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Protective Effect of Docosahexaenoic Acid on Aluminium Induced Autonomic and Gross Behavioural Changes in Male Albino Rats

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ABSTRACT

Essential omega-3 polyunsaturated fatty acids are crucial for development and function of the brain. It may help to ameliorates neurotoxicity. Aluminium (Al) is a potent neurotoxic element, which plays 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 evaluated autonomic response, gross behavioural and sensory response in rats following 100 mg / kg b.w. of $AlCl_3$ treated rats for 90 days. 50mg, 100mg and 200mg of Docosahexaenoic acid (DHA) were treated along with $AlCl_3$ with for 90 days. Different behavioural responses like autonomic responses, gross behavioural and sensory were evaluated in rats. Catalepsy, posture righting reflex, nocieceptive response of rats were changed and significant altered gross behavioural and autonomic response were found in Al treated rats. 100mg of DHA shows maximum reversal in Al induced behavioural changes. On the basis of results it may conclude that DHA ameliorate Al behavioural changes in rats. 100mg DHA dose were considered significant in the study. Further study may require in different animal model for depth knowledge.

KEYWORDS: Aluminium neurotoxicity; Docosahexaenoic acid; Gross Behaviour; Autonomic Response

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INTRODUCTION

The docosahexaenoic acid (DHA, 22:6n-3) is an omega (n)-3 polyunsaturated fatty acid (PUFA) and it is a major component of membrane phospholipids in brain, retina, and spermatozoa[1,2]. In the brain, has taken on a central role as a target for therapeutic intervention in Alzheimer's disease (AD) as well as other neurodegenerative disorders. DHA also keeps the membranes surrounding each synapse- the communication gap between to nerve cells in a more fluid state. This help nerve cells release chemicals in to the gap more quickly and for the detector sites (receptors) on the side of the gap. Brain cells whose membranes are rich in DHA therefore communicate more quickly with each other.

Aluminium (Al) is a ubiquitous neurotoxic element having a very easy access to central nervous system (CNS)[3]. It is access through blood brain barrier and distributed within the neurons and glial cells thus causing various neurological alterations. Al promotes the development and accumulation of insoluble amyloid β protein and the aggregation of hyperphosphorylated tau protein[4]. It is also associated with cortical cholinergic neurotransmission deficits and iron induced increased oxidative stress. Al toxicity could lead to progressive dementia or Alzheimer's. Our previous studies reported that Al increases oxidative burden in the brain and alters post synaptic density and cognitive impairment in the animals [5,6,7,8] . Moreover, Bolla and assosiated[9] also observed that decline in visual memory in hemodialyzed patients who exhibited higher serum Al.

In an attempt to model human neurobehavioral changes in rodents, a wide range of behavioural testing paradigms have been developed. Many of these tests induce a fearful response like maze test for learning and memory, rota rod, anxity effect and avoidance response etc. Others integrate an approach–avoidance conflict designed to inhibit an ongoing behavior that is characteristic for the animal, such as contrasting the tendency of mice to engage in exploratory activity or social investigation against the aversive properties of an open, brightly lit, or elevated space. In this study we have try to develop in vivo grass behaviour, autonomic and sensory responses for non invasive characterization of neurological responses.

In view of the aforementioned considerations, the present study was designed to assess Al induced cognitive dysfunction in rats and dose dependent protective efficacy of docosahexaenoic acid was tested. To achieve these objectives we administered 100 mg / kg body weight of aluminum Chloride ($AlCl_3$)[7,8] and three different doses of DHA 50 mg, 100mg and 200 mg were given per os intrapharyngially via a feeding cannula to rats for 90 days. Moreover, vitamin E was used for standard antioxidant.

MATERIAL AND METHODS

ANIMALS

Seventy two male Rattus norigious, Wistar strain rats (weight 220 ±10 gram) 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 by the Institutional Animal ethical committee (1302/ac/09/CPCSEA), NIMS Univesity, Jaipur, India.

