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Aluminium Acetate Induced Oxidative Stress in Brain of Albino Mice

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Abstract: Recently, aluminium (Al) has been identified as one of the environmental factors responsible to cause certain neurodegenerative diseases, particularly Alzheimer's Disease (AD). However, the relationship between Al and AD is controversial. We examined aluminium induced oxidative stress in the brain of mice when aluminium acetate was administered. Albino mice were divided into four groups containing 10 animals each. The first group of animals were treated as controls and administered distilled water, to the second group of animals single dose of aluminium acetate (3.5 mg kg^{-1} body weight) was given. To the third group of animals double doses (7 mg kg^{-1} body weight) were given with 72 h interval. To the 4th group of animals multiple doses (14 mg kg^{-1} body weight) with 72 h interval were given. Exposure to sublethal dose (3.5 mg kg^{-1}) of aluminium acetate has revealed significant variations in detoxification enzymes like xanthine oxidase (XOD), superoxide dismutase (SOD), catalase (CAT) and Lipid Peroxidation (LP) in different regions of brain (cerebral cortex, cerebellum, striatum, medulla) of albino mice. Present results revealed that aluminium acetate exposure increased the activities of XOD, SOD, CAT and LP in all the brain regions in a dose dependent manner, so that it induced the oxidative stress in brain of albino mice.

Key words: Aluminium acetate, detoxification enzymes, lipid peroxidation, mouse brain

Introduction

The toxic consequences in human after aluminium exposure are well established (Nayak, 2002). The brain is the most sensitive organ to show aluminium-induced disorders (Nayak and Chatterjee, 1998). Aluminium salts administered by different routes can produce toxic effects in animal models (Liu *et al*., 1996), some of which involve alterations in enzymatic activities (Zatta *et al*., 1995). Aluminium is considered a non essential metal and its increased biological availability has been linked with both acute and chronic disease in humans (Exley, 2001). The pathology of laboratory animals that have been experimentally intoxicated with aluminium invariably shows many indices of oxidative stress. These include changes in the levels of antioxidants, such as SOD and catalase, as well as higher levels of biomarkers of peroxidation such as MDA and lipid hydroperoxides (Moumen *et al*., 2001; Esparza *et al*., 2005). Xanthine oxidase pathway is one of the important sites of free radical production. Xanthine oxidase generates oxygen radicals and uric acid from xanthine (Bast *et al*., 1991). Superoxide dismutase catalyzes the dismutation of superoxide radical (O_2^-) to form hydrogen peroxide. Catalase is a major antioxidant enzyme. It is found mainly in peroxisomes and it removes hydrogen peroxide produced during oxidation in that organelle. It reduces the toxic hydrogen peroxide into water and protects the cell from possible oxidative damage. Lipid peroxidation is associated with cellular injury and is commonly used as an indicator of oxidative damage in cells and tissues. Cellular damage mediated by free radicals could be involved in aluminium neurotoxicity (Patricia *et al*., 1993).

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In vitro and *in vivo* experimental studies have implicated the formation of reactive oxygen species in the potential neurotoxic effect of aluminium, particularly in alzheimer's disease (Zaman, 1994) showed that aluminium stimulated NADPH oxidation and takes part in the process of free radical formation. Experimental animal models and cell culture studies reveal that aluminium affects the expression of superxode dismutase, catalase, glutathione peroxidase and glutathione (Campbell *et al*., 1999). Aluminium acetate is harmful by inhalation, in contact with skin and if swallowed it causes possible risk of irreversible effects and it is generally used in astringents.

Therefore, the present study was designed to determine the aluminium acetate induced alterations in free radical formation and its detoxification in different regions like cerebral cortex, cerebellum, striatum and medulla of brain of albino mice by selecting specific enzymes such as superoxide dismutase, catalase, xanthine oxidase and lipid peroxidation.

Materials and Methods

Chemicals

Chemicals used in this study namely aluminium acetate was obtained from Sigma, USA. All the other chemicals were obtained from Qualigens and Loba Chemie, India.

Animal Exposure

Healthy adult albino mice (wistar) of same age group 60±2 days and weight 25±5 g were taken from Veterinary College, Bangalore, India. The animals were housed at constant temperature (28±2°C) and relative humidity (60±10%) with a 12 h light : 12 h dark cycle. The toxicity of aluminium acetate was estimated as per Finney (1964) and was found to be 35 mg kg^{-1} body weight (John Sushma and Jayantha Rao, 2005). Ten fold lower concentration of LD_{50} (3.5 mg kg^{-1} body weight) was selected as sublethal dose. The animals were divided into 4 groups. The first group of animals were considered as controls, received only distilled water without aluminium. To the animals of second group single dose i.e., 3.5 mg kg^{-1} body weight of aluminium actetate was given. Double doses (7 mg kg^{-1}) were given with 72 h interval to the third group of animals on 1st and 4th days. To the 4th group of animals multiple doses (14 mg kg^{-1}) were given with 72 h interval i.e., on 1st, 4th, 7th and 10th days. After 72 h both control and experimental animals were sacrificed and the cerebral cortex, cerebellum, striatum and medulla were isolated in cold conditions.

