, Vitamin E antidote.

INTRODUCTION

The widespread distribution of fluoride in the environment through drinking water and food often results in adverse health effects. Reports from our laboratory have elucidated alterations in the structure and metabolism of some organs of rats and mice treated with NaF.[1-5] However, the induced effects were transient and reversible by feeding ascorbic acid, calcium, or vitamin E individually or in combination during the withdrawal period.[2-5]

Aluminium is one of the most abundant metals in the earth's crust and is present in air, water, and soil. It occurs naturally only in combination with oxygen, fluoride, silicate, etc. Nutritionally, it is nonessential, but it is used

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in the treatment of drinking water, in several pharmacological preparations, in numerous processed foods, and in the manufacture of cooking utensils.[6] A dose of aluminium chloride (400 mg/kg body weight) for 15 days and chronic treatment (200 mg/kg body weight) of male mice for 60 days cause alterations in their reproductive organs.[7-8] Similar results were reported by Llobet *et al* using 200 mg/kg body weight aluminium nitrate on male mice for 4 weeks.[9] High concentrations of aluminium chloride are known to cause serious effects on several body functions[6] as well as maternal toxicity, embryolethality and resorption[10] and to inhibit the action on Ca^{++} ATPase in brain cells in rats.[11] Aluminium lactate also produces developmental toxicity in mice including poor ossification, skeletal deformities, and cleft palate.[12]

The toxicity of aluminium is potentiated by fluoride which promotes its absorption in the gastrointestinal tract and accumulation in bone of male chickens when both fluoride and aluminium are administered together.[13] The chronic administration of aluminium fluoride and sodium fluoride in the drinking water of rats resulted in distinct morphological alterations in the brain, including effects on neurons and the cerebrovasculature.[14] Patients with toxicity from both fluoride and aluminium showed osteoporosis in cortical bone and osteosclerosis in cancellous bone since, together, fluoride and aluminium stimulated osteoclastic activity and the parathyroids resulting in bone resorption and skeletal transformation.[15]

There is paucity of data on the toxic effects of aluminium chloride and sodium fluoride administered in combination on the liver and gastrocnemius muscle of mice and reversal of toxicity by some antidotes. Hence the present study was undertaken.

MATERIALS AND METHODS

Adult male albino 5- to 6-week-old mice (*Mus musculus*) weighing between 25 and 30 g were obtained from Cadila Pharmaceutical Company, Ghodasar, Ahmedabad, India, and were housed at a temperature of 26 ± 2ºC and exposed to a 12-14 hr daylight regimen. They were maintained on standard chow, and water (0.6-1.0 ppm F^-) was given *ad libitum*. All treatments were given orally using a hypodermic syringe and a bent-tip canula. The animals of the Group IA were provided standard diet and served as untreated controls. Group IB animals were administered olive oil (dosage shown in experimental protocol table) and served as vehicle treated control, while Groups IC to IE were given ascorbic acid, calcium phosphate and vitamin E (α-tocopherol) orally and were positive controls. Animals of Group II were administered sodium fluoride (Loba Chemie, 99% purity) in 0.2 mL of water at a dose of 10 mg/kg body weight. Animals of Group III were administered aluminium chloride (SD Fine Chemicals Ltd, Boisar, 401 501, purity 99.5%) in 0.2 mL of water at a dose of 200 mg/kg body weight. The animals of Group IV received a combination of NaF + $AlCl_3$ (same dose as in Groups II

and III). Group V mice were treated as in Group IV for 30 days, and then the treatment was withdrawn for another month to study any reversibility of the induced effects. Additionally, during the 30-day withdrawal period, Group VI mice were administered ascorbic acid (AR Grade 98% purity), Group VII mice received calcium phosphate (Glaxo India, 99% purity), Group VIII mice received vitamin E (α-tocopherol) (Roche Products Ltd, Mumbai) and Group IX animals received a combination of ascorbic acid, calcium and vitamin E. The dosages of NaF and $AlCl_3$ were selected on the basis of LD_{50} values, which for fluoride in male mice is 54.4 mg/kg body weight,[16] while for $AlCl_3$ it is 4 g/kg body weight.[8] The dosages of ascorbic acid, calcium phosphate and vitamin E were based on earlier work.[2,4,5] After respective treatments, the animals were sacrificed by cervical dislocation. The serum was obtained by collecting the blood by cardiac puncture, kept at room temperature for 1 hr, and then stored in the refrigerator. The serum was separated after 24 hr by centrifugation. The liver and gastrocnemius muscle were dissected out carefully, blotted free of blood, and weighed on a Roller Smith Torsion Balance (USA) to the nearest milligram and utilised for the study.

