EFFECTS OF SODIUM FLUORIDE AND ALUMINIUM CHLORIDE ON OVARY AND UTERUS OF MICE AND THEIR REVERSAL BY SOME ANTIDOTES

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SUMMARY: A study was made of the effects on ovary and uterus of administering sodium fluoride (10 mg/kg body weight) or aluminium chloride (200 mg/kg body weight) alone and in combination to female albino mice (*Mus musculus*) for 30 days. The reversibility of the induced effects by withdrawal of NaF + $AlCl_3$ treatment and by administering ascorbic acid (AA), calcium (Ca), or vitamin E alone and in combination were also investigated. All treatments (NaF, $AlCl_3$, and NaF + $AlCl_3$) resulted in a significant decline of ovarian protein and 3β- and 17β-hydroxysteroid dehydrogenase activities which could be related to increased cholesterol levels in the ovary suggesting altered steroidogenesis. The treatment also caused a hypercholesterolemic effect in serum. Accumulation of glycogen in uterus could be related to inhibition of phosphorylase activity affecting carbohydrate metabolism. The withdrawal of combined treatment for 30 days brought about an incomplete recovery. However, AA, Ca, or vitamin E supplementation alone and in combination produced an additive effect for recovery of most of the parameters almost to control levels. Hence the effects of NaF and/or $AlCl_3$ are transient and reversible.

Keywords: Aluminium chloride, Ascorbic acid antidote, Calcium antidote, Estradiol level, Female mice, Glycogen increase, Hydroxysteroid dehydrogenase, Hypercholesterolemia, Ovarian protein, Phosphorylase activity, Sodium fluoride, Toxicity reversal, Uterine glycogen, Vitamin E.

INTRODUCTION

Adverse reproductive effects of fluoride and their amelioration have been investigated earlier in rodents. These studies revealed that ingestion of NaF by female mice caused alterations in structure and metabolism (protein, carbohydrate, oxidative, energy, and nucleic acid) in some of their organs.[1-4] These changes were mainly caused by increased tissue burden of fluoride which resulted in increased lipid peroxidation, formation of oxygen free radicals and decrease in superoxide dismutase activity in the ovary. Occupational exposure to organic fluoride has been reported to induce abnormal menstruation, increase in the frequency of miscarriages and pregnancy complications among female workers in fluorine factories.[5]

Aluminium is found in relatively high concentrations in the earth's crust, in some drinking water, in several pharmacological preparations, and in many processed foods. Recently, increased attention is being paid to aluminium due to its serious effects on the central nervous system, energy metabolism and haematology.[6,7] Chinoy and Bhattacharya[8,9] reported that a single dose of aluminium chloride (400 mg/kg body weight) for 15 days or

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chronic treatment (200 mg/kg body weight) for 60 days administered to male mice caused alterations in the metabolism of testis, epididymis, and vas deferens that led to poor sperm motility and reduction in fertility rate.

Dai *et al*[10] found that fluoride promoted the absorption of aluminium in the gastrointestinal tract of male chickens and accumulated in bone when the animals were administered aluminium and fluoride in combination. On the other hand, aluminium is known to protect against fluoride toxicosis in hens by forming a stable complex between Al^{3+} and F^-, thereby increasing fecal fluoride excretion.[11]

There are contradictory reports regarding the combined treatment of fluoride and aluminium in animals and humans. The present study was therefore undertaken to investigate the effects of oral feeding of sodium fluoride or aluminium chloride alone or in combination on the ovary and uterus of mice. The reversal of induced effects by the use of some antidotes was also studied.

MATERIALS AND METHODS

Healthy Swiss strain, 5- to 6-week-old adult female albino mice (*Mus musculus*), weighing between 30 and 35 g were obtained from the National Institute of Occupational Health (NIOH), Ahmedabad, India. They were housed (5 mice per cage) in an air-conditioned animal housing unit at a temperature of 26 ± 2°C and exposed to 10-12 hr of light/day. They were maintained on standard chow, and water (0.6-1.0 ppm F^-) was given *ad libitum*.