TREATMENT

Animals were randomly divided into five groups (n = 18) viz. Group 1 served as control treated with normal saline, Group 2 treated with 100mg / kg body weight of $AlCl_3$ and three other groups treated with DHA (50mg, 100mg and 200mg) along with 100mg / kg $AlCl_3$ for 90 days. Moreover, Vitamin E (100mg/ kg b.w.) was also given as standard drug.

DOSE

Each solution were prepared 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 groups and an equivalent volume of physiological saline was given to control groups for 90 days.

EXPERIMENTAL PROCEDURE

After 90 days rat were allow to move for assessment of gross behavioural, autonomic response and sensory responses as per described previously[10,11].

I. GROSS BEHAVIORAL RESPONSES

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. The SMA was assigned as present / absent
2. **Posture/position**: Normal upright posture (+: present or –: absent)

3. **Ataxia**: Any in-coordination in movements occurring in absence of involuntary movements. (+: Present or –: absent)
4. **Tremor**: Involuntary movements (jerking of entire body or limbs) due to alternating contraction of muscles. (+: Present or –: absent)
5. **Convulsions**: Alternate contraction (flexion) and extension [clonic], or sustained extension [tonic] of limbs resulting into loss of upright posture (+: present or –: absent).
6. **Straub's tail**: A sustained (> 30 sec) raising of tail (making an angle >60° with the body) (+: present or –: absent)
7. **Catalepsy**: A condition in which body or limbs remain passively in any position in which they were placed. It was tested by the placing forepaws on a metallic rod placed at height of 6 cm and if forepaws was not withdrawn within 10 sec catalepsy were considered to be positive (+: present or –: absent).
8. **Abnormal (Bizarre) behavior**: Stereotypy, head shaking, head searching, upright walking and circling (+: present or –: absent).

II. AUTONOMIC RESPONSES

1. **Piloerection:** Erection of body hairs (+: present or –: absent).
2. **Salivation**: Secretion of saliva were observed as dripping of saliva from mouth, which will be scored as 1 = mild, 2 = moderate and 3 = severe as comprised to control (0 = no dripping).
3. **Lachrymation**: Secretion and discharge of tears observed as shedding of tears around the lower eyelid, which was scored as 1 = mild, 2 = moderate and 3= severe as compared to control (0 = no shedding).
4. **Palpebral closure**: Ptosis (drooping of upper eyelids) present or absent
5. **Defaecation**: scored as 1 = mild, 2 = moderate and 3= severe as compared to control
6. **Urination**: is scored as 1 = mild, 2 = moderate and 3= severe as compared to control.

III. SENSORY

1. **Righting Reflexe**:

Rats were placed on their back to see whether the animal could quickly right itself and assume a normal posture. Neurological deficits were indicated by an inability to regain normal body posture within 5 sec.

2. **Nociceptive response**:

Reaction time to nociceptive response was observed by Eddy hot plate analgesia meter. Rats were placed on the plate heated at 55 °C and jump (all the limbs lifted from the plate) were taken as reaction to pain. The time taken for jump (pain reaction time) was recorded.

RESULT AND DISCUSSION

In the present study several cognitive and non-cognitive parameters were investigated in the male rats following Al exposure. In the present study, we evaluated non invasive method of behavioural changes following Al induced neurotoxicity. Moreover, protective potential of different doses of DHA were tested. It was reported that Al could induces brain damage which may impair memory and learning as seen in Alzheimer disease[12]. This element (Al) appears mainly in food products and in drinking water from both natural sources and treatment methods[13]. Our previous study showed that Al promotes oxidative stress by increased rate of lipid peroxidation[6,7].

Several studies demonstrated that Al alters memory and learning ability. In this study first time we evaluated gross behavioural and autonomic responses in Al treated rats. The behavioural profiles exhibited gross behavioural response, autonomic and sensory responses following Al treatment. The gross behavioral response i.e., spontaneous motor activity (SMA), posture position, Atexia, tremer, convulsions, straub's tail, catalepsy and Bizzare behavior are shown in the table 1.