BIOCHEMICAL STUDIES

Protein: Protein concentration in liver and gastrocnemius muscle of control and all treated Groups of mice were determined by the method of Lowry *et al*[17] and expressed as mg/100 mg fresh tissue weight.

Succinate dehydrogenase (E.C.1.3.99.1): Succinate dehydrogenase (SDH) activity was assayed in liver and gastrocnemius muscle of control and all the treated animals by the method of Beatty *et al*[18] and expressed as μg formazan formed/mg protein/15 minutes.

Glycogen: Glycogen levels were estimated in liver and muscle of control and all treated animals by the method of Seifter *et al.*[19] The concentration was expressed as μg/100 mg fresh tissue weight.

Phosphorylase (E.C.2.4.1.1): The activity of phosphorylase was assayed in liver and gastrocnemius muscle of control and all treated Groups by the method of Cori *et al*[20] and expressed as μg phosphorus released/mg protein/15 minutes.

Cholinesterase (ChE) (E.C.3.1.1.7): The activity of cholinesterase in gastrocnemius muscle and liver of control and all treated Groups was estimated by the method of Heurga *et al*[21] and expressed as ChE activity/100 mg fresh tissue weight.

Serum transaminases (serum glutamic oxaloacetic and glutamic pyruvic transaminases) (SGOT and SGPT) (E.C.2.6.1.2): Determination of serum transaminases of control and treated groups was carried out by the method of Reitman and Frankel[22] and expressed as mU/mL.

EXPERIMENTAL PROTOCOL

Group	Treatment and dose	Duration (days)	Day of autopsy	No. of animals
IA	*Control, untreated*	-	*	20
IB	*Vehicle treated* Control, olive oil (0.2 mL/animal/day)	30	31st	20
IC	*Positive control* Control, Ascorbic acid (15mg/animal/day)	30	31st	20
ID	Control + Calcium as phosphate (25 mg/animal/day)	30	31st	20
IE	Control + Vitamin E (2 mg/animal/day)	30	31st	20
II	Sodium fluoride (NaF) (10 mg/kg body weight)	30	31st	20
III	Aluminium chloride (200 mg/kg body weight)	30	31st	20
IV	Sodium fluoride + aluminium chloride (dosage as in Gr. II and III)	30	31st	20
V	NaF and $AlCl_3$ as in Gr. II and III and withdrawal for another 30 days	30 + 30	61st	20
VI	NaF + $AlCl_3$ as in Gr. IV + ascorbic acid (15 mg/animal/day) for another 30 days	30 + 30	61st	20
VII	NaF + $AlCl_3$ as in Gr. IV + calcium as phosphate (25 mg/animal/day) for another 30 days	30 + 30	61st	20
VIII	NaF + $AlCl_3$ as in Gr. IV + Vitamin E (2 mg/animal/day) for another 30 days	30 + 30	61st	20
IX	NaF + $AlCl_3$ as in Gr. IV + AA + Ca (as phosphate) + Vitamin E for another 30 days	30 + 30	61st	20

*Sacrificed with treated

RESULTS

Protein levels, activities of SDH, phosphorylase, and cholinesterase were significantly decreased ($P<0.001$) in liver and gastrocnemius muscle of the mice after 30 days of treatment with NaF, $AlCl_3$, and NaF + $AlCl_3$ (Groups II, III and IV) (Tables 1, 2, 4, and 5). The SDH activity in muscle was comparatively more affected than in liver. On the other hand, a significant ($P<0.001$) increase of glycogen occurred in liver and gastrocnemius muscle as well as in SGOT and SGPT activities after the three treatments (Tables 3 and 6).

Withdrawal of combined treatment for 30 days (Group V) resulted in insignificant recovery in protein and glycogen levels and activities of SDH and phosphorylase in both tissues (Tables 1-4), while significant recovery was obtained in activities of cholinesterase (liver, $P<0.001$; muscle $P<0.01$), SGPT and SGOT ($P<0.01$) (Tables 5 and 6). However, all the therapeutic treatments given alone (Groups VI to VIII) resulted in significant recovery ($P<0.001$) in all the parameters studied (Tables 1-6). Out of all the antidotes

used individually, ascorbic acid treatment (Group VI) was comparatively more effective than the others. In Group IX (AA + Ca + Vit. E) complete recovery occurred in all parameters and became comparable to the controls.