The animals were divided into nine groups as shown in the following protocol table. All treatments were given orally with a hypodermic syringe with an attached angular needle. Groups IA to E of animals served as controls (untreated, vehicle treated, and positive controls).

To Group II animals, sodium fluoride (NaF) (Loba Chemie, Mumbai, 99% purity) was administered at a dose of 10 mg/kg body weight (in 0.2 mL of water) for 30 days.

To Group III animals, aluminium chloride ($AlCl_3$) (SD Fine Chemicals Ltd Boisor 401501, 99.5% purity) was administered at a dose of 200 mg/kg body weight (in 0.2 mL of water) for 30 days. The above doses were established on the basis of LD_{50} values, *viz*, 51.6 mg/kg body weight for F^- in female mice[12] and 4 g/kg body weight for $AlCl_3$,[9] respectively.

The Group IV animals were orally given NaF and $AlCl_3$ in combination (doses as above) for 30 days. To study the reversibility of the effects induced by the combined doses of NaF and $AlCl_3$, treatment was withdrawn for 30 days in another group of mice while they were maintained on standard food and water *ad libitum*. These were Group V animals.

Animals of Group VI, VII, VIII and IX were administered ascorbic acid (AA) (15 mg/0.2 mL of distilled water/animal/day) (Loba Chemie, Mumbai, 99% purity), calcium as a suspension of calcium phosphate (25 mg/0.2 mL

of distilled water/animal/day) (Glaxo, India, 99% purity) or vitamin E (2 mg/0.2 mL olive oil/animal/day) (tocopherol acetate, E Merck, India Ltd. Mumbai, 99% purity) alone and in combination (Group IX) during the 30-day withdrawal period. The doses of ascorbic acid, calcium or vitamin E were based on earlier work.[13,14,15]

At the end of each treatment, the animals were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation. For serum, the blood was collected by cardiac puncture and kept at room temperature for 1 hr. It was stored in the refrigerator and serum was separated after 24 hr by centrifugation. The uteri and ovaries were dissected out carefully, blotted free of blood and weighed on a Roller Smith Torsion Balance (USA) to the nearest milligram and utilized for the study.

EXPERIMENTAL PROTOCOL

Group	Treatment and dose	Duration (days)	Day of autopsy	No. of animals
IA	*Control, untreated*			
	Control + Distilled water	-	*	20
	Vehicle Treated			
IB	Control + Olive oil (0.2 mL/animal/day)	30	31st	20
	Positive Control			
IC	Control + ascorbic acid (AA) (15 mg/animal/day)	30	31st	20
ID	Control + Calcium phosphate (25 mg/animal/day)	30	31st	20
IE	Control + vitamin E (2 mg/animal/day)	30	31st	20
II	NaF-treated (10 mg/kg body weight/mice/day)	30	31st	20
III	$AlCl_3$-treated (200 mg/kg body weight/mice/day)	30	31st	20
IV	NaF + $AlCl_3$ (doses as in Group II and III)	30	31st	20
V	NaF + $AlCl_3$ as in Group IV, on 31 day withdrawal for 30 days	30 + 30	61st	20
VI	Treatment withdrawal of Group IV + AA (dose as in Group IC)	30 + 30	61st	20
VII	Treatment withdrawal of Group IV + calcium phosphate (dose as in Group ID)	30 + 30	61st	20
VIII	Treatment withdrawal of Group IV treatment + Vitamin E (dose as in Group IE)	30 + 30	61st	20
IX	Treatment withdrawal of Group IV + AA + calcium phosphate + Vit. E (doses as in Group IC, ID, IE)	30 + 30	61st	20

*Sacrificed with treated

BIOCHEMICAL STUDY

Protein levels of ovary and uterus: Levels for control and all treated animals were estimated by the method of Lowry *et al*[16] and expressed as mg/100 mg fresh tissue weight.