Posture position and catalepsy were found to be significantly changed when compared with the controls while it was reverses in DHA treatment. Significant ($p<0.01$) altered posture position in Al treated rats it is only 33% when compared with the controls. While, the maximum reversible changes were found in DHA-100 treated rats (77%) groups, and least in DHA-50 (55%) rats shows normal posture when compared with Al treated rats. It is reported that brain learns to relax some muscles like the front of our shoulders and hamstrings and therefore, postural development obviously depends on muscular development as well as on neural control. Muscles which are important for maintaining a stable posture involve trunk muscles as well as so called postural muscles in the extremities. It is suggested that Al alters the posture position in rats it may be due to Al induced alteration in central and peripheral nervous system. Catalepsy is the position that the animals are unable to correct externally imposed postures[14]. There is pharmacological evidence for stimulation of dopaminergic neurons by noradrenergic neurons in the brain[15]. In this study, 55% rats showed catalepsy as compared to control rats. There were 22% rats in DHA-100 and 33% Vit-E treated, DHA-200 and DHA-50 treated rats exhibited catalepsy when compared with the Al treated rats. It has been reported that Catalepsy is a symptom of certain nervous disorders or conditions such as Parkinson's disease and epilepsy and

Protein kinase- C has been suggested as a mediator of cataleptic behavior. It has also been demonstrated that Al alters the protein kinase activity in both adult and old rat brain[16]. Other causes of catalepsy may include reuptake inhibitors of adrenergic neurotransmitters such as Respirine[17] or may be due to elevated deposition of lactae during lactic acidosis. Previously, it has been reported that, Al accelerate the deposition of lactate during metabonomic changes in glycolysis[8].

Autonomic bodily responses symbolize dynamic modification to the auto regulatory brain functions. Various autonomic responses were measured they include piloerection, lacrimation salivation, palpibral clousers, urination and deification etc. that are automatically generated by physical, cognitive and emotional behaviours and that clinical index of toxicity. Sympathetic nervous system causes certain muscles to contract and hair follicles to protrude outwards from the skin. It is commonly called piloerection. It is a physiological response to intense emotions and fear. Al treated rats showed 11% of severe, 44% of moderate and 33% mild piloerection in Al treated groups as compared with the controls (fig-1). While, a highly reverse response was observed in DHA 100 treated group (in this group only 33% rats showed mild piloerection), followed by Vit E treated groups (28% mild and 16% moderate), DHA 200 (22% mild, 22% moderate and 11% severe) and DHA 50 (44% mild, 33% moderate). Previously, it has been reported that 90 days of Al treatment induces piloerection as sign of toxicity[7]. The salivation (fig.2) was significantly changed between groups. In Al treated groups, 33% rats of showed moderate and mild characters of salivation, while a highly reverse was observed in DHA 100 treated groups (only 44% rats showed mild salivation), followed by DHA 200 (22%), Vit E treated groups (28%) and DHA 50 (44%). Saliva is produced from salivary glands and mucous membranes and, as a biological fluid and controlled by autonomic nervous system. It is reported that salivary levels may reflect changes in CSF[18]. Sayer et al[19] showed association between neurodegeneration and the activity of salivary acetyl cholinesterase (AChE). During neurodegenerative changes like amyloid-beta protein accumulated in the saliva. Saliva has also used for the non invasive detection of AD. Lachrymation (fig.3) was significantly changed between groups. In Al treated group, 11% rats showed moderate and mild characters of lachrymation, while a highly reverse was observed in DHA 100 treated groups (100% normal), followed by DHA 200 (22% rats of mild) and DHA 50 (22% of mild) and Vit E treated groups (11% of mild and 5.5% moderate). It is reported that lacrimation from the lacrimal glands modulated by specific neurotransmitters and parasympathetic nerves. Al alteration in neurotransmitter by Al toxicity is well known[20,21]. Furthermore, Xing et al[22] also reported that increased blink rates in Huntington's disease (HD) is the symptoms of neuro-ophthalmologic abnormalities. It may also prove to be sensitive and quantifiable biomarkers of