Table 1. Protein levels (mg/100 mg fresh tissue weight) in liver and gastrocnemius muscle of control and treated groups of mice

Group	Treatment	Liver	Muscle
IA	Control, untreated	26.44 ± 0.32	26.66 ± 0.15
IB	Control + Olive oil	26.48 ± 0.51	26.58 ± 0.26
IC	Control + Ascorbic acid	26.98 ± 0.28	26.64 ± 0.18
ID	Control + Calcium phosphate	26.79 ± 0.19	26.44 ± 0.50
IE	Control + Vitamin E	26.45 ± 0.45	26.66 ± 0.12
II	NaF	18.96 ± 0.57*	16.10 ± 0.59*
III	$AlCl_3$	16.53 ± 0.38*	11.60 ± 0.50*
IV	NaF + $AlCl_3$	17.29 ± 0.53*	11.92 ± 0.37*
V	Withdrawal of Group IV	18.29 ± 0.31†	12.54 ± 0.28†
VI	Withdrawal of Group IV + Ascorbic acid	26.36 ± 0.75*	25.72 ± 0.27*
VII	Withdrawal of Group IV + Calcium phosphate	25.93 ± 0.92*	26.03 ± 0.43*
VIII	Withdrawal of Group IV + Vitamin E	25.53 ± 0.75*	25.44 ± 0.64*
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	26.24 ± 0.62*	25.72 ± 0.26*

Values are Mean ± S.E. *P<0.001. †Nonsignificant.
Comparison between: Group I and Groups II, III, and IV.
Group IV and Groups V, VI, VII, VIII, and IX.

Table 2. Succinate dehydrogenase activity (µg formazan formed/mg protein) in liver and gastrocnemius muscle of control and treated groups of mice

Group	Treatment	Liver	Muscle
IA	Control, untreated	18.29 ± 0.35	20.23 ± 0.64
IB	Control + Olive oil	18.73 ± 0.20	21.00 ± 0.71
IC	Control + Ascorbic acid	18.64 ± 0.28	22.82 ± 0.51
ID	Control + Calcium phosphate	18.72 ± 0.26	20.54 ± 0.52
IE	Control + Vitamin E	18.40 ± 0.24	21.53 ± 0.38
II	NaF	13.59 ± 0.47*	14.33 ± 0.37*
III	$AlCl_3$	10.38 ± 0.31*	6.74 ± 0.26*
IV	NaF + $AlCl_3$	8.26 ± 0.26*	7.29 ± 0.37*
V	Withdrawal of Group IV treatment	8.84 ± 0.28†	8.23 ± 0.23†
VI	Withdrawal of Group IV + Ascorbic acid	18.90 ± 0.27*	20.78 ± 0.55*
VII	Withdrawal of Group IV + Calcium phosphate	17.64 ± 0.21*	20.04 ± 0.62*
VIII	Withdrawal of Group IV + Vitamin E	17.92 ± 0.14*	19.18 ± 0.70*
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	18.94 ± 0.2*	21.26 ± 0.55*

Values are Mean ± S.E. *P<0.001. †Nonsignificant.
Comparison between: Group I and Groups II, III, and IV.
Group IV and Groups V, VI, VII, VIII, and IX.

Table 3. Glycogen levels (mg/100 mg fresh tissue weight) in liver and gastrocnemius muscle of control and treated groups of mice

