

## IMPACT OF COEXPOSURE TO ALUMINUM AND ETHANOL ON PHOSPHOESTERASES AND TRANSAMINASES OF RAT CEREBRUM

### UTICAJ DVOSTRUKE IZLOŽENOSTI ALUMINIJUMU I ETANOLU NA FOSFOESTERAZE I TRANSAMINAZE U MOZGU PACOVA

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**Summary:** Ubiquitous presence along with uncontrolled use of aluminum and increasing trends of ethanol consumption in India increased the chance of coexposure to aluminum and ethanol. Possibilities are there, that both of them follow common mechanisms to produce neurotoxicity. The phosphomonoesterases and glutamate transaminases are studied in rat brain cerebrum after combined exposure to aluminum and varied doses of ethanol for 4 weeks. Dose dependent decreases in growth have been observed. The impact of aluminum on cerebral acidic and alkaline phosphomonoesterases activities were shown to be altered in a dose dependent fashion by the coexposure to ethanol. Aspartate aminotransferase and alanine aminotransferase of the cerebrum were responding differentially to aluminum exposure in the presence of different doses of ethanol exposure. It has been suggested that the ethanol-induced augmentation of impacts of aluminum on the cerebrum is dose dependent and there might be a critical level of ethanol exposure for the observed effect on cerebrum.

**Keywords:** aluminum, ethanol, cerebrum, phosphomonoesterase, transaminase

### Introduction

Multifaceted health problems have been documented to originate from aluminum exposure, the brain and bone being the major target organs (1). Although the role of aluminum in the neuropatho-

**Kratak sadržaj:** Sveprisutnost aluminijuma uz njegovu nekontrolisanu upotrebu kao i trend sve veće konzumacije etanola u Indiji povećali su mogućnost dvostruke izloženosti aluminijumu i etanolu. Moguće je da oba prate zajedničke mehanizme u proizvodnji neurotoksičnosti. Fosfomonoesteraze i glutamat-transaminaze ispitivane su u velikom mozgu pacova posle kombinovane izloženosti aluminijumu i različitim dozama etanola tokom 4 nedelje. Uočena su smanjenja rasta zavisna od doze. Pokazano je da se uticaj aluminijuma na aktivnosti moždane kisele i alkalne fosfomonoesteraze menja u zavisnosti od doze usled dvostruke izloženosti etanolu. Aspartat-aminotransferaza i alanin-aminotransferaza u velikom mozgu davale su različit odgovor na izloženost aluminijumu u prisustvu različitih doza izloženosti etanolu. Pokazano je da pojačanje uticaja aluminijuma na mozak izazvano etanolom zavisi od doze i da za opaženi efekat na mozak može postojati kritičan nivo izloženosti etanolu.

**Ključne reči:** aluminijum, etanol, veliki mozak, fosfomonoesteraza, transaminaza

logical disorders is a subject of long-standing and controversial debate among the researchers (2), the association between them cannot be undermined. Recently, Bihari et al. (3) have suggested that almost all the metabolic pathways are vulnerable to be altered by aluminum exposure because of (a) the capability of aluminum to displace calcium and magnesium from their binding sites and (b) the ability of a variety of biomolecules to bind aluminum. On the other hand, the complex and poorly understood biochemistry of aluminum (4) poses additional impediment to the prognosis of aluminum exposure.

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Because of its omnipresence, exposure to aluminum is unavoidable. Elimination of all but traces of the absorbed aluminum, in addition to restricted bioavailability, made it a poorly assimilated metal in the body. However, it has been documented that its presence in the body might amount to a significant level over several years of exposure. The amount of body aluminum burden is decided by the level (including duration) of aluminum exposure itself, coexposure beverages, and functional status of the kidney. Aluminum is associated with neurotoxicity, and has been evidenced to produce biochemical, cytological and functional impairment of neuronal and glial cells (1, 2). The brain is generally protected from aluminum insult because of the blood-brain barrier. However, a compromise in the blood-brain barrier function or higher gastrointestinal absorption or decreased urinary excretion may enhance the chance of neurotoxicity, even with lower levels of aluminum exposure. All these risk enhancement factors could be possible in the case of alcoholism.

Nearly ten years back, a WHO report suggested that India is likely to face a heavy burden of medical and social problems due to increased alcohol consumption (5) and the alcohol itself is a neurotoxicant. Consumption of alcohol among Indian men ranges from 16.7 % to as high as 58.3 %, varying by the degree of urbanization as well as by region. Using an average of 60 per cent male abstinence and almost total female abstinence, per capita consumption of adult drinkers in India is approximately nine litres of absolute alcohol (6).

