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DOCOSAHEXAENOIC ACID REVERSES ALUMINIUM INDUCED ALTERATION IN VITAMINS AND LIPID PROFILES IN RAT SERUM

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ABSTRACT

Aluminium (Al) is the third most widespread metal in the environment. It is known to be toxic for human and animals. We have studied the effect of simultaneous oral treatment of docosahexaenoic acid on aluminum induced changes in serum lipid profile and vitamins. A total of twenty four male albino rats were taken and divided in to four groups (N=6). First control group was treated with normal saline, second was treated with 100mg DHA, third was treated with 100mg $AlCl_3$ and fourth group was treated with both $AlCl_3$ and DHA. Aluminum treated rats showed a significant increase in serum low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride (TG), Cholesterol (TC) and lipid peroxide level (LPx) while decreased concentration of high density lipoprotein (HDL) and vitamins (A,C and E). DHA administration with Al showed reversal changes near to control in serum lipoprotein ie., LDL, VLDL, HDL, vitamins (A, C and E) and TG level. Moreover, DHA reduces the rate of lipid peroxidation. The results indicate that DHA has some beneficial effect in preventing Al induced toxicity.

KEYWORDS: Aluminum, Docosahexaenoic acid, lipid peroxidation, lipoproteins, vitamins

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INTRODUCTION

Aluminum (Al) is a third most abundant element in the earth's crust followed by oxygen and silicon. It is used extensively in modern daily life. It is found in our food products, medicines and also added to drinking water for purification purposes[1]. The high Al diet may lead to increase the deposition of Al in different tissues of the body especially in bones and brain[2,3]. It is known to be a potential contributing factor in the aetiology of severe neurodegenerative disorders such as Alzheimer's disease (AD)[4,5]. Al promotes the formation of amyloid-β protein and neurofibrillary tangles plaques by aggregating tau proteins in Alzheimer's disease[6]. Several studies reported that Al increases production of reacting oxygen species[2]. The possible mechanism of Al induced toxicity has been related to cell damage via free radical production and increased lipid peroxidation (LPx) is one of the major consequences of oxidative stress[2]. The primary targets of reactive oxygen species are cell-membrane polyunsaturated fatty acids, which, in turn, lead to damage in the cell structure and function. Some authors suggest that AL interacts with cell membrane directly and facilitate lipid degeneration[7]. There is an abundant evidence that Docosahexanoic acid (DHA) may have a protective role against oxidative burden by decreased lipid peroxidation rate[8.] Naturally, DHA is a major component of brain gray matter and of the retina in most mammalian species and is considered essential for normal neurological and cellular developments. DHA can also enter the blood by consuming food naturally containing DHA (eg: fish, fish oils, eggs, liver) or foods fortified with DHA-rich fish oils. Taking into consideration the above facts, the present study was conducted with an objective to explore the protective potential of DHA on AL induced biochemical deterioration in male albino rats and its correlate them with the time dependent severity of the disease process.

MATERIALS AND METHODS

Twenty four male Rattus norigious, Wistar strain rats (weight 220 ±10 grams) were taken from NIMS University animal house. 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 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 four groups (n = 6) viz. Group 1 served as control treated with normal saline, Group 2 treated with 100mg / kg body weight of DHA[9], Group three treated with 100mg /kg body weight of $AlCl_3$ and Group four treated with 100mg $AlCl_3$ + 100mg DHA for 90 days.

Dose

Each solution was prepared with 1% gum acacia formed an aqueous suspension which was directly introduced into the rat pharynx via a feeding cannula (The sharp edged 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 groups and an equivalent volume of physiological saline was given to control groups for 90 days.

Blood collection and serum separation

After 45 and 90 days of treatment the blood was collected from the retro-orbital plexus of the rats in plain vial for serum separation. The vial was kept for some time. Serum from blood after clotting separated out and collected in clean centrifuge tube and again centrifuged for 5-minutes at 3000 rpm. The serum thus obtained was used for further use.