Group No.	Treatment	Liver	Muscle
IA	Control, untreated	1114.30 ± 24.38	819.27 ± 15.05
IB	Control + Olive oil	1106.77 ± 14.25	791.25 ± 11.82
IC	Control + Ascorbic acid	1135.01 ± 19.12	802.63 ± 12.73
ID	Control + Calcium phosphate	1038.52 ± 14.05	821.33 ± 31.33
IE	Control + Vitamin E	1053.10 ± 21.06	815.64 ± 18.70
II	NaF	1412.28 ± 85.42‡	1217.05 ± 68.53‡
III	$AlCl_3$	1995.74 ± 70.72‡	1438.92 ± 74.36‡
IV	NaF + $AlCl_3$	1630.16 ± 48.96‡	1628.11 ± 61.94‡
V	Withdrawal of Group IV treatment	1476.10 ± 35.33*	1570.23 ± 20.55†
VI	Withdrawal of Group IV + Ascorbic acid	1140.00 ± 23.33‡	918.84 ± 30.58‡
VII	Withdrawal of Group IV + Calcium phosphate	1164.32 ± 37.42‡	886.51 ± 18.64‡
VIII	Withdrawal of Group IV + Vitamin E	1157.90 ± 50.43‡	859.75 ± 24.22‡
IX	Withdrawal of Group IV + AA + Calcium phosphate +Vitamin E	1075.40 ± 47.51‡	903.46 ± 37.51‡

Values are Mean ± S.E. *P<0.02. †Nonsignificant. ‡P<0.001.
Comparison between: Group I and Groups II, III, and IV.
Group IV and Groups V, VI, VII, VIII, and IX.

Table 4. Phosphorylase activity (mg phosphorus released/mg protein/15 min) in liver and gastrocnemius muscle of control and treated groups of mice

Group No.	Treatment	Liver	Muscle
IA	Control, untreated	9.34 ± 0.29	9.01 ± 0.13
IB	Control + Olive oil	9.36 ± 0.18	8.85 ± 0.13
IC	Control + Ascorbic acid	9.55 ± 0.15	9.38 ± 0.21
ID	Control + Calcium phosphate	9.21 ± 0.15	9.35 ± 0.24
IE	Control + Vitamin E	9.02 ± 0.13	9.03 ± 0.24
II	NaF	6.12 ± 0.35*	3.89 ± 0.16*
III	$AlCl_3$	4.88 ± 0.28*	2.43 ± 0.12*
IV	NaF + $AlCl_3$	5.57 ± 0.22*	3.112 ± 0.12*
V	Withdrawal of Group IV treatment	6.21 ± 0.33†	3.77 ± 0.41†
VI	Withdrawal of Group IV + Ascorbic acid	7.53 ± 0.42*	8.82 ± 0.18*
VII	Withdrawal of Group IV + Calcium phosphate	8.58 ± 0.16*	8.39 ± 0.31*
VIII	Withdrawal of Group IV + Vitamin E	8.95 ± 0.24*	8.53 ± 0.30*
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	9.36 ± 0.29*	8.88 ± 0.45*

Values are Mean ± S.E. *P<0.02. †Nonsignificant.
Comparison between: Group I and Groups II, III, and IV.
Group IV and Groups V, VI, VII, VIII, and IX.

Table 5. Cholinesterase activity (ChE/mg protein) in liver and muscle of control and treated groups of mice

Group	Treatment	Liver	Muscle
IA	Control, untreated	4.71 ± 0.17	7.62 ± 0.09
IB	Control + Olive oil	4.48 ± 0.13	7.39 ± 0.13
IC	Control + Ascorbic acid	4.73 ± 0.17	7.61 ± 0.05
ID	Control + Calcium phosphate	4.91 ± 0.14	7.48 ± 0.04
IE	Control + Vitamin E	4.50 ± 0.11	7.57 ± 0.08
II	NaF	3.17 ± 0.14†	6.44 ± 0.11†
III	$AlCl_3$	2.54 ± 0.09†	6.48 ± 0.06†
IV	NaF + $AlCl_3$	1.93 ± 0.07†	5.33 ± 0.11†
V	Withdrawal of Group IV treatment	3.25 ± 0.18†	6.28 ± 0.11*
VI	Withdrawal of Group IV + Ascorbic acid	4.78 ± 0.05†	7.40 ± 0.11†
VII	Withdrawal of Group IV + Calcium phosphate	4.71 ± 0.07†	7.44 ± 0.12†
VIII	Withdrawal of Group IV + Vitamin E	4.59 ± 0.04†	7.40 ± 0.09†
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	4.96 ± 0.03†	7.65 ± 0.14†

Values are Mean ± S.E. *P<0.01. † P<0.001.
Comparison between: Group I and Groups II, III, and IV.
Group IV and Groups V, VI, VII, VIII, and IX.