Estimation of Xanthine Oxidase (XO) Activity

Xanthine oxidase activity was estimated by the method given in Worthington Manual (2004). The activity was expressed as μm of urate/mg protein/h.

Estimation of Superoxide Dismutase Activity

Superoxide dismutase activity was measured as the inhibition of photoreduction of nitroblue tetrazolium (NBT) by the enzyme as per the method of Beachamp and Fridovich (1971). One unit is equal to the amount of enzyme activity 50% inhibition of the photoreduction.

Estimation of Catalase (CAT) Activity

Catalase activity was measured following the method of Beers and Sizer (1952). The enzyme activity was expressed as μm of H_2O_2 composed/mg protein/h.

Estimation of Lipid Peroxidation

The lipid peroxides were determined by the TBA method of Ohkawa *et al*. (1979). The trimethoxy pentane (TMP) was used as external standard.

Protein Estimation

The total protein content of tissue homogenate was determined according to the method of Lowry *et al.* (1951).

Statistical Analysis

Standard statistical procedures such as student t-test and ANOVA were used to analyze the data for the significance level.

Results

In the present study, profound changes were observed in all the enzymes related to detoxification mechanism (Table 1-4). In the case of XOD, cerebral cortex has recorded more in its activity followed by cerebellum, striatum and medulla (Table 1) in control regions of brain of albino mice. Under experimental conditions, the levels of XOD increased and it is more in double and multiple doses animals.

The SOD activity was maximum in cerebral cortex followed by striatum, cerebellum and medulla in control mice (Table 2). In experimental animals, the significant change was observed in double and multiple doses animals.

The catalase activity in both control and experimental brain regions was recorded (Table 3). Cerebral cortex has recorded maximum activity followed by cerebellum, striatum and medulla both in control and aluminium acetate administered mice.

In the present study, the malondialdehyde formation was recorded both in control and experimental brain regions of albino mice (Table 4). Maximum in its formation was observed in cerebral cortex followed by striatum, cerebellum and medulla. The malondialdehyde or lipid peroxidation was enhanced as a function of aluminium acetate administration in experimental brain regions. In the present investigation, double and multiple doses of aluminium acetate administered mice have recorded significant increase in all parameters in every region of brain. Such dose dependent elevation have been recorded under aluminium acetate intoxication in the present study.

Table 1: Changes in xanthine oxidase (μ moles of formazon formed/mg protein/h) activity levels in brain of control and aluminium acetate treated albino mice

	Control	Single dose	Double dose	Multiple dose
Cerebral cortex	0.838±0.005	1.098±0.004 (31.026)	1.638±0.007 (95.465)	1.992±0.004 (137.708)
Striatum	0.787±0.007	1.008±0.005 (27.891)	1.529±0.006 (93.994)	1.889±0.006 (139.691)
Cerebellum	0.806±0.008	0.838±0.006 (3.927)	1.482±0.005 (83.836)	1.811±0.007 (124.596)
Medulla	0.713±0.005	0.792±0.007 (11.072)	1.509±0.006 (111.516)	1.796±0.009 (151.810)

Values are mean±SD of six individual observations. Tissue was pooled from six to eight animals (Values in parentheses indicate percent change)

Table 2: Changes in superoxide dismutase (μg/mg protein) activity levels in brain of control and aluminium acetate treated albino mice

	Control	Single dose	Double dose	Multiple dose
Cerebral cortex	5.438±0.005	5.875±0.006 (8.041)	6.415±0.007 (17.965)	7.387±0.005 (35.851)
Striatum	5.097±0.007	5.432±0.005 (6.582)	6.051±0.005 (18.709)	7.458±0.005 (46.325)
Cerebellum	3.754±0.005	3.997±0.006 (6.486)	4.786±0.007 (27.494)	5.398±0.005 (43.787)
Medulla	3.441±0.006	3.792±0.004 (10.195)	4.451±0.005 (29.364)	5.582±0.005 (62.224)

Values are mean±SD of six individual observations. Tissue was pooled from six to eight animals (Values in parentheses indicate percent change)

Table 3: Change in catalase (μ moles of H_2O_2 decomposed/mg protein/h) activity in brain of control and aluminium acetate treated albino mice