presymptomatic disease severity and progression of HD[23, 24]. The palpebral closures (fig.4) were found to be significantly changed between groups. 11% and 44% rats of Al treated groups showed moderate and mild palpebral closure respectively, while high recovery was observed in DHA 100 treated groups (22% mild), followed by DHA 50 (16% mild and 5.5 % rats of moderate) and DHA 100 (22% of mild and 5.5% moderate) and Vit E treated groups (11% of mild and 5.5% moderate). Defecation (fig.5) was also found significantly changed by 11%, 44% and 11% rats of Al treated groups showed mild, moderate and severe defecation respectively, while high recovery was observed in DHA 100 treated groups (16% mild and 5.5% moderate) followed by DHA 200 (16% mild and 5.5 % moderate) and DHA 50 (22% of mild and 22% moderate) and Vit E treated groups (22% of mild and 11% moderate). It is known that nervous system control of the urinary bladder and it is lost during toxic insults. Loss of nervous system control, in turn, means that, while the bladder fills with urine, the message that the bladder is full cannot reach the brain. As a result, do not longer able to feel the urge to urinate. As in our study, urination (fig.6) was also found significantly changed between groups. A 11%, 56% and 11% rats of Al treated groups showed mild, moderate and severe urination respectively, while high recovery was observed in DHA 100 treated groups (22% mild) followed by DHA 200 (22% mild and 11% moderate) and DHA 50 treated groups (22% of mild and 22% moderate).

The type of motor response to a sensory input that occurs within the brain stem is always a reflex reaction. A reflex is an automatic response to a stimulus it is always an unconscious reaction, the sensory nerve in direct connection with a motor nerve, sensory/motor loop. Reflexes control the vital functions of life, breathing, heartbeat, blood pressure control. In the present study righting reflex (fig.7) was found to be significantly increased by 78% in Al treated rats as compared with the controls. While, both DHA 100 and DHA 200 treated rats exhibited 37% reduction when compared with the Al treated rats. These results reflect impairment in neuromuscular coordination and sensorimotor reflex. Dam *et al*[25] showed impairment of righting reflex with ACh metabolism arising from brain AChE inhibition. These observations substantiated that aluminium might be affecting various steps in the metabolic pathway of the neurotransmitters through end-product inhibition. It has been reported that acetylcholine is synthesized in a single reaction from the precursors, acetyl CoA and choline, catalyzed by the enzyme choline acetyl transferase. Acetylcholine accumulates during acute and chronic Al exposure[26, 27]. Al passes through the blood brain barrier due to its small size and could affect the cholinergic system [28]. The nociceptive responses (fig.8) were found to be significantly increased by 132% in Al treated groups when compared with the controls. Moreover DHA 100 reflects maximum reduction by 48% as compared with the Al treated rats. On the other hand Vit E exhibited only and

31% reversal changes as compared with Al treated rats. The neural processes of encoding and processing noxious stimuli called nociception. All primary sensory nociceptors connected to the synapses with neurons in the grey matter (dorsal horn) of the spinal cord. Primary sensory nociceptors and the spinal circuits that they engage contribute differentially to the main classes of clinically relevant pains, namely, inflammatory pain resulting from tissue injury and neuropathic pain resulting from nerve injury[29,30,31].

