Research article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Alteration in Thyroid Hormones and Vitamins As Early Markers of Aluminum Induced Neurodegeneration in Rats

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ABSTRACT

Aluminum (Al) is a potent neurotoxic element, which has been suggested to play an important role in the degeneration of nerve cells of experimental animals as well as human brain. It is associated with Alzheimer's like symptoms leading to cognitive decline. In the present study we attempt to investigate the early markers of aluminum induced neurodegeneration in rats. The effects of Al on thyroid function and vitamins were evaluated in adult wistar rats and its correlates with the Al induced cognitive dysfunction. 100 mg /kg body weight of $AlCl_3$ was given orally to rats for 90 days. Time dependent study was carried out on the 30, 60 and 90 days of treatment. The T3, T4, FT4 and Vitamins A, C, E was estimated on 30, 60 and 90 days along with behavioral test. We observed that serum concentration of total T3 and T4 was decreased and FT4 was insignificant changed while vitamins C and E were markedly changed on 60 and 90 days of treatment. It has been observed that the concentration of T3 and T4 levels and vitamin E and C is significantly correlates with the behavioral activity. On the basis of results it may conclude that the concentration of T3, T4, Vitamin E and Vitamin C may be used as a marker of cognitive decline for the detection of aluminum induced neurobehavioral changes.

KEYWORDS: Thyroid hormone; Vitamins; Aluminum; Neurodegeneration; Cognitive impairment

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INTRODUCTION

Aluminum (Al) is a third most abundant element in the earth's crust and used extensively in modern daily life. It is found in our food products, medicines and also added to drinking water for purification purposes. The high Al diet may lead to increase the deposition of Al in the CNS[1]. It is known to be a potential contributing factor in the etiology of severe neurodegenerative disorders such as Alzheimer's disease (AD)[2,3]. Al promotes the formation of amyloid-β protein plaques[4,5] by aggregating tau proteins in Alzheimer's disease[6]. Our previous studies[1, 7,8] reported that Al increases oxidative burden in the brain and alters post synaptic density and cognitive impairment in the animals[9]. Moreover, Bolla and collogues[10] also observed that decline in visual memory in hemodialyzed patients who exhibited higher serum Al.

In addition, It is reported that plasma triiodothyronine (T3) and thyroxine (T4) concentrations were increased in sublethally Al-stressed brown trout (Salmo trutta)[11]. Experimental studies reported that changes in thyroid hormones induce deposition of amyloid-β, the major component of the amyloid deposits found in the brain of cases of AD. Thyroid hormone plays a significant role in adult brain function, but the precise mechanisms still unknown[12,13].

Vitamins in the body act as potent antioxidant. Vitamin like A, C, E and beta Carotene is a naturally occurring antioxidant nutrient that plays an important role in animal health by inactivating harmful free radicals that are produced during normal metabolic process or induced by external intoxicants such as Al. Vitamin (ascorbic acid) is an essential micronutrient required for normal metabolic functioning of the body and it ameliorates glial activation and reduces release of proinflammatory cytokines induced by Al[14]. Previously, it is known that antioxidant nutrients, like vit-C, are important for neurological function[15-17]. Retinoic acid (RA), the active metabolite of vitamin A (retinoid), has also been shown to control the expression of genes related to APP processing[18,19] in AD. Deprivation of vitamin A results in Aβ accumulation[20], loss of hippocampal long-term potentiation (LTP)[21] and memory deficits in rodents[22,23], all of which are hallmarks of AD. The impairment in spatial learning and memory and the depression of synaptic plasticity that occurs in vitamin A-deprived rodents also occur during aging in rodents[24].

Taking into consideration the above facts, the present study was conducted with an objective to evaluate the thyroid status, serum vitamin levels and behavioural activity in AL toxicity and its correlate them with the time dependent severity of the disease process.

MATERIAL AND METHODS

ANIMALS

Albino Wistar rats (weight 220 ±10g, n = 20) were purchased from Industrial Toxicology Research Institute, Lucknow, (UP) India. The animals were separately housed in polypropylene cages in a room, which was maintained at a temperature of 22±2 ^{0}C, relative humidity of 50±10 % and 12h light dark cycles. They were fed a commercial pellet diet (Dayal Industries, Barabanki, UP, India) and allowed access to water ad libitum. The Institutional Animal Ethics Committee approved the study prior to the initiation of the experiment and also approved all experimental protocols.