Improper control over use of aluminum as additives and medicine, excessive use of aluminum-made cooking utensils in the low-income group and lack of awareness keep a high proportion of the general population in India vulnerable to aluminum insult. Regarding the occupational exposure, recently, Dhatrak and Nandi (7) estimated that more than 90% of the miners working in metallic mines in India are at the risk of aluminum intoxication. Considering the licit as well as illicit alcohol use in India, a significant portion of the Indian population is under the threat of aluminum and alcohol coexposure. On the other hand, aluminum has already been identified as a probable etiological contributor to alcoholic amnesia and dementia (8). However, Krewski et al. (9) reviewed that only two studies are available regarding aluminum and ethanol coexposure. They also mentioned that although these studies suggest that ethanol can affect the toxicokinetics of aluminum, the mechanism is not known (9).

In the present study, certain biochemical parameters of the rat brain cerebrum have been checked to find out the impact of aluminum along with different doses of ethanol exposure.

## Methods

### Subjects

Male albino rats of the Wistar strain weighing 100–120 g were used for the present study. The animals were maintained on a semisynthetic diet as reported elsewhere (10) and water *ad libitum*. Rats were housed in polypropylene cages with bottles having a stainless steel nozzle and kept in a room with regulated temperature ( $22 \pm 2$  °C) and 12 hour light – dark cycle. After one week of acclimatization rats were divided into groups as shown in Table 1.

### Procedure

The treatment was continued for four weeks. Both ethanol and aluminum treatment were carried out through orogastric intubation. Ethanol (0.8–2.0 g/kg body weight) was given at 9 AM while aluminum (10 mg/kg body weight) was given at 5 PM daily.

After the period of treatment, overnight fasted rats were sacrificed by cervical dislocation. The whole brain was removed, washed with ice-cold saline, blotted dry and immediately transferred to the ice chamber. The homogenized cerebrums were used for estimation of protein, phosphomonoesterases (11) and glutamate transaminases (12).

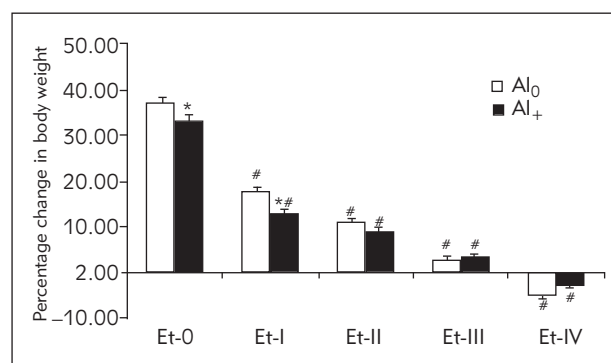
The collected data were statistically analyzed by two-way ANOVA with replication and the differences between the means were calculated by Tukey's honestly significant difference (HSD) test. The HSDs were calculated accepting  $q_{0.05,10,50} = 4.69$  (extrapolated from the table) and source of variations (within mean square) as obtained from the ANOVA table.

## Results

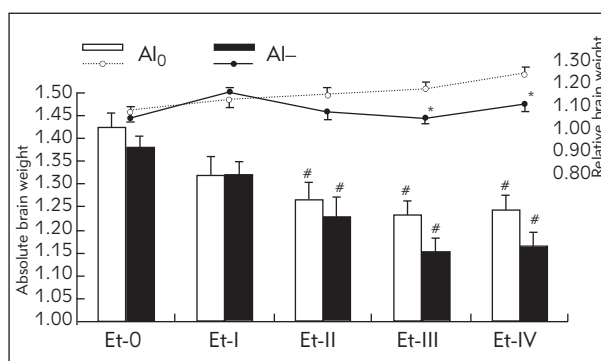
Changes in the body weight of animals during the period of treatment are presented in Figure 1 as percentage change of the body weight of the respective animals at the onset of the experiment. Figure 1 indicates that the final body weight changes at the end of 4 weeks were dependent on the doses of ethanol exposure. Both aluminum-exposed ( $Al_+$ ) and aluminum-unexposed ( $Al_0$ ) animals showed significant negative correlation with the dose of ethanol exposure. Two way ANOVA with replication showed that both aluminum ( $F_{1,50} = 7.91, P < 0.01$ ) and ethanol ( $F_{4,50} = 617.5, P < 0.001$ ) exposures have significant impact on the changes in body weight. The interaction between the aluminum exposure and ethanol exposure was also found to be significant ( $F_{4,50} = 6.60, P < 0.001$ ). However, when the body weight changes in  $Al_+$  animals were compared with those of their counterpart in each ethanol regimen, only the groups without any ethanol exposure (Et-0) and the group with the least level of ethanol exposure (Et-I) showed significant difference between the  $Al_+$  and  $Al_0$  animals' body weight gain.