Table-1. Gross Behavioral Responses after 90 days of Al and DHA treatment

	Control	Aluminum	DHA 50	DHA 100	DHA 200	Vit-E	χ2- test, (DF= 15)	P- value
SMA	100	61	61	78	67	72	χ2 =9.4	P=0.0801
Posture position	100	33	54	77	66	66	χ2 =20	**P=0.0012**
Atexia	ND	33	33	22	28	28	χ2 =7.7	P=0.1736
Tremer	ND	28	11	11	22	17	χ2 =6.8	P=0.2399
Convulsion	ND	39	33	22	33	28	χ2 =9.1	P=0.1065
Straub,s tails	100	61	67	78	67	72	χ2 =9.2	P=0.1075
Catalepsy	ND	55.3	33	22	33	28	χ2 =14.3	**P=0.0136**
Bizzare Behavior	ND	44	33	22	33	22	χ2 =10.2	P=0.055

Table shows Gross behavioral responses after 90 days of treatment in each groups (n=18). Data were analyzed by χ2 test and express as % of rats represented characters

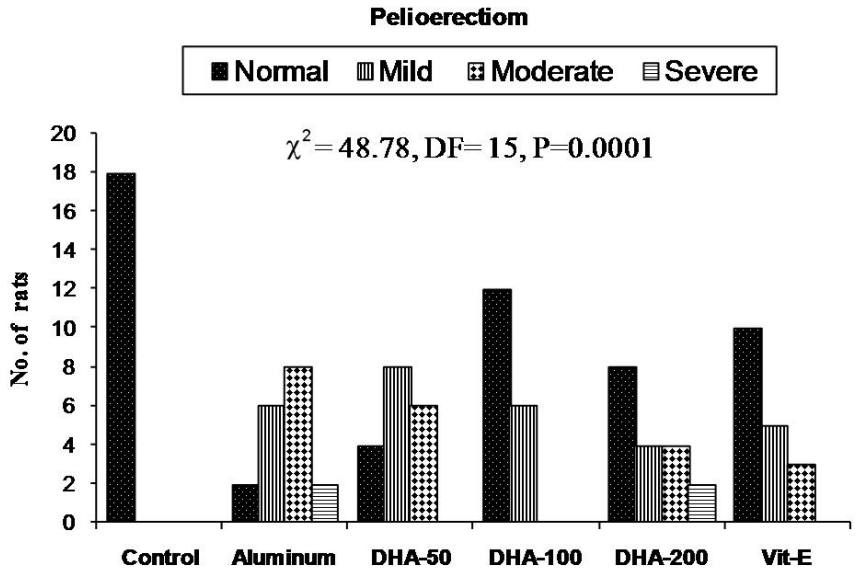

Fig.1. Effect of aluminum on piloerection. Data are presented as number of rats in each group (n=18).

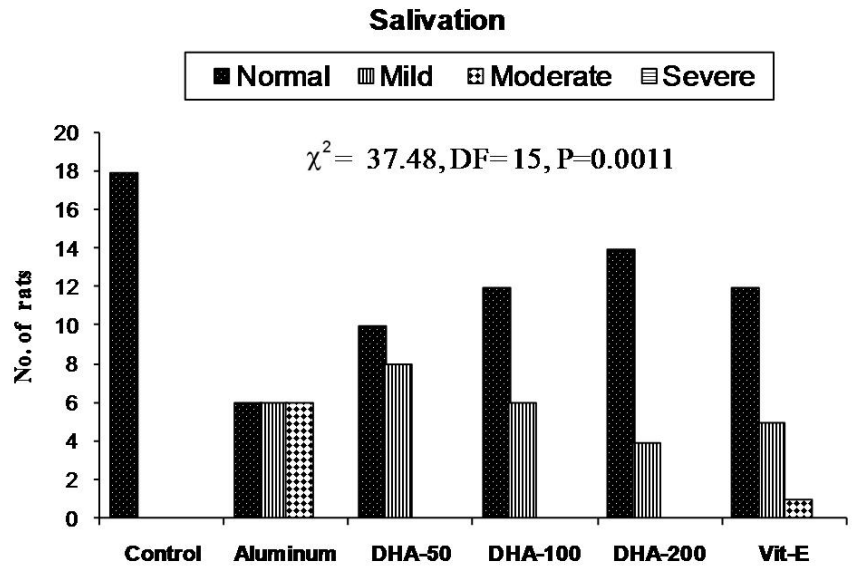

Fig.2. Effect of aluminum on salivation. Data are presented as number of rats in each group (n=18).