TREATMENT

Animals were randomly divided into two groups (n = 10) viz. control and experimental. Aluminum chloride 100 mg/ kg body weight mixed with 1% gum acacia formed an aqueous suspension which was directly introduced into the rat pharynx via a feeding cannula (The sharp age of the tip of a hypodermic needle no. 16 was blunted by grinding on a stone and thereafter bent to 120° so that the curved needle could easily be introduced into the rat pharynx via oral cavity without the pointed tip lacerating the passage) to experimental group and an equivalent volume of physiological saline was given to control groups for 90 days.

BEHAVIORAL TEST

1. **Spontaneous motor activity** [SMA]: SMA was measured by scored on a scale of 0 – 9 in which SMA in control group was assigned score 4.
2. **Muscle coordination test (Rota rod)**: The period of stay on rotating rod (speed: 5 rotations / min; Total duration of test 2 min) for each control and treated rat were recorded by Rotamex (Techno Electronics, India). The rats were trained to stay for period of 2 min on rotating rod and only trained rats were included in the study. Motor was measured using Rota Rod at least 5s and it was rotated at speed of 10 rpm for 2 consecutive days on third day the time duration of each rotation speed was also recorded.
3. **Y-maze learning and memory test**

A test was used to assess the short term memory ‘in vivo’ was carried out in both Al treated and normal rats by Y maze (techno co. 40 cm long x 13 cm height x 10 cm width).Y maze test is a gross test for spatial memory. This test was used to see if the mouse remembers the arm it had just explored and therefore enters in one of the other arms of the maze. Rats were placed at the bottom

of the Y (middle arm) in the y maze and are allowed to explore freely all three arms for an eight-minute session. The first two minutes are for habituation and for the last six minutes the alteration between arms was recorded via photo beam breaks. The acquisition time was noted to determine the short-term memory.

BLOOD COLLECTION

After 30, 60 and 90 days of treatment the blood was collected from the tail vein of the rats in heparinized vial and in plain vial for hemolysate preparation and for serum separation respectively.

HEMATOLOGY

The hematological tests were carried out using commercially available Qualigens kit. The hematological parameters namely hemoglobin (Hb; mg/dl), red blood corpuscles (RBC; X 10^6 cells/ mm^3 of blood), white blood corpuscles (WBC; X 10^3 cells/ mm^3 of blood), packed cell volume (PVC; percent), prothrombin time (PTT; sec) and erythrocyte sedimentation rate (ESR; mm/h) were carried out in blood samples of Al treated young and old rats and their controls.

SERUM ENZYME LEVELS

Serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatine and glucose were assayed using commercially available Qualigens kits (Mumbai, India).

HORMONAL ASSAY

Serum T3 (triiodothyronine), T4 (thyroxine), and FT4 (free tetraiodothyronine) were analysed using commercially available kit (DPC-USA) through solid phase radioimmuno assay.

VITAMIN ESTIMATIONS IN SERUM

Vitamin A was estimated by the previously described procedure of Craft et al[25] using high pressure liquid chromatography (HPLC) system (Waters Limited Mulford USA). The retinyl acetate was used as a internal standard and retinol levels were expressed as µg/dl. Serum vit-C was measured according to method of Lowry et al[26] using 2,4 dinitrophenyl hydrazine in presence of thiourea as a mild reducing reagent to form a red coloured compound bis-hydrazone, which was measured at 520nm in spectrophotometer. Serum vitamin-E was estimated by method of Baker & Frank after xylene

extraction & reduction of ferric to ferrous ions, which then forms a red coloured complex with α-α' Dipyridyl[27]. Absorbance was read at 460nm by spectrophotometer and a correction for the carotenes was made after adding ferric chloride and measured at 520 nm.

RESULT AND DISCUSSION

In the present study we observed that Al induced neurobehavioral changes in rats and correlates with the time dependent association of haematological, hormonal and biochemical findings. The effect of Al on body weight gain, food intake and feed efficiency was progressively increased during the experimental period of both the groups. The final body weight of intoxicated rats (Fig.1) with Al was significantly lower than that of the health normal group. These results clearly indicate that Al cause a significant decrease in body weight. This harmful effect of Al on the body weight gain was elevated paralleled with increase of Al dose. The amount of food intake of both groups was unchanged significantly. This means that the value of food intake was not paralleled to the rate of growth and feed efficiency. The obtained results are in agreement with the findings of our previous study[7]. It is suggestive that loss of body mass indicated that Al induces severe toxicity and it may be due to loss of lipid, protein and other biomolecules.