Serum Lipid profiles

Serum total cholesterol, high-density lipoprotein, triacylglycerol were determined using Randox Laboratory kit reagents and

VLDL was calculated using the formula TG/2.2 mmol/l. Low density lipoprotein (LDL) cholesterol was determined by differential substraction of the sum of the cholesterol fractions from the total cholesterol.

Vitamin estimations in serum

Vitamin A was estimated by the previously described procedure using high pressure liquid chromatography (HPLC) system (Waters Limited Mulford USA) [10]. 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[11] 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 after xylene extraction & reduction of ferric to ferrous ions, which then forms a red coloured complex with α-α' Dipyridyl[12]. Absorbance was read at 460nm by spectrophotometer and a correction for the carotenes was made after adding ferric chloride and measured at 520nm.

RESULT AND DISCUSSION

In the present study, we evaluated Al induced time dependent changes in serum vitamins and lipid profiles in male albino rats. Protective potential of DHA was also tested in time course changes. The concentration of vitamins A was found to be significantly ($p<0.05$) reduced in Al treated rats on the day 45 and 90 when compared with their respective controls (Fig-1). While, DHA treatment found to be reverses these changes near to control on day 90 as compared with Al treated rats. Retinoic acid (RA), the active metabolite of vitamin A (retinoid), has also been shown to control the expression of genes related to Amyloid precursor protein (APP) processing in AD. Vitamin A is a hydrophobic antioxidant[13,14], which is decreased in diabetes causes retinopathy. It has also been reported that omega 3 fatty acid increases the concentration of vitamin A[15]. Our results are similar to that of Berson et al[16]. He also observed that omega 3 fatty acid restore the vitamin A and increase the visual activity. Vitamin C concentration was diminished on the 45th and 90th days. Administration of Al caused a significant decrease in vitamin C levels in the serum (Fig-2). 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[17]. Vitamin E is the potent antioxidant. It is found to be reduced in both 45 and 90 days of treatment (Fig-3). Vit E (ά- Tocopherol and its derivatives) is a predominant chain breaking lipid - soluble antioxidant and is believed to be the primary free radical scavenger and prevent lipid peroxidation[18]. Antioxidants are known to reduce oxidative radical induced reaction. α tocopherol is an important antioxidant in biological systems. It inhibits peroxidation of membrane lipid by scavenging lipid peroxyl radical with formation of tocopheroxyl radical as a consequence[19]. In addition, α-tocopherol maintained the levels of antioxidant membrane-bound enzyme and the activities of antioxidant enzymes near the normal value thus emphasizing their effect as antioxidant[20,21]. Chinoy and Memon[22] demonstrated that Vit E has a very significant bearing on the amelioration of Al toxicity in human.

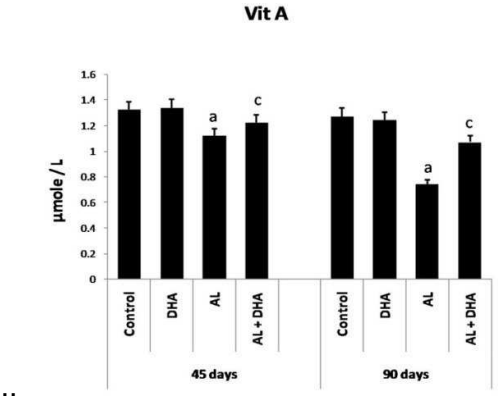

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Figure.1

The concentration of vitamin A in serum of control and experimental group on 45 and 90 days. The results are expressed as Mean ± SD six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control Vs AL treated group (a) and AL treated Vs AL + DHA treated rats (c).