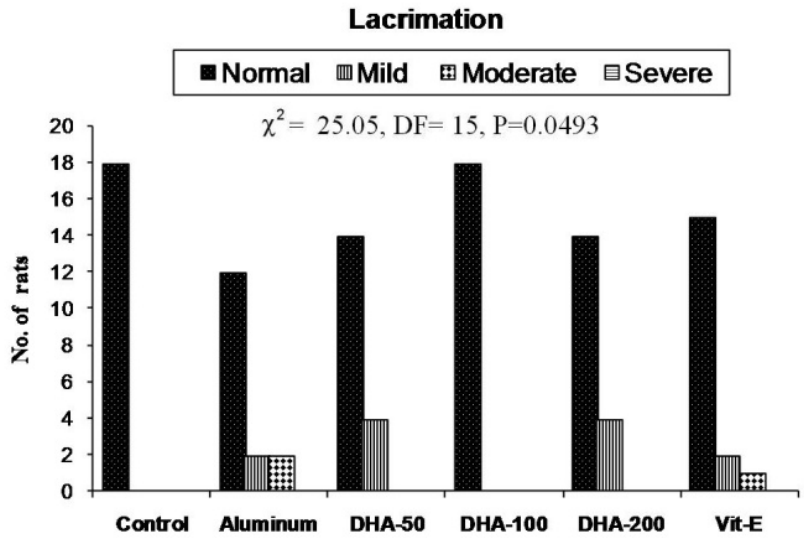

Fig.3. Effect of aluminum on Lachrymation. Data are presented as number of rats in each group (n=18).

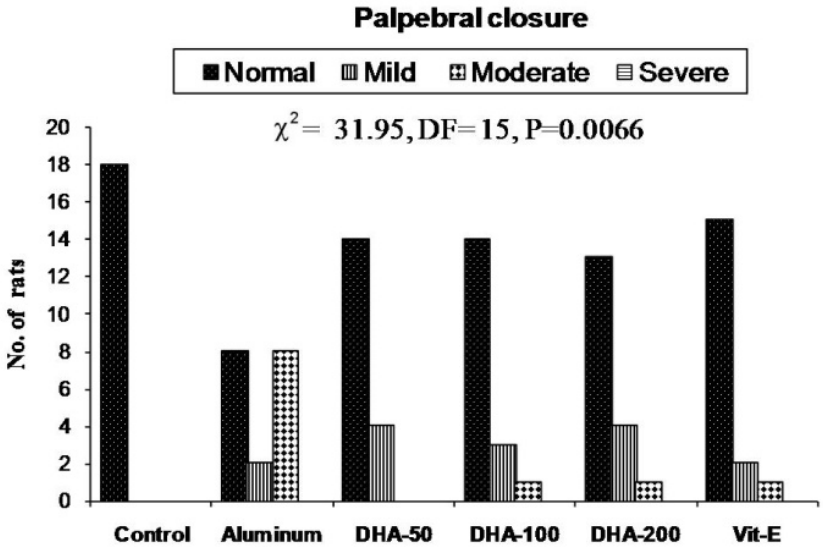

Fig.4. Effect of aluminum on palpebral closure. Data are presented as number of rats in each group (n=18).