**Table 1** Different ethanol groups and their aluminum subgroups (containing 6 animals each) along with their regimen of aluminum and ethanol exposure.

Ethanol Groups	Aluminum Subgroups	Rats exposed to		
		Dist. water, mL	Aluminum, mL	Ethanol, mL
Et-0	Without aluminum (Al <sub>0</sub> )	0.2	–	–
	With aluminum (Al <sub>+</sub> )	–	0.2	–
Et-I (0.8 g/Kg bw)	Without aluminum (Al <sub>0</sub> )	0.2	–	0.2
	With aluminum (Al <sub>+</sub> )	–	0.2	0.2
Et-II (1.2 g/Kg bw)	Without aluminum (Al <sub>0</sub> )	0.2	–	0.2
	With aluminum (Al <sub>+</sub> )	–	0.2	0.2
Et-III (1.6 g/Kg bw)	Without aluminum (Al <sub>0</sub> )	0.2	–	0.2
	With aluminum (Al <sub>+</sub> )	–	0.2	0.2
Et-IV (2.0 g/Kg bw)	Without aluminum (Al <sub>0</sub> )	0.2	–	0.2
	With aluminum (Al <sub>+</sub> )	–	0.2	0.2



**Figure 1** Comparison of changes in body weights between aluminum-exposed and aluminum-nonexposed animals at different levels of ethanol exposure. Each bar represents the mean of six observations ± SEM. \* indicates significant change when compared with respective without aluminum (Al<sub>0</sub>) group. # indicates significant change when compared to respective animals of Et-0 group.



**Figure 2** Comparison of absolute (g) and relative (g/100g body weight) brain weights between aluminum-exposed and aluminum-nonexposed animals at different levels of ethanol exposure. Each bar and each dot represents the mean of six observations ± SEM. \* indicates significant change when compared with respective without aluminum (Al<sub>0</sub>) group. # indicates significant change when compared to respective animals of Et-0 group.

Absolute and relative brain weights of Al<sub>0</sub> and Al<sub>+</sub> animals of all the ethanol exposure groups were depicted in Figure 2. The absolute brain weights of both Al<sub>0</sub> and Al<sub>+</sub> animals were found to be decreased in a dose-dependent fashion, like the changes in body weight (Figure 1). The significant negative correlation between the absolute brain weights and doses of ethanol is evidenced by high *r* values (–0.95 for Al<sub>+</sub> and –0.96 for Al<sub>0</sub>). The absolute brain weights of Al<sub>+</sub> animals were found to be lesser in comparison to those of Al<sub>0</sub> animals for all the doses of ethanol exposure, however, none of the differences were found to be statistically significant. Nevertheless, in the ANOVA, the influence of either aluminum ( $F_{1,50} = 5.37, P < 0.05$ ) or ethanol ( $F_{4,50} = 15.01, P < 0.001$ ) was found to be significant, while the

interaction between them was found to be insignificant. On the contrary, the relative brain weights of both Al<sub>0</sub> and Al<sub>+</sub> animals were found to be comparable for all the doses of ethanol exposure (Figure 2), though the ANOVA test showed significant influences of aluminum ( $F_{1,50} = 14.93, P < 0.001$ ), ethanol ( $F_{4,50} = 4.89, P < 0.01$ ) as well as the interaction between them ( $F_{4,50} = 3.01, P < 0.05$ ). The relative brain weights of Al<sub>+</sub> animals of higher doses of ethanol exposure groups (Et-III and Et-IV) showed significantly lower values in comparison to those of Al<sub>0</sub> of the same groups (Figure 2).

The phosphomonoesterases activities of the cerebrum of Al<sub>0</sub> and Al<sub>+</sub> animals for all the ethanol doses are tabulated in the form of Table II. Changes in cerebrum acidic phosphomonoesterase (ACP) activities

**Table II** Changes in phosphomonoesterases activities (units/100 mg wet weight of brain) of cerebrum upon exposure to graded doses of ethanol in presence or absence of aluminum coexposure.