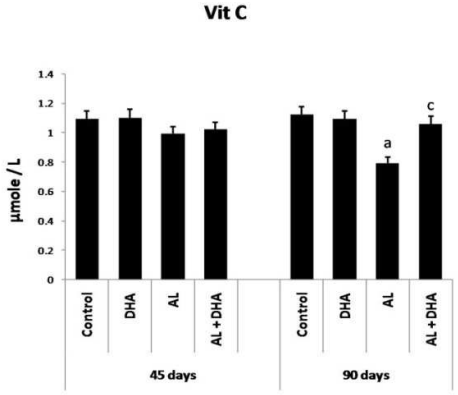

Figure.2

The concentration of vitamin C in serum of control and experimental group on 45 and 90 days. The results are expressed as Mean ± SD six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control Vs AL treated group (a) and AL treated Vs AL + DHA treated rats (c).

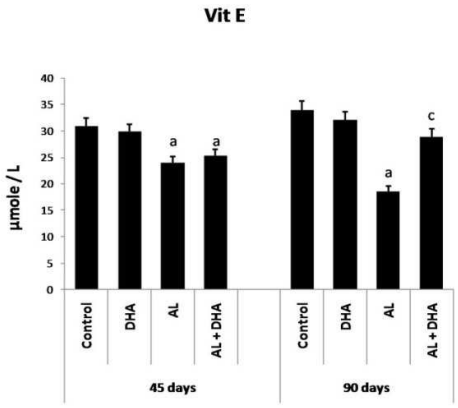

Figure.1

The concentration of vitamin E in serum of control and experimental group on 45 and 90 days. The results are expressed as Mean ± SD six rat of each group (N=6). Superscripts relate significant (p< 0.05) comparison with Control Vs AL treated group (a) and AL treated Vs AL + DHA treated rats (c).

Table-1

Effect of Aluminum on lipid profiles in rats on 45 and 90 days of treatment

	45 days				90 days			
	Control	DHA	AL	AL + DHA	Control	DHA	AL	AL + DHA
TL (mg/ml)	360.5± 38.4	371.5 ± 43.4	276.7 ± 33.8[a]	319.7 ± 31.3[c]	405±27.5	419.5 ± 27.5	347.4 ± 33.2[a]	394.6 ± 36.4[c]
TC (mg/ml)	87.55 ± 9.7	91.5 ± 12.7	117.3 ± 23.9[a]	101.2 ± 13.9[c]	96.6 ± 12.8	101.3 ± 14.2	137.2 ± 32.7[a]	127.6 ±23.4[c]
TG (mg/ml)	75.6 ± 2.5	69.6 ± 2.5	83.57 ± 2.2[a]	73.1 ± 1.8[c]	65.17 ±7.2	68.34 ± 5.9	79.4 ± 12.7[a]	74.7 ± 19.7[c]
LPx (nmole/ml)	1.27±0.1	1.22 ± 0.1	1.98 ± 0.2[a]	1.41 ± 0.2[c]	1.34 ± 0.1	1.33 ± 0.2	2.57 ± 0.2[a]	1.83 ± 0.2[c]

Values are expressed as mean ± SD for six animals (N=6) in each group. The values of total lipid (TL), total cholesterol (TC), triglycerides (TG) and lipid peroxide levels (LPx) in control and experimental groups on 45 and 90 days. Sstatistical significance was determined by one way ANOVA followed by Neumann Keules post hoc test between groups. Superscripts relate significant (p< 0.05) comparison with AL treated vs control (a) and AL Vs AL + DHA treated rats (c).

Table-2

Effect of Aluminum on lipoproteins in rats on 45 and 90 days of treatment

	45 days				90 days			
	Control	DHA	AL	AL + DHA	Control	DHA	AL	AL + DHA
LDL (mg/dl)	46.6 ± 6.9	49.3 ± 6.8	62.9 ± 4.3[a]	57.8 ± 3.3	43.6 ± 5.9	46.6 ± 6.8	102.9 ± 5.6[a]	77.5 ± 6.6[c]
VLDL (mg/dl)	27.4 ± 1.2	29.2± 1.3	36.1 ± 1.1[a]	30.2 ± 1.2	22.4 ± 1.7	23.6 ±1.7	42.4 ±1.4[a]	29.4 ± 1.7[c]
HDL (mg/dl)	59.8 ± 4.3	57.3 ± 5.3	43.4 ± 2.1[a]	53.2 ±2.14[a]	57.12 ± 1.5	55.3 ± 3.1	29.9 ± 2.7[a]	41.5 ± 4.4[c]

Values are expressed as mean ± SD for six animals (N=6) in each group. The values of low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and high density lipoprotein (LDL) in control and experimental groups on 45 and 90 days. Sstatistical significance was determined by one way ANOVA followed by Neuman Keules post hoc test between groups. Superscripts relate significant (p< 0.05) comparison with AL treated vs control (a) and AL Vs AL + DHA treated rats (c).