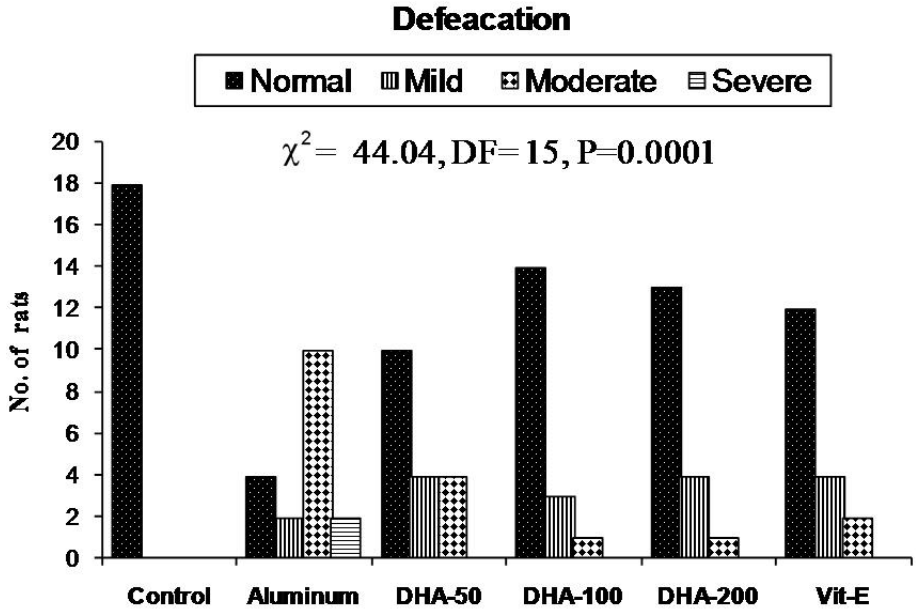

Fig.5. Effect of aluminum on defecation. Data are presented as number of rats in each group (n=18).

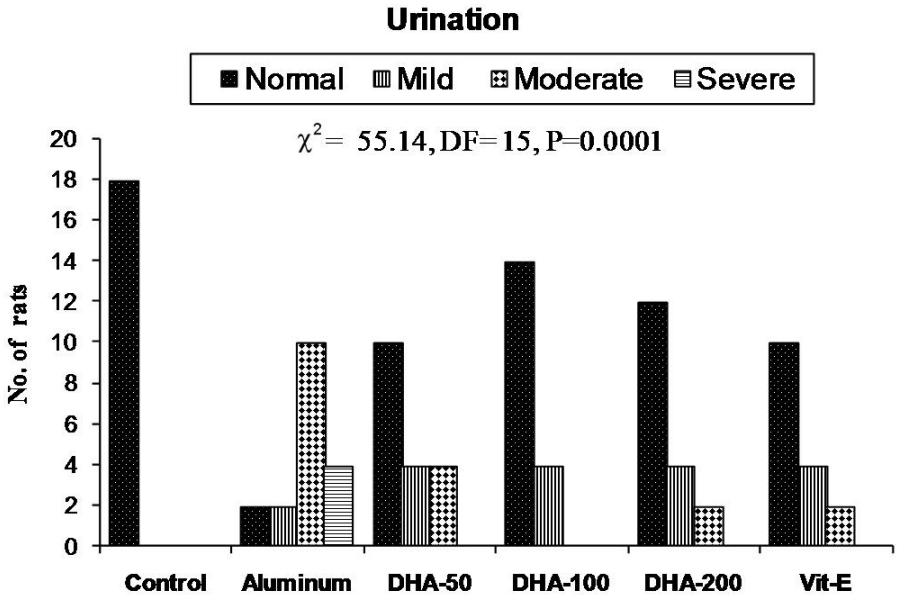

Fig.6. Effect of aluminum on urination. Data are presented as number of rats in each group (n=18).