Table 6. SGPT and SGOT activities (mU/mL) in serum of control and treated groups of mice

Group	Treatment	SGPT	SGOT
IA	Control, untreated	16.8 ± 1.78	27.2 ± 1.90
IB	Control + Olive oil	15.4 ± 1.88	29.2 ± 1.12
IC	Control + Ascorbic acid	16.0 ± 1.33	26.0 ± 1.14
ID	Control + Calcium phosphate	15.8 ± 0.9	22.8 ± 4.3
IE	Control + Vitamin E	15.2 ± 1.87	25.2 ± 2.5
II	NaF	27.9 ± 1.40†	38.2 ± 1.6†
III	$AlCl_3$	38.2 ± 2.23†	44.6 ± 2.05†
IV	NaF + $AlCl_3$	48.0 ± 2.38†	49.0 ± 4.8†
V	Withdrawal of Group IV treatment	35.6 ± 2.98*	35.6 ± 2.29*
VI	Withdrawal of Group IV + Ascorbic acid	20.6 ± 3.21†	24.4 ± 3.1†
VII	Withdrawal of Group IV + Calcium phosphate	22.8 ± 2.8†	28.6 ± 2.29†
VIII	Withdrawal of Group IV + Vitamin E	24.4 ± 2.18†	24.0 ± 1.74†
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	16.2 ± 1.98†	23.4 ± 3.3†

Values are Mean ± S.E. *P<0.01. † P<0.001.
Comparison between: Group I and Groups II, III, and IV.
Group IV and Groups V, VI, VII, VIII, and IX.

Table IA. Liver and muscle protein

Source of Variation	SS	Df	MS	F–Cal	F–Tab
Liver (protein)					
Between Groups	2104.497	12	175.3747	58.2064	1.835815
Within Groups	352.5187	117	3.012981		
muscle (protein)					
Between Groups	4995.586	12	416.2988	252.2905	1.835815
Within Groups	193.0591	117	1.650078		

SS–Sum of squares; df–degree of freedom; MS–Mean of squares;
F–Cal = Fisher calculated; F–Tab = Fisher tabulated.

Table 2A. Liver and muscle SDH

Source of Variation	SS	Df	MS	F–Cal	F–Tab
SDH (liver)					
Between Groups	1869.952	12	155.8293	188.3336	1.835815
Within Groups	96.80709	117	0.827411		
SDH (muscle)					
Between Groups	4213.766	12	351.1472	140.5669	1.835815
Within Groups	292.2751	117	2.498078		

SS–Sum of squares; df–degree of freedom; MS–Mean of squares;
F–Cal = Fisher calculated; F–Tab = Fisher tabulated.

Table 3A. Liver and muscle glycogen

Source of Variation	SS	Df	MS	F–Cal	F–Tab
Glycogen (liver)					
Between Groups	10183929	12	848660.7	42.29988	1.835815
Within Groups	2347366	117	20062.96		
Glycogen (muscle)					
Between Groups	11909885	12	992490.4	63.07917	1.835815
Within Groups	1840883	117	15734.04		

SS–Sum of squares; df–degree of freedom; MS–Mean of squares;
F–Cal = Fisher calculated; F–Tab = Fisher tabulated.

Table 4A. Liver and muscle phosphorylase

Source of Variation	SS	Df	MS	F–Cal	F–Tab
Phosphorylase (liver)					
Between Groups	344.9303	12	28.74419	44.83084	1.835815
Within Groups	75.01689	117	0.64117		
Phosphorylase (muscle)					
Between Groups	894.0393	12	74.50328	149.4714	1.835815
Within Groups	58.31806	117	0.498445		

SS–Sum of squares; df–degree of freedom; MS–Mean of squares;
F–Cal = Fisher calculated; F–Tab = Fisher tabulated.

Table 5A. Muscle and liver cholinesterase

Source of Variation	SS	Df	MS	F–Cal	F–Tab
Cholinesterase muscle					
Between Groups	83.29345	12	6.941121	123.8767	1.835815
Within Groups	6.5558	117	0.056032		
Cholinesterase liver					
Between Groups	122.5758	12	10.21465	59.86231	1.835815
Within Groups	19.96438	117	0.170636		

SS–Sum of squares; df–degree of freedom; MS–Mean of squares;
F–Cal = Fisher calculated; F–Tab = Fisher tabulated.