3β- and 17β-Hydroxy steroid dehydrogenases (3β- and 17β-HSD) (E.C. 1.1. 1.51): Ovarian 3β- and 17β-HSD activities in control and all treated groups were assayed by the method of Talalay[17] and expressed as nanomoles of androstenedione formed/mg protein/min.

Cholesterol in ovary and serum of control and treated groups of mice were estimated by the method of Zlatkis *et al*[18] and expressed as mg/100 mg fresh tissue weight and mg/100 mL serum.

Glycogen concentration in uterus of control and treated groups of mice was estimated by the method of Seifter *et al*[19] and expressed as μg/100 mg fresh tissue weight.

Phosphorylase (E.C.2.4.1.1) activity in uterus of control and all treated groups was assayed by the method of Cori *et al*[20] and the enzyme activity was expressed as μg phosphorus released/mg protein/15 min.

Endocrine investigations The level of Estradiol (E_2) was assayed from the serum using the radioimmunoassay technique of Carlström *et al.*[21]

For all biochemical estimations, a minimum of eight replicates were done for each parameter. The data were analysed statistically using Student's 't' test and Analysis of Variance (ANOVA).

RESULTS

Protein levels in ovary and uterus and estradiol in serum, as well as activities of 3β- and 17β-HSD in ovary and phosphorylase in uterus, decreased significantly ($P<0.001$) after the treatment with NaF, $AlCl_3$ and their combination (Groups II-IV) for 30 days (Tables 1, 2, 4, 5). The levels of glycogen in uterus and cholesterol in ovary and serum were found to increase significantly ($P<0.001$) following the same treatments of Groups II-IV (Tables 3, 4, and 5).

Withdrawal of the combined treatment (Group IV) for 30 days (Group V) showed nonsignificant recovery (as in 3β-HSD) or significant recovery in different parameters (Tables 1-5) compared with the combined treatment.

The values of all the parameters were comparable to control values, and recovered significantly ($P<0.001$), after administration of therapeutic agents alone and in combination (Group VI-IX) during the withdrawal period. The combined treatment of ascorbic acid, vitamin E and calcium phosphate (Group IX) was more effective in promoting recovery than the individual treatments with these antidotes (Groups VI-VIII).

Table 1. Protein levels (mg/100mg tissue weight) in ovary and uterus of control and treated mice (Groups IA-IX)

Group No.	Treatment	Ovarian protein	Uterine protein
IA	Control + Distilled water	12.05 ± 0.09	18.59 ± 0.10
IB	Control + Olive oil	12.03 ± 0.05	18.26 ± 0.20
IC	Control + Ascorbic acid	12.09 ± 0.13	18.29 ± 0.16
ID	Control + Calcium phosphate	12.11 ± 0.09	18.22 ± 0.12
IE	Control + Vitamin E	12.11 ± 0.15	18.18 ± 0.09
II	NaF	10.23 ± 0.12†	14.31 ± 0.16†
III	$AlCl_3$	8.32 ± 0.06†	11.02 ± 0.10†
IV	NaF+$AlCl_3$	7.85 ± 0.09†	9.28 ± 0.13†
V	Withdrawal of Group IV treatment	9.62 ± 0.09*	12.87 ± 0.13†
VI	Withdrawal of Group IV + Ascorbic acid	11.92 ± 0.09†	18.18 ± 0.15†
VII	Withdrawal of Group IV + Calcium phosphate	12.04 ± 0.06†	18.39 ± 0.07†
VIII	Withdrawal of Group IV + Vitamin E	12.04 ± 0.03†	18.32 ± 0.07†
IX	Withdrawal of Group IV + AA + calcium phosphate +Vitamin E	12.11 ± 0.05†	18.18 + 0.07†

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

Table 2. 3β- and 17β-HSD activity (nanomoles of androstenedione formed/mg protein/min) in ovary of control and treated mice (Groups IA-IX)