Groups of animals	Acidic phosphomonoesterases		Alkaline phosphomonoesterases	
	Al <sub>0</sub>	Al <sub>+</sub>	Al <sub>0</sub>	Al <sub>+</sub>
ET-0	85.42 ± 3.75	97.46 ± 2.14	61.34 ± 4.06	59.73 ± 3.41
Et-I	89.56 ± 4.13	102.01 ± 5.66	55.42 ± 2.21	48.02 ± 2.86 <sup>#</sup>
Et-II	88.10 ± 6.50	103.07 ± 4.89	54.64 ± 4.10	43.36 ± 3.48 <sup>**#</sup>
Et-III	97.27 ± 2.63	108.04 ± 6.79	48.25 ± 2.54 <sup>#</sup>	42.39 ± 2.19 <sup>#</sup>
Et-IV	110.28 ± 7.52 <sup>#</sup>	106.35 ± 5.86	40.34 ± 3.24 <sup>#</sup>	42.17 ± 4.71 <sup>#</sup>

Values are presented as mean ± standard error of mean. \* indicates significant change when compared with respective without aluminum (Al<sub>0</sub>) group. <sup>#</sup> indicates significant change when compared to ET-0 group.

**Table III** Changes in aminotransferase activities (mg or pyruvate produced/hr/mg of protein) of cerebrum upon exposure to graded doses of ethanol in presence or absence of aluminum coexposure.

Groups of animals	Aspartate aminotransferase		Alanine aminotransferase	
	Al <sub>0</sub>	Al <sub>+</sub>	Al <sub>0</sub>	Al <sub>+</sub>
ET-0	8.78 ± 0.49	8.33 ± 0.25	4.24 ± 0.18	5.37 ± 0.41*
Et-I	10.31 ± 0.96	7.22 ± 0.41*	4.93 ± 0.22	5.07 ± 0.51
Et-II	10.28 ± 0.53	7.79 ± 0.72*	4.73 ± 0.24	5.66 ± 0.23
Et-III	9.97 ± 0.61	7.31 ± 0.28*	5.02 ± 0.18	4.45 ± 0.15
Et-IV	8.33 ± 0.45	7.70 ± 0.73	4.79 ± 0.36	4.58 ± 0.21

Values are presented as mean ± standard error of mean. \* indicates significant changes when compared with respective without aluminum (Al<sub>0</sub>) group.

were found to be increased gradually with the increase in ethanol doses in either of the groups ( $r$  for Al<sub>0</sub> was 0.86 and for Al<sub>+</sub> was 0.94). The influences of aluminum ( $F_{1,50} = 7.60$ ,  $P < 0.01$ ) and ethanol ( $F_{4,50} = 31.92$ ,  $P < 0.001$ ) exposures on the cerebrum ACP activities were also found to be significant, while the interaction between the aluminum and ethanol was not found to be significant. On the other hand, the cerebrum ACP activities of Al<sub>+</sub> animals were found to be higher in comparison to those of Al<sub>0</sub> animals in all the ethanol groups, except for the highest dose of ethanol; however, the differences were not statistically significant in either of the groups (Table II). Contrary to these, the alkaline phosphomonoesterase (ALP) of the cerebrum was found to be higher in the Al<sub>+</sub> animals in comparison to that of Al<sub>0</sub> animals in all the ethanol groups, except Et-IV groups (Table II). However, the difference between the activities of the cerebrum ALP of Al<sub>0</sub> and Al<sub>+</sub> was statistically significant only in the Et-III group of ethanol exposure. Like those of cerebrum ACP activities, the changes in cerebrum ALP activities also followed the changes of ethanol doses ( $r$  for Al<sub>0</sub> was -0.95 and  $r$  for Al<sub>+</sub> was -0.93), while the significant influences of both aluminum ( $F_{1,50} = 5.19$ ,  $P < 0.05$ ) and ethanol ( $F_{4,50} = 9.31$ ,  $P < 0.001$ ) were found without any significant interaction.

The percentage alterations of both acidic and alkaline phosphomonoesterases of the cerebrum in Al<sub>+</sub> animals in relation to those of Al<sub>0</sub> animals of each

ethanol group are presented in Figure 3. The graphical representation showed that the tendencies of changes in both types of phosphomonoesterases were being altered with the higher doses of ethanol exposure.