Table 6A. Serum Transaminases

Source of Variation	SS	Df	MS	F–Cal	F–Tab
SGPT					
Between Groups	6877.815	12	573.1513	19.0757	1.943619
Within Groups	1562.4	52	30.04615		
SGOT					
Between Groups	4669.815	12	389.1513	10.08164	1.943619
Within Groups	2007.2	52	38.6		

SS–Sum of squares; df–degree of freedom; MS–Mean of squares;
F–Cal = Fisher calculated; F–Tab = Fisher tabulated.

DISCUSSION

The treatments of Groups II to IV caused a significant decline of protein levels ($P<0.001$) in liver and gastrocnemius muscle of male mice, which might be due to changes in protein synthesis and/or metabolism. Earlier reports[1,7,8,23-25] on individual NaF and $AlCl_3$ treatments in rats, mice, and guinea pigs corroborate results of our present study. Aluminium accumulation is known to occur in liver of aluminium-treated rats.[26] Thus it is likely that the tissue burden of aluminium might have caused disturbances in protein metabolism.

The activity of succinic dehydrogenase, an oxidative enzyme involved in the Krebs cycle, was significantly decreased in liver and gastrocnemius muscle after treatments in Groups II – IV, corroborating earlier findings.[1,2,23,24,27] This decrease would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. Other tricarboxylic acid (TCA) cycle enzymes like isocitrate dehydrogenase and aconitase are also known to be affected by NaF treatment.[28]

The activity of cholinesterase was decreased by NaF, $AlCl_3$ and their combined treatments (Groups II - IV). Fluoride ions alone or when complexed with aluminium are known to cause an inhibitory effect on this enzyme *in vitro*[29,30] and *in vivo* in rats and guinea pig.[31] Our results corroborate these findings by others.

The elevation in serum transaminase activities by all the three treatments may be correlated with the hepatic cellular alterations and liver damage.[32] Fluoride and aluminium alone cause a similar increase in these serum transaminases.[33-34]

All the treatments in Groups II - IV for 30 days caused a significant enhancement in the levels of glycogen in liver and gastrocnemius muscle which was accompanied by a significant decrease in the activity of phosphorylase. This accumulation of glycogen probably resulted from its decreased utilization, thereby affecting functions of both muscle and liver and lead to hypoglycemia. These results corroborate earlier data on muscle, liver, vas deferens, and uterus[1,2,5,7,8,35] in rats and mice.

NaF and $AlCl_3$ treatment alone or in combination caused significant effects not only on protein and oxidative metabolisms but also on carbohydrate metabolism in liver and gastrocnemius muscle as well as cholinesterase activity in muscle, which correlated with structural alterations in these organs.[32,36]

The mechanism of action of combined NaF + $AlCl_3$ toxicity has not been established clearly. But it is known that high levels of fluoride in water leach greater amounts of aluminium from low-quality aluminium utensils.[37] In experiments with rats, F has been shown to increase Al absorption and cause dental fluorosis and renal damage by the combined toxicosis of F^- and Al.[13]

Withdrawal of treatment was not conducive for significant recovery in all the parameters. However, the toxicity was almost completely reversed in both liver and muscle after treatments with therapeutic agents (Groups VI-IX). On the whole, ascorbic acid treatment alone (Group VI) or in combination with calcium and vitamin E (Group IX) resulted in better recovery than with calcium or vitamin E alone (Groups VII and VIII). Similar findings have been reported earlier from our laboratory[5,24,27] and by Colomina *et al*[38] who administered aluminium hydroxide along with ascorbic acid to mice and did not observe any maternal or developmental toxicity, thereby indicating a beneficial effect of ascorbic acid.

Recovery due to ascorbic acid ingestion could have resulted from its powerful reducing action in several oxido-reduction reactions and as a supplementary source of energy by activating several enzymes and metabolic processes.[39] Similarly, calcium may play a role in recovery of NaF and $AlCl_3$-induced toxicity since it is also known to activate many enzymes and is recognized, along with ascorbic acid, as a potent inhibitor of phosphodiesterase (PDE).[40] Hence the levels of c-AMP would increase, which could result in growth and differentiation of cells.[40] Vitamin E is also known for its possible therapeutic role especially in oxidation-related events and is one of the most potent biological antioxidants.[5]

The present study demonstrates therapeutic effects of ascorbic acid, calcium, and vitamin E administered alone or in combination to reverse sodium fluoride and aluminium chloride toxicity. Clearly, these results have a very significant bearing on the amelioration of human suffering in individuals exposed to combined fluoride and aluminium toxicity.

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