Group No.	Treatment	3β-HSD	17β-HSD
IA	Control + Distilled water	0.91 ± 0.03	0.37 ± 0.006
IB	Control + Olive oil	0.90 ± 0.01	0.35 ± 0.006
IC	Control + Ascorbic acid	0.87 ± 0.02	0.34 ± 0.009
ID	Control + Calcium phosphate	0.84 ± 0.01	0.33 ± 0.009
IE	Control + Vitamin E	0.92 ± 0.01	0.34 ± 0.02
II	NaF	0.63 ± 0.01*	0.20 ± 0.006*
III	$AlCl_3$	0.40 ± 0.02*	0.17 ± 0.003*
IV	NaF+$AlCl_3$	0.20 ± 0.01*	0.09 ± 0.003*
V	Withdrawal of Group IV treatment	0.31 ± 0.02†	0.20 ± 0.006*
VI	Withdrawal of Group IV + Ascorbic acid	0.78 ± 0.01*	0.33 ± 0.006*
VII	Withdrawal of Group IV + Calcium phosphate	0.77 ± 0.01*	0.32 ± 0.006*
VIII	Withdrawal of Group IV + Vitamin E	0.79 ± 0.01*	0.34 ± 0.006*
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	0.91 ± 0.02*	0.36 ± 0.01*

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

Table 3. Cholesterol levels in ovary (mg/100mg fresh tissue weight) and serum (mg/100 mL of serum) of control and treated mice (Groups IA-IX)

Group No.	Treatment	Ovarian cholesterol	Serum cholesterol
IA	Control + Distilled water	1.79 ± 0.03	1.20 ± 0.03
IB	Control + Olive oil	1.73 ± 0.03	1.18 ± 0.02
IC	Control + Ascorbic acid	1.73 ± 0.05	1.17 ± 0.03
ID	Control + Calcium phosphate	1.75 ± 0.06	1.19 ± 0.01
IE	Control + Vitamin E	1.74 ± 0.07	1.19 ± 0.02
II	NaF	2.67 ± 0.08†	1.79 ± 0.02†
III	$AlCl_3$	3.54 ± .08†	2.00 ± 0.02†
IV	$NaF+AlCl_3$	4.12 ± 0.03†	2.26 ± 0.02†
V	Withdrawal of Group IV treatment	2.90 ± 0.05†	2.11 ± 0.02*
VI	Withdrawal of Group IV + Ascorbic acid	1.91 ± 0.02†	1.20 ± 0.01†
VII	Withdrawal of Group IV + Calcium phosphate	1.91 ± 0.03†	1.17 ± 0.02†
VIII	Withdrawal of Group IV + Vitamin E	1.86 ± 0.03†	1.17 ± 0.02†
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	1.80 ± 0.02†	1.19 ± 0.02†

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

Table 4. Glycogen concentration (µg/100mg fresh tissue weight) and phosphorylase activity (µg phosphorus released/mg protein/15 min) in uterus of control and treated mice (Groups IA-IX)

Group No.	Treatment	Glycogen	Phosphorylase
IA	Control + Distilled water	169.86 ± 4.91	4.22 ± 0.10
IB	Control + Olive oil	171.44 ± 3.53	4.06 ± 0.10
IC	Control + Ascorbic acid	169.77 ± 3.35	4.07 ± 0.12
ID	Control + Calcium phosphate	169.41 ± 3.16	4.11 ± 0.12
IE	Control + Vitamin E	168.67 ± 2.75	4.02 ± 0.08
II	NaF	229.52 ± 2.22†	2.97 ± 0.11†
III	$AlCl_3$	277.85 ± 2.40†	1.80 ± 0.04†
IV	$NaF+AlCl_3$	299.85 ± 2.40†	1.17 ± 0.03†
V	Withdrawal of Group IV treatment	216.22 ± 2.76†	1.64 ± 0.03*
VI	Withdrawal of Group IV + Ascorbic acid	174.65 ± 0.89†	3.84 ± 0.07†
VII	Withdrawal of Group IV + Calcium phosphate	177.58 ± 0.89†	3.78 ± 0.09†
VIII	Withdrawal of Group IV + Vitamin E	175.38 ± 1.85†	3.88 ± 0.11†
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	169.91 ± 1.92†	4.22 ± 0.14†