Changes in activities of aspartate and alanine aminotransferases of the cerebrum of Al<sub>0</sub> and Al<sub>+</sub> animals with different doses of ethanol exposures are presented in Table III. The aspartate aminotransferase (ASAT) activities of the cerebrum of Al<sub>+</sub> animals of all the ethanol regimes were found to be decreased in comparison to those of respective Al<sub>0</sub> animals. Except for the highest dose of ethanol exposure (Et-IV), the alterations in ASAT activities were found to be statistically significant in the case of all other groups of ethanol exposures. The aluminum-induced alteration in cerebrum ASAT activities of the animals which were not exposed to ethanol was also found to be statistically insignificant. Two-way ANOVA with replication also showed that the influence of aluminum on the alteration of cerebrum ASAT activities was significant ( $F_{1,50} = 25.75$ ,  $P < 0.001$ ), while the same was not significantly influenced either by ethanol exposure or by the interaction of aluminum and ethanol exposures.

The influence of aluminum on the cerebrum alanine aminotransferase (ALAT) activities with different doses of ethanol exposure showed that neither aluminum nor ethanol had statistically significant

influence. However, the interaction between the exposures was found to have significant ( $F_{4,50} = 3.13, P < 0.05$ ) influence on the alterations of ALAT activities of the cerebrum. The difference in cerebrum ALAT activities between the  $Al_+$  and  $Al_0$  animals was found to be statistically significant only in the Et-0 group (Table III). Figure 4 depicts the aluminum-induced alterations (in percentage) of ASAT and ALAT activities of the cerebrum with graded doses of ethanol exposure.

## Discussion

Ethanol is the most abused psychoactive substance after caffeine. Chronic alcoholism is a major public health problem and causes multi-organ disease and toxicity. Chronic alcohol intake is associated with several degenerative and inflammatory processes in the central nervous system (13). As Davis (8) suggested the aluminum could potentiate the degenerative changes in brain, Rajasekaran (14) found that combined exposure to aluminum and ethanol is enhancing the neurotoxic effects of either of them. They observed that both body weight gain and food intake were reduced in either the aluminum-exposed group or aluminum-ethanol coexposed group, without producing any effect on body weight gain in the only ethanol-exposed group (14). We have also found a decrease in body weight gain with significant impact from aluminum exposure, ethanol exposure as well as their interaction (Figure 1). Reduction in food intake could be the acceptable cause for the reduction in body weight gain. There are several reports available in support of aluminum- or ethanol-induced reduction in food intake or body weight gain (reviewed in reference 14). The observed changes in absolute brain weight (Figure 2) can also be attributed to aluminum and/or ethanol exposure. However, the observed changes in relative brain weight (Figure 2) suggest that the brain was protected from the aforementioned impact when the animals were exposed to only aluminum (Et-0) or a low dose of ethanol coexposure (Et-I). Whereas, the recorded differences between relative brain weights of  $Al_+$  and  $Al_0$  animals (insignificant in Et-II and significant in Et-III and IV) suggest that higher doses of ethanol exposure can make the brain more vulnerable to aluminum intoxication. Das et al. (15) have reported that ethanol exposure in rats was able to produce oxidative stress in the brain even before the pathophysiological changes were there. This prooxidative change in the microenvironment of brain could be the detrimental for aluminum exposure to brain. Bharathi et al. (16) have reviewed that the phosphate groups of various biomolecules are the possible binding targets for aluminum. The binding of aluminum may render the phosphated biomolecules protection from lytic activity of different phosphoesterases, which in turn may cause unwanted accumulation of these phosphated biomolecules. Strong and Jakowec (17) have

reported that aluminum exposure prevents ALP to digest the neurofilament. Ochmanski and Barabasz (18) reported that aluminum may inhibit the activities of acid and alkaline phosphatases. On the other hand, Sallam et al. (19) have reported that plasma ACP and ALP activities are enhanced on exposure to aluminum. These reports suggest that activities of both ACP and ALP are being altered by the aluminum exposure. However, as there is no conclusive evidence available, the observed results could be attributed to modulation of enzyme activities themselves and / or alteration of enzyme contents.