	Control	Single dose	Double dose	Multiple dose
Cerebral cortex	0.319±0.006	0.335±0.004	0.348±0.005	0.363±0.004
		(5.060)	(9.077)	(13.720)
Striatum	0.287±0.005	0.299±0.006	0.318±0.005	0.326±0.004
		(4.355)	(10.080)	(13.821)
Cerebellum	0.296±0.004	0.302±0.005	0.317±0.003	0.338±0.004
		(2.082)	(7.0343)	(14.294)
Medulla	0.248±0.005	0.259±0.004	0.267±0.003	0.283±0.003
		(4.765)	(7.919)	(14.496)

Values are mean±SD of six individual observations. Tissue was pooled from six to eight animals. (Values in parentheses indicate percent change)

Table 4: Change in malondialdehyde Content (nm g^{-1}. wet wt. of tissue) in brain of control and aluminium acetate treated albino mice

	Control	Single dose	Double dose	Multiple dose
Cerebral cortex	120.39±1.414	122.54±0.751	129.62± 0.746	133.73±0.782
		(1.791)	(7.667)	(11.080)
Striatum	104.62±1.232	105.98±0.189	116.82±0.248	122.11±0.211
		(0.979)	(11.307)	(16.347)
Cerebellum	105.62±0.368	108.12±0.505	114.21±0.508	128.13±0.382
		(2.368)	(8.134)	(21.256)
Medulla	93.65±0.142	95.43±0.278	106.97±0.281	119.78±0.060
		(1.919)	(14.241)	(27.928)

Values are mean ±SD of six individual observations. Tissue was pooled from six to eight animals. (Values in parentheses indicate percent change)

Discussion

This study shows that aluminium acetate interferes with detoxification enzymes and lipid peroxidation in different regions of brain of albino mice. Aluminium is known to cause neurotoxic effects. In rats, aluminium has a pro-oxidant effect and thus acts as a neurotoxin (Nehru and Anand, 2005). It would appear from the Table 1 that, XOD activity was increased in aluminium acetate administered mice. This increased XOD might be due to the conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase is produced for the nitrogen balance of the tissue (Dellacorte and Stripe, 1972). Enhancement of XO may lead to the accumulation of intermediate toxic compounds such as hydrogen peroxide and hydroxyl radicals, since SOD activity is increased as well (Moumen *et al*., 2001). In the present investigation, more XOD activity was observed in brain of albino mice treated with aluminium acetate also suggest that these regions of brain favour detoxification of ammonia by channeling the same towards uric acid synthesis thereby maintaining nitrogen balance in the tissues under toxic stress.

In the present study, significant increase in SOD activity was observed in all brain regions when multiple doses of aluminium acetate was given. The elevated levels of xanthine oxidase leads to the formation of superoxide anion (O_2^-) in the tissues of mice in response to aluminium acetate treatment. The increased activity of SOD may be to detoxify the superoxide anion radicals produced from XOD reaction in order to arrest the radical damage to cellular organization. A significant increase in lipid peroxidation and decrease in the activities of antioxidant enzymes, SOD and CAT was observed (Dua and Gill, 2001) in cerebrum, cerebellum and brain stem of rat exposed to aluminium phosphide. Aluminium influences the activity of superoxide dismutase which plays a major part in the destruction of oxygen free radicals in the body and the other oxidative enzymes, peroxidase and catalase (Serra *et al*., 1991). Increased catalase activity might be due to its active involvement in decomposition of hydrogen peroxide (H_2O_2) generated during dismutation of superoxide anion radicals by SOD. This is in consonance with the reports of Pritosos *et al*. (1986). Lipid peroxidation in the biological system

is known to produce cellular damage in brain (Slater, 1979). The elevated levels of xanthine oxidase might result in generation of free radicals of oxygen and there by increased lipid peroxidation. The *in vivo* effects of aluminium on lipid peroxidation were studied in mouse brain homogenates and purified brain sub cellular fractions (Patricia *et al*., 1993). The concentration of lipid peroxidation products increased in the aluminium lactate exposed brain of rat (Ogasawara *et al*., 2003). Oxidative damage to brain cell components may be an important mechanism mediating the neurotoxicity of aluminium. Significant increase in lipid peroxidation was observed in cerebrum and cerebellum of pup brains as well as in the adult group when rats were treated with aluminium chloride for 8 weeks (Nehru and Anand, 2005). Aluminium exposure promotes oxidative stress in different neural areas and acts as a pro-oxidant (Esparza *et al*., 2005). The increase in lipid peroxidation rates in brain membranes contribute to the etiology of aluminium neurotoxicity. Lipid peroxidation was influenced by aluminium ingestion (Sugawara and Sugawara, 1992). Thus these parameters have shown dose dependent elevation in the present investigation.

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