We observed alterations in the lipid profiles following AL treatment (Table-1). The concentration of lipid was found to be significantly reduced in Al treated rats on both 45 and 90 days of treatment. The reduction of lipid may be correlate with the Al induced increased lipid peroxidation. Although biological mechanism of Al is not fully understood[23] it has been reported that Al promote the production of free radicals resulting in Al-Fe interdependence reaction[24]. Total cholesterol and triglycerides were found to be elevated in AL treated rats when compared with the controls on the day 45 and 90. The increase in serum TG may be due to hypoactivity of lipoprotein lipase in blood vessels which breaks up TG. High serum cholesterol level may results from the hepatic dysfunction[25]. Previously it has been reported that Al exposure accelerates neurodegeneration through lipid peroxidation via free radical production[26]. In the present experiment, there was a significant increase in LPx after Al administration as we were found that significant ($p<0.05$) elevated levels of MDA in the rat serum on the day 45 and 90. It is suggested that Al is responsible for oxidative burden. AL produces a subtle rearrangement in the membrane structure that facilitated the oxidative action of iron[27]. Effects of Al on lipid peroxidation in various tissues such as liver, kidney, testis and brain of different animals were investigated and Al increased the rate of lipid peroxidation in some studies[28,29,30,31], while these changes were not observed in some studies[23,32]. The concentrations of lipoproteins were found to be reduced significantly following AL exposure (Table-2). Increased levels of LPO or VLDL and LDL (*ß*-lipoproteins) in treated rats are known to be cytotoxic to cells and tissues. Previously it is reported that during hyperlipemia, lipoproteins may initiate and maintain atheromatous lesions by endothelial cell injury and lipid accumulation[33]. Recently it has been emphasized that hyperlipemic *ß*-lipoproteins are cytotoxic to cells and tissues presumably due to enhanced levels of associated LPO[34]. Lipids play significant role in various disorders like cardiovascular, hyperlipidemia fatty liver. It modifies the composition, structure, and stability of cellular membranes. Excess lipid in the blood is considered to accelerate the development of arteriosclerosis. An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death. High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage. Recent reports indicate that cytotoxicity induced by LDL and very low density lipoprotein can result from lipoprotein oxidation. Oxidation in these studies is indicated by the relative amount of MDA equivalents or thiobarbituric acid-reacting substances (TBARS) residing on the lipoprotein. The present data showed that the presence of DHA with Al reduces its harmful effect on all above measured biochemical parameters and their levels become near to the normal values of control. These results are in good accordance with other investigators[35,36] who found that DHA maintained the levels of antioxidant membrane-bound enzyme and the activities of antioxidant enzymes near the normal value thus emphasizing their effect as antioxidant. Restoration of lipoproteins was found after the treatment of DHA. These results were agreed with the finding of Pan et al[37]. He showed that the hypolipidemic actions of dietary PUFAs. A separate study shows that rats initially maintained on a diet deficient in fish oil have poor learning and memory skills. When they are transferred to a diet supplemented with DHA, however, they demonstrate dramatic improvement in learning and memory[38,39].

CONCLUSION

On the basis of result, it can be suggested that AL produces more free radicals and in turn this decreases the level of vitamins in the serum and deleterious effect in lipid profiles. Moreover, DHA ameliorate these changes. From these results, we could suggest that, DHA should be taken to compensate the deleterious effect of AL and potential risk for Alzhiemr's Disease.

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