Within the cell, aluminum is not evenly distributed and it generally accumulates inside the lysosome (20) and nucleus (21). Intracellular acidic phosphomonoesterase (ACP) is largely confined to lysosomes, which primarily respond to cellular injury (22). In the present investigation, aluminum exposure was found to enhance, though statistically insignificant, the ACP activities of cerebrum of all the ethanol groups (Table II). Aluminum-induced increase in ACP activities in different regions has been already reported (11). This observation is in agreement with the earlier observations of the altered activities of specific lysosomal hydrolytic enzymes in non-neuronal (23) and neuronal tissues (24) due to aluminum administration. It has been suggested that aluminum-induced increased ACP activity of brain may be an indication of lysosomal proliferation (11). The elevated activity of lysosomal enzymes in various conditions was suggested to be due to increased synthesis of the enzymes (24); that may be true for ACP also. In the present investigation, appreciably higher ACP activities were observed in the cerebrum of alcohol-exposed animals when they were not exposed to aluminum ( $Al_0$  animals of Et-I to IV groups, Table II); though significant elevation of ACP activity was observed only in Et-IV group of animals. However, simultaneous exposure to aluminum and ethanol did not cause significant increase in ACP activities in either of the ethanol treated groups (Table II). Thus, the detrimental effect of both aluminum and alcohol can be suggested. The insignificant contrasting alteration in response to coexposure of aluminum and highest employed dose of ethanol (Et-IV groups of animals) could be due to the already raised cerebrum ACP activity in response to either aluminum or ethanol. The raised ACP activity could be the early indication of the cerebral lesion.

Alkaline phosphomonoesterase (ALP) is a membrane-associated enzyme, predominantly concentrated in the vascular endothelium of the brain. There is a more or less continuous sheath of ALP covering all internal and external surfaces of the central nervous system including the spinal cord (25) and thus it may functionally be a part of the blood-brain barrier mechanism (22). In the present investigation, aluminum exposure lowered the cerebrum ALP activity significantly or insignificantly in most of

the groups (Table II). This observation indicates that aluminum may not only impel ALP resistance to the neurofilaments H and M (17), but may also cause inhibition of the ALP activity; which could be a region-specific observation as suggested by an earlier study (11). With the higher doses of ethanol exposure (Et-I and II), cerebral ALP activities were found to be significantly lower than those of animals in the without-ethanol (Et-0) group. At the same time, cerebral ALP activities were found to be decreased significantly upon coexposure to aluminum and ethanol in the groups Et- III and IV. These observations (Table II) suggest that the impact of aluminum exposure on the cerebral ALP activity was worsened by the coexposure of ethanol and the effect is more obvious with higher doses of ethanol exposure.

Although changes in ACP activity are often associated with parallel changes in ALP activity (25), the observed differential responses of ACP and ALP to the aluminum insult may be due to the difference in localization of these enzymes (11).

Aluminum-induced neuron damage increases the permeability of membranes thus increasing the ALP activity in the soluble portions of the nerve cells (11). This process may well be autocatalytic with an initial release of small amount of hydrolases, which then attack lysosomal membranes releasing additional ACPs, and thus in turn can attack various other cellular structures and damage them (22). This may also be true for the alcohol-induced alterations in these phosphoesterases. As both aluminum and alcohol are altering the enzyme activities in the same direction, the dose dependency is well observed to cause significant alterations in the cerebrum of different groups.

The observation that aspartate aminotransferase (ASAT) is present in rat cerebral homogenate at about the same concentration as in liver suggests its importance in the brain amino acid pool homeostasis (26). The ASAT activity is found in both mitochondrial and soluble fractions of the brain. The enzyme in mitochondria is latent and can be 'activated' by various procedures that disrupt the mitochondrial structure. The critical role of mitochondrial dysfunction in the pathogenesis of age-related nerve cell degeneration has been suggested on several occasions (27, 28). On the other hand, coexposure of aluminum and glutamate is reported to produce morphological abnormalities of mitochondrial structures in pyramidal neurons of hippocampal formation, which are not observed when exposed to aluminum or glutamate alone (29). Toninello et al. (30) also showed that aluminum is an inducer of mitochondrial permeability transition.