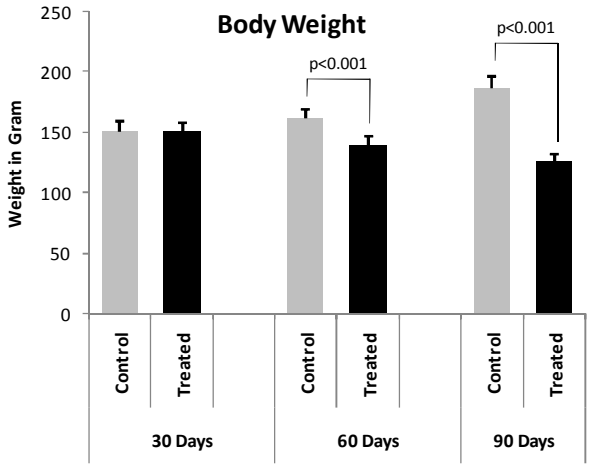

Fig.1. Time dependent effect of Al administration on the body weight of rats.

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant of control and treated groups on different intervals.

Earlier Studies in animal models shows the loss of cognitive function under Al toxicity. The behavioral profiles (Fig.2) exhibited spontaneous motor activity (SMA) was found to be significantly

increased in Al treated rats as compared with the controls on the day 60 and 90 (Fig-2a). On the other hand muscle in-cordination was found to be increased at 90 days (Fig. 2b). The Y maze learning and memory test showed (Fig. 2c) that there was gradually decreased in acquisition time in Al treated rats as comparison with control rats. These results were significantly appeared from the day 60.

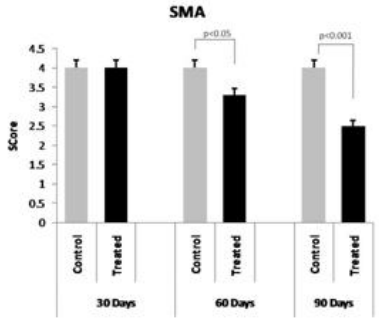

Fig-2 (a)

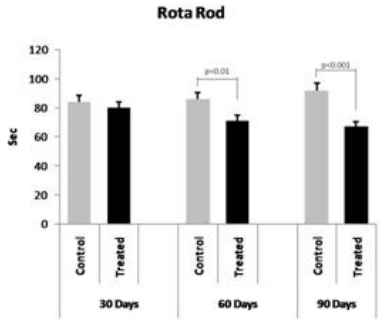

Fig-2 (b)

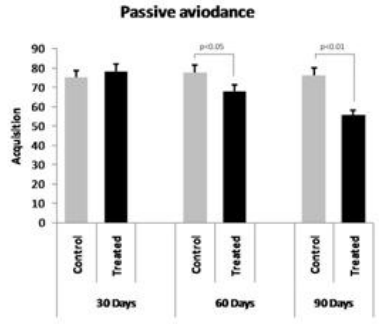

Fig-2 (c)

Fig. 2: Time dependent effect of Al administration on behavioural activity (spontaneous motor activity, rota rod and passive avoidance).

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant of control and treated groups on different intervals.

Table-1. Time dependent effect of aluminum on haematological parameters

Parameters	30 Days		60 Days		90 days	
	Control	Al treated	Control	Al treated	Control	Al treated
Hb	12.8 ± 0.2	12.1 ± 0.1	12.9 ± 0.13	10.3 ± 0.12*	11.7 ± 0.34	9.5 ± 0.4*
RBC	4.54 ± 0.1	4.22 ± 0.1	4.71 ± 0.06	3.41 ± 0.08	3.94 ± 0.14	2.66 ± 0.1*
WBC	6.43 ±0.1	7.32 ± 0.1	6.12 ±0.11	8.02 ± 0.12	6.71 ±0.14	8.34 ±0.1*
PCV	64.8 ±2.1	65.2 ± 2.2	63.6 ±3.12	55.6 ± 2.12*	56.2 ±2.18	42.3 ±2.6*
PTT	14.8 ±0.1	15.1 ± 0.2	14.3 ±0.15	13.2 ± 0.23	13.4 ±0.09	20.4 ±2.1*
ESR	4.12 ±0.1	5.1 ± 0.1	4.4 ±0.12	7.8 ± 0.14*	5.67 ±0.1	10.27 ±1.1*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Haemoglobin (Hb; mg/dl), red blood corpuscles (RBC; X 10^6 cells/ mm^3 of blood), white blood corpuscles (WBC; X 10^3 cells/ mm^3 of blood), packed cell volume (PVC; percent), prothrombin time (PTT; sec) and erythrocyte sedimentation rate (ESR; mm/h) concentration on 30, 60 and 90 day of treatment of aluminum. Statistical significance was determined by Mann-Whitney *p*-test. Probability, ($p< 0.05$) was considered statistically significant control and treated groups on different intervals.