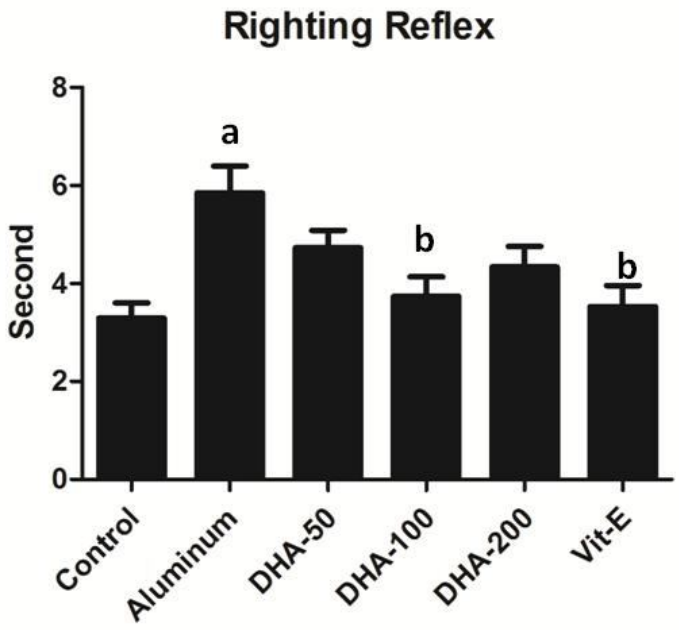

Fig -7. Showing Righting reflex and data are presented as Mean ±SE. The acceptance of significance is p>0.05 and compared with control and Al treated groups (a) and drug (DHA and Vit E) treated with Al treated rats (b).

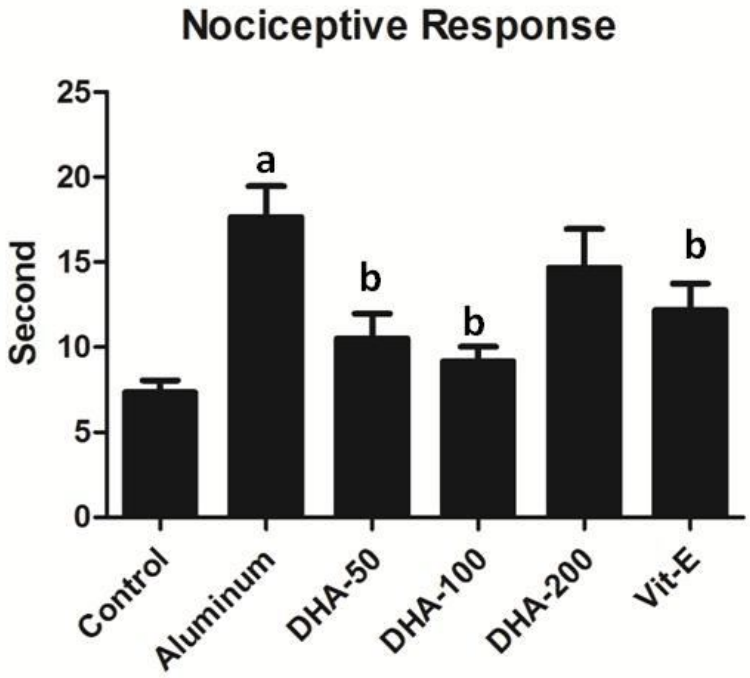

Fig -8. Showing Noceceptive response and data are presented as Mean ±SE. The acceptance of significance is p>0.05 and compared with control and Al treated groups (a) and drug (DHA and Vit E) treated with Al treated rats (b).

CONCLUSION

The results of the present study conclude that a prolonged oral Al exposure produces behavioral effects in rats. Al neurotoxicity affects the neurobehavioral changes consist of facilitating autonomic, gross behavioral and sensory response. These non invasive predictive markers facilitate Al induced neurodegeneration in animals and it could be used as a clinical indices in human study. The docosahexaenoic acid reduces the risk neurotoxicity. DHA 100mg dose were appropriate to ameliorates Al neurotoxicity. Consequently, the exposure to Al should be reduced and attention paid to sources of aluminium in foods, water and personal-care products. Furthermore, using diets rich in DHA could be beneficial in neurodegenerative disorders such as AD. In addition, further study is required for the increment of the knowledge regarding the mechanism of action of DHA protection over Al toxicity.

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