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

Table 5. Estradiol level (pg/mL) in serum of control and treated female mice (Groups IA-IX)

Group No.	Treatment	Estradiol
IA	Control + Distilled water	17.14 ± 0.17
IB	Control + Olive oil	17.20 ± 0.06
IC	Control + Ascorbic acid	17.05 ± 0.05
ID	Control + Calcium phosphate	17.04 ± 0.19
IE	Control + Vitamin E	17.39 ± 0.08
II	NaF	12.88 ± 0.07†
III	$AlCl_3$	11.25 ± 0.09†
IV	NaF+$AlCl_3$	9.42 ± 0.13†
V	Withdrawal of Group IV treatment	10.93 ± 0.10*
VI	Withdrawal of Group IV + Ascorbic acid	17.05 ± 0.10†
VII	Withdrawal of Group IV + Calcium phosphate	16.94 ± 0.06†
VIII	Withdrawal of Group IV + Vitamin E	17.08 ± 0.11†
IX	Withdrawal of Group IV + AA + Calcium phosphate + Vitamin E	17.22 ± 0.09†

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

ANOVA OF VARIOUS PARAMETERS

Table 1A. Protein

Source of Variation	SS	df	MS	F–CAL	F–TAB
Ovary					
Between Groups	294.52914	12	24.544095	300.38558	1.8358151
Within Group	9.55991	117	0.0817086		
Uterus					
Between Groups	1270.4604	12	105.8717	1108.9258	1.8358151
Within Groups	11.17026	117	0.0954723		

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

Table 2A. 3β and 17β-HSD (Ovary)

Source of Variation	SS	df	MS	F–CAL	F–TAB
3β-HSD					
Between Groups	7.5447477	12	0.628729	265.71771	1.8358151
Within Groups	0.27684	117	0.0023662		
17β-HSD					
Between Groups	0.9209031	12	0.0767419	93.344474	1.8358151
Within Groups	0.09619	117	0.0008221		

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

Table 3A. Cholesterol

Source of Variation	SS	df	MS	F–CAL	F–TAB
Ovary					
Between Groups	78.075852	12	6.506321	122.5726	1.8358151
Within Groups	6.21052	117	0.0530814		
Serum					
Between Groups	15.224156	12	1.2686797	480.70722	1.8784831
Within Groups	0.2058571	78	0.0026392		

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

Table 4A. Glycogen and phosphorylase (uterus)

Source of Variation	SS	df	MS	F–CAL	F–TAB
Glycogen					
Between Groups	242380.63	12	20198.386	288.79436	1.8358151
Within Groups	8183.0236	117	69.940373		
Phosphorylase					
Between Groups	145.06424	12	12.088687	145.37019	1.8358151
Within Groups	9.72948	117	0.0831579		

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

Table 5A. Estradiol (serum)

Source of Variation	SS	df	MS	F–CAL	F–TAB
Between Groups	848.04777	12	70.670648	917.21648	1.8601227
Within Groups	7.0114625	91	0.077049		

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

DISCUSSION

As found in our earlier studies,[3,9] the present investigation demonstrated that protein levels declined in various reproductive tissues of rodents treated with either NaF or $AlCl_3$. This decline with NaF might be related to impairment of protein synthesis by fluoride ions.[22] Similarly, the decrease in uter-

ine and ovarian protein levels from treatment with $AlCl_3$ might be due to altered enzyme activities and reduced secretions, especially in the uterus.

Fluoride or aluminium treatment alone or in combination revealed a decline in the activities of both 3β- and 17β-HSDs in ovary suggesting a block in the steroidogenic pathway. This finding is supported by the significant accumulation of cholesterol in the ovaries of treated mice indicating that its metabolism might be altered. Some earlier studies in male rats and mice[9,23] after sodium fluoride or aluminium chloride treatments corroborate the above findings. The serum cholesterol also showed a significant increase after treatment which indicates a probable hypercholesterolemic effect.