The haematological modifications induced by Al demonstrate that altered peripheral blood composition (Table-1) is a reflection of disrupted haematopoietic process. Blood, which rapidly and constantly flows through the tissues and play in important role in the transportation of nutrients, antioxidants, hormones and some other chemicals. These chemical are required to for the physiological functioning of the body. Al supplementation caused decrease in RBCs and WBCs count and it is time dependent effect of Al intoxication. After long term exposure it is found that detritus alteration was observed these finding concomitant with the observation of Mahieu et al[28] and Zaman et al[29]. Moreover, the haemoglobin concentration and PVC after Al treatment were also found to be gradually decreased with the increasing of exposure time. There is evidence that anaemia is associated with Al accumulation in plasma and/or bone tissue in patients with chronic renal insufficiency[30]. The present results indicated that aluminium treatment resulted in a significant ($P < 0.05$) decline in haemoglobin (Hb), total erythrocytic count (TEC) and packed cell volume (PCV), while total leukocyte count (WBC) increased. Vittori et al[30] reported that erythrocyte morphological changes were induced by aluminium and cells lost their typical biconcave shape. Simultaneously, an increased membrane protein breakdown compatible with band 3 degradation was detected. They also suggested that aluminium may disturb erythropoiesis through combined effects on mature erythrocytes and

cellular metabolism in late erythroid progenitors. Also, the inhibition in erythropoiesis and iron metabolism due to aluminium treatment probably hinders haemoglobin synthesis and erythroid

Humans are uniformly exposed to Al that is present in the soil, food and drinking water. There are several data linking elevated Al levels to neurological pathologies such as multiple sclerosis, Guam Parkinson dementia, Parkinson's disease and Alzheimer's disease[31]. It was reported that prolonged Al sulphate intake accelerate features of senescence in the adult mice liver. In our study we found that the elevated concentration of serum ALP, GOT and GPT followed by Al administration on days 30, 60 and 90 (Table 2).

Table-2. Time dependent effect of aluminum on clinical enzymes

Test	30 days		60 Days		90 days	
	Control	Treated	Control	Treated	Control	Treated
ALP (KA)	25.6 ±1.0	46.3 ±4.2*	27.9 ±2.21	77.7 ±3.3*	26.3 ±3.4	85.81±3.5*
SGOT(U/ml)	17.3 ±1.8	20.3 ±1.3*	16.4 ±1.64	28.2 ±2.1*	17.9 ±1.3	23.2 ±1.7*
SGPT(U/ml)	13.2 ±1.2	12.4 ±1.9	11.9 ±1.65	16.4 ±1.91*	12.9 ±1.37	19.2 ±1.1*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Alkaline phosphate (ALP; KA), serum oxaloacetic acid transaminase (SGOT; U/ml) and serum glutamine pyruvate transaminase (SGPT; U/ml) activity on 30, 60 and 90 day of aluminum treatment. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant control and treated groups on different intervals.

The Al toxicity was characterized by markedly elevated serum ALP, GOT and GPT on days 60 and 90 as compared with their respective controls. Transaminase i.e. SGOT and SGPT are excellent markers of tissue damage. Elevated in serum level has also been reported in the Al induced neurodegeneration[8]. On the other hand, investigation of the subcellular distribution of Al in postmortem examination of the dialysis dementia patient indicates deposition of Al in brain and liver.

Prolonged Al exposure accelerates ageing changes in the adult rat brain[32] and enhanced Al deposition in the brain is a shared characteristic of progressive neurological diseases that are common in aged populations[33]. The deposition of Al in non nervous organs and its subsequent effects are less known. It was previously described that the effects of Al in the rat kidney and liver, where it induces lysosomal activation, increases iron deposition[34] and hence the liver was involved in Al absorption and excretion through biliary flux[35].