No significant change in ASAT activity has been observed in cerebrum in response to either aluminum exposure or ethanol exposure. The lack of impact of aluminum exposure on cerebral ASAT (12) and

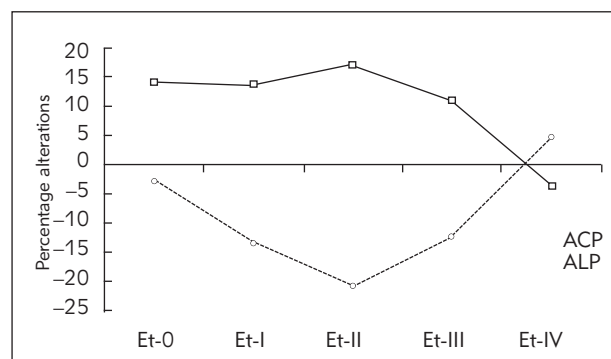
serum ASAT (31) activities were already reported. The impact of aluminum exposure becomes statistically significant in the ethanol-exposed groups (Et- II, III and IV). The present observation is also suggestive of the fact that aluminum and ethanol coexposure is having more impact than exposure to either of them alone. It has been suggested that the increased ASAT activity may be connected with an increased transport of NADH from the cytosol to mitochondria (32). Therefore, the observed insignificant change in increase in ASAT activities in response to ethanol exposure could be an indication of the suggested transport of NADH. Sedman et al. (33) showed that ethanol causes a significant increase in protein turnover in all sections of the brain. As amino acid incorporation into brain protein has been shown to reduce upon alcohol administration (34), an altered amino acid distribution pattern within the brain might be expected in response to aluminum exposure. Alteration in glutamate level as well as its metabolism in response to aluminum exposure is already evidenced (11, 12).

Though alanine aminotransferase (ALAT) is indicated to be distributed in both mitochondrial and soluble fractions of the brain, unlike ASAT, it is less active in the brain. However, Matthews et al. (35) reported that both these enzymes degrade glutamate; though only ALAT was able to reduce toxic (500  $\mu\text{mol/L}$ ) levels of glutamate to the physiologic (<20  $\mu\text{mol/L}$ ) range. It has been already reported that aluminum exposure causes significant increase in glutamate content in different regions of the brain (11). In the present investigation, the ALAT activity increased significantly in the cerebrum of Et-0 group. In ethanol-exposed animals (Et-I, II, III and IV) the variation in the aluminum-induced alteration of ALAT activities in cerebrum suggested that the changes are depending on the other inducers. In general, the enzyme activity was raised due to alcohol, aluminum, as well as both of these (Table II).

These transaminase reactions are reversible, but the equilibrium of the ASAT and ALAT reactions favour formation of aspartate and alanine respectively (36). Increased aminotransferase activities might participate in the enhanced synthesis of excitatory amino acid neurotransmitters in the nervous system (37). Thus, the aluminum insult in presence of higher doses of ethanol exposure could produce significant alteration in cellular microenvironment amino acid homeostasis which may lead to glutamate accumulation, as it has been suggested earlier in other regions of the brain (12).

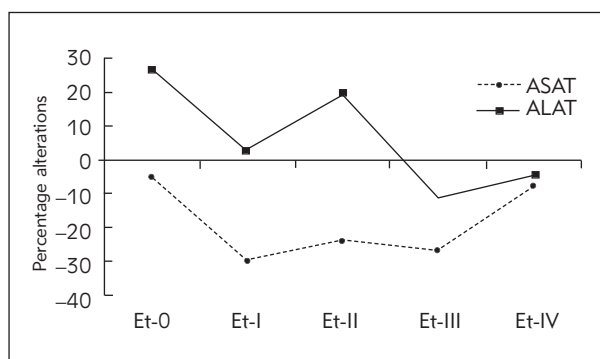
## Conclusion

In the present study, it has been revealed that though intermediate exposure to aluminum was itself unable to disturb the amino acid homeostasis in



**Figure 3** Percentage alteration of cerebral phosphoesterase activities in aluminum-exposed animals in comparison to aluminum-nonexposed animals at different levels of ethanol exposure.

cerebrum by altering the measured transaminase activities, the same can be possible with higher doses of ethanol coexposure (Figure 4). Similarly, the observed changes in phosphatase activities of cerebrum (Figure 3) also corroborate the statement regarding the impact of ethanol coexposure on aluminum-induced cerebral toxicity. Therefore, it can be concluded that coexposure of ethanol augments the toxic impacts of aluminum in the cerebrum,



**Figure 4** Percentage alteration of glutamate transaminase activities in cerebrum of aluminum-exposed animals in comparison to aluminum-nonexposed animals at different levels of ethanol exposure.

however, there is a possibility of having a critical level of ethanol exposure to potentiate the impact of aluminum neurotoxicity.

### Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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