Aluminium as well as fluoride affects carbohydrate metabolism.[24,25] Aluminium salts when ingested at toxic levels cause profound disorders in phosphate metabolism, while fluoride is known to act as an inhibitor of glycolysis[26] by decreasing the activity of isocitrate dehydrogenase.[27] The present study revealed an accumulation of glycogen in the uterus on administration of NaF, $AlCl_3$ or NaF + $AlCl_3$, which could be correlated with the decline in the activity of phosphorylase in the uterus as also reported earlier for other tissues in male and female mice.[1,2,9]

The principal action of estradiol is the manifestation of oestrus, changes in the reproductive tract and mammary glands, and regulation of the secretion of gonadotropins. In the present study, a decline in estradiol levels resulted from all the three treatments (Groups II-IV). Tokar and Savchenko[27] also observed low testosterone levels in individuals afflicted with fluorosis.

Upon withdrawal of the combined treatment of sodium fluoride and aluminium chloride, a significant recovery was obtained in all the parameters studied in the ovary, uterus and serum. The recovery was, however, more significant when antidote treatments were given in the withdrawal period (Group V), especially treatment in Group IX.

Experimental studies have shown that the dietary factors such as calcium, amino-acids and vitamins C, E, and D can mitigate the toxic effects of individual treatments of aluminium and fluoride.[9,14,15,28] In the present study, treatment with ascorbic acid, calcium, or vitamin E, alone and in combination, brought about complete recovery almost to control levels in all the parameters studied. The beneficial effects of ascorbic acid might be due to its antioxidant and detoxification properties in suppressing fluoride and aluminium toxicity. It is known that the activity of 3β-HSD is altered in the ascorbic acid deficient rats as compared to the controls.[29] Moreover, ascorbic acid is known to activate adenyl cyclase and inhibit phosphodiesterase (PDE), resulting in higher c-AMP levels[30] which are needed for tissue metabolism. The increased levels of c-AMP might have resulted in the recovery in the activities of 3β- and 17β-HSDs and phosphorylase, since c-AMP is known to activate several kinases.

Calcium likewise activates several enzymes. Earlier workers have demonstrated that calcium ingestion by fluoride intoxicated male and female mice and rats brought about a significant regain in the NaF-inhibited enzyme activities.[3,13,14] It is known that Ca and c-AMP interact with each other for various metabolic reactions in different tissues.[30,31] Thus, additional calcium supplementation might have helped in recovery of the enzymes involved in carbohydrate metabolism and steroidogenesis. Ca, like AA, also inactivates PDE and hence would help in recovery of enzymes by an increase in the levels of c-AMP.

Various adverse health affects of vitamin E deficiency in vertebrates are well documented, including disorders of the reproductive organs.[32] Chinoy and Sharma[15] reported that ingestion of vitamin E by fluorotic male mice brought about a significant recovery in NaF-induced reproductive failure. In the present study also, vitamin E exerted a significant protective effect of all the parameters studied, bringing them almost to control values. Vitamin E is recognized to be a potent biological anti-oxidant, and for its ability to prevent cell injury by maintaining sulphydryl groups of membrane-binding proteins.[33] It has also been related to changes in calcium homeostasis in the cardiac tissue.[34] Hence vitamin E was found to be very effective in the complete recovery of all parameters in the present study.

From the foregoing data, it is evident that the administration of sodium fluoride or aluminium chloride alone induced toxicity in female mice. This toxicity was enhanced by their combined treatment (Group IV) in affecting steroidogenesis in ovary, carbohydrate metabolism in uterus, and causing a hypercholesterolemic effect in mice. These effects, however, were transient and reversible upon withdrawal of the treatments. On the other hand, AA, Ca, or vitamin E supplementation, each given alone or in combination, manifested significant therapeutic effects.

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