In this study, the thyroid hormone (i.e. T3, T4 and FT4) were assessed and found that significantly altered concentration of T3 and T4 levels. However, FT4 were found to be insignificant change in different intervals (Table.3). Both T3 and T4, changes were observed on day 60 and 90 of Al treatment

when compared with their respective controls. In healthy individuals the relationship between T3 and T4 is finely controlled in such a way that differences in hormone activity due to the wide range of serum T4 among normal's may be buffered by systematic adjustment of the T3/T4 ratio. Deficiency of thyroid hormone during environmental exposure of brain is associated with profound, and often irreversible morphological defects that contribute to severe cognitive and neurological impairment[36]. Although the developmental effects of thyroid hormone have been well established, its influence on the adult brain is relatively poorly understood. The adult mammalian brain does not exhibit the severe morphological defects associated with developmental hypothyroidism, however, hypothyroidism in adulthood has been clearly linked to cognitive dysfunction and depressed mood[37,38]. Neurophysiological stress has been reported to decrease plasma levels of vitamins A, E, and Vit C[39,40]. During the past several years, major epidemiological studies have addressed the role of the antioxidant vitamins A, C, and E in the protection against different diseases.

Table-3. Time dependent effect of aluminum on thyroid hormone (T3, T4 and FT4) levels.

Test	30 days		60 days		90 days	
	Control	Treated	Control	Treated	Control	Treated
T3	1.86 ± 0.22	2.02 ± 0.1	2.06 ± 0.22	3.12±0.1*	2.33 ± 0.19	4.3 ± 0.23*
T4	68.7 ± 1.2	73.5 ± 2.0	62.5 ± 1.2	42.4 ±2.9*	69.8 ± 1.3	41.2 ±1.7*
FT4	11.5 ±1.2	10.03 ±1.6	11.50 ±1.2	12.03±1.6	17.24 ± 2.08	15.93 ±1.6

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Alkaline phosphate (ALP; KA), serum oxaloacetic acid transaminase (SGOT; U/ml) and serum glutamine pyruvate transaminase (SGPT; U/ml) activity on 30, 60 and 90 day of aluminum treatment. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant control and treated groups on different intervals.

Al caused a significant decrease in the level of vitamins in blood serum of rats. Vitamin C concentration was diminished on the 60th and 90th days. Administration of Al caused a significant decrease in vitamin A levels in the blood serum on the 60th and 90th days. Al, similar to the case of other vitamins, caused a significant decrease in vitamin E on the 30th, 60th and 90th days (Table.4).

Vitamin E (VE) (alpha-tocopherol) is a naturally occurring antioxidant nutrient that plays an important role in animal health by inactivating harmful free radicals that are produced through normal cellular activity and from various stressors. Fatma and El-Demerdash[41] reported that elevated Al toxicity can be prevents by the supplementation of vitamin E

Table-4. Time dependent effect of aluminum on vitamins level (Vit A, VitC and Vit E) levels.

Test	30 days		60 days		90 days	
	Control	Treated	Control	Treated	Control	Treated
Vit A (umole/l)	1.32 ± 0.03	1.1 2 ± 0.02	1.41 ± 0.03	1.22 ±0.02*	1.27 ± 0.03	0.74 ±0.01*
Vit C (umole/l)	1.09 ± 0.09	0.96 ± 0.08	1.17 ± 0.07	0.85 ±0.08*	1.12 ± 0.07	0.79 ±0.07*
Vit –E (umole/l)	30.9 ±0.1	23.9 ±0.1*	33.6 ±0.3	18.5 ±0.2*	31.2 ±0.3	16.6 ± 0.2*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Vitamin A, C and E on 30, 60 and 90 day of aluminum treatment. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant control and treated groups on different intervals.

On the other hand, Yousef[42] has shown the protective effect of Vitamin C on Al induced changes in haemato-biochemical parameters. The results of the present study, the reduced concentration of vitamin particularly, Vit E and C may be responsible for the excessive generation of free radical in the brain. The reduced form of the vitamin, ascorbic acid, is an especially effective antioxidant owing to its high electron-donating power and ready conversion back to the active reduced form. There is great interest in the clinical roles of Vitamin C because of evidence that oxidative damage is a root cause of several neurodegenerative disorders. Population studies show that individuals with high intakes of Vitamin C have lower risk of a number of chronic diseases, including heart disease, cancer, eye diseases, and neurodegenerative conditions.

CONCLUSION

On the basis of results it may be safely conclude that Al induced changes in haematological parameters, clinical enzymes, thyroid hormones and vitamins are correlates with the neurodegenerative disorders as evident by the neurobehavioral changes following 90 days of Al treatment. Time dependent changes may be used as the markers of neurodegenration. Further study is also required to get depth knowledge relating to assess aluminium induced neuronal changes non- invasively.

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