SPIRULINA ATTENUATES ALUMINUM INDUCED NEUROTOXICITY IN SWISS ALBINO MICE (MUS MUSCULUS L)

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ABSTRACT

Protective role of diet supplement Spirulina (@ 230 mg/kg body) has been explored in Swiss albino mice exposed orally to sub-acute dose (78.4mg/kg body weight) of aluminum. Standard protocols were followed for studying brain histopathology. The general health of aluminum treated mice deteriorated, more particularly of those reared only on standard chow. Molecular, granular and purkinje layer were closely placed to each other in the cortical region of the cerebellum in controls were lying apart in aluminum treatments. Nerve cells in the molecular layer were necrosed and there was reduction in the granular cell counts. The lysis of purkinje cells disrupted continuity of purkinje layer. Other abnormal features noted in purkinje cells were alteration in their size, vacuolization, enucleated condition and reduction in number (19-30.5%). The medullary nerve fibers present in between granular layers were compact, distinct and form a clear network in controls but these were loose and hazy in Al treatments. Such toxic effects on histopathology were comparatively lesser in the diet modulated Spirulina group.

INTRODUCTION

Aluminum is the third most abundant trivalent light metal in earth’s crust. It makes up about 8% of the earth’s crust in undecomposed rock fragments and in secondary aluminum silicate clays (Verstraeten et al. 2008, ATSDR 2006). The possible major sources of human consumption of aluminum are through food, drinking water and drugs (Becaria et al. 2002, Pournourmohammadi et al. 2008). Total dietary intake has been estimated at 4-9 mg/day (Yokel and Mcnamara 2001). Other source of aluminum to humans is the two most commonly used defluoridation techniques viz. Nalgonda and Activated Alumina process associated with high free residual aluminum content (Gupta 1997).

Brain possess complexities as it has variety of levels (local modules of neurons, single neurons and their geometry, synapses and neuromodulators) and information processing powers (in memory, perception, concept, creation, action control and so on) (Taylor et al.2005). Neurons are the basic functional unit of the brain. They have electrochemical connection with each other to exchange information, forms a network in body. Cerebellum is located at the bottom of the brain, with the large mass of cerebral cortex.

Aluminum accumulates in all the regions of brain with maximum accumulation in cortex region especially in hippocampus. Aluminum brain concentration should be lower than 2ug/g (Andrasi et al. 2005). The elimination half-life of aluminum from the human brain is 7 years. Tissue accumulation of aluminum in human beings has been associated with the occurrence of several clinical disorders including memory loss resulting in Alzheimers disease (Gupta et al. 2005).

Toxic agents badly affect brain histology as number of nerve cells and brain tissue was reduced. Reduction in number of purkinje cells was recorded when exposed to alcohol (Phillips et al. 1987), nicotine (Wei-Jung et al. 2003), aluminum phosphide (Tripathi and Pandey 2007) and oxidative stress (Bhalla and Dhawan 2009).

In stressed conditions purkinje cells were dead and hyperchromatic called ‘Dark’ neurons were increased in stressed condition in rats (Rani et al. 2001). Oxidative stress caused structural damage to brain, cause disorganization in layers of cerebrum and increase vascular spaces in rat brain (Bhalla and Dhawan 2009). Cerebellar cortical region showed infiltration in round cells in molecular layer and disappearance of processes of purkinje cells and subcortical zone showed degeneration of nerve fibers and the
appearances of necrotic patches in aluminum phosphide toxicity (Tripathi and Pandey 2007).

Spirulina (SP) is a blue-green algae used in the daily diet of natives of Africa and America (Ciferrri 1983). Recent studies have documented role of Spirulina as a therapeutic supplement in health management, besides being a rich protein source in diet. Owing to maximum protein content among both plant and animal kingdoms, it reduces metal toxicity when supplemented in diets (Jeyprakash and Chinnaswamy 2005). It also has phytotherapeutic role that is assigned to its rich content of protein (60-70% by weight), vitamins, especially vitamin B₁₂ and pro-vitamin-A (β-carotene) (Careri et al. 2001), minerals, especially iron and antioxidants like phycocyanin and phycocyanobilin (Reddy et al. 2000). One of the few sources of dietary γ-linolenic acid (GLA), it also contains a host of other phytochemicals that have potential health benefits. It has been reported as an immunomodulator (Ishii et al. 1999) and antioxidant (Reddy et al. 2000). It also possesses medicinal properties such as anti-cancerous (Mishra et al. 1998), anti-viral (Hayashi et al. 1996), hyperlipidemia (Torres-Duran et al. 1998), probiotic (Parda et al. 1998), and effective against diabetes, obesity and retarded blood circulation (De Caire et al. 1995).

The objective of the present study was to analyze the affect of aluminum on general health of animals and on brain histology and their protection with Spirulina.

MATERIALS AND METHODS

Animals

Healthy, mature Swiss albino male mice (age about 75 days) weighing 25-30g were acclimatized one week prior to entry into the experimental protocol. Animals were housed in a well ventilated animal house of Zoology Department, University of Rajasthan, Jaipur, as per guidelines of Institutional Ethical committee (Temperature = 25 ± 3°C, humidity = 40-60%, 12hr light: dark cycle) and were fed standard chow (Ashirwad Ltd., Chandigarh) and potable water ad libitum through out study.

Test chemicals

Reagent grade aluminum sulphate [Al₂(SO₄)₃·16H₂O: (Merck Ltd., Mumbai, India)) was dissolved in the distilled water. Spirulina capsules (Trade name: Sunova, Dabur India Ltd.) purchased from the drug store served as diet supplement @230mg/kg body weight/day.

Sub-acute Toxicity

To test sub-acute toxicity of aluminum, 40 male mice were separated into 4 groups having 10 mice each; Group I as standard feed control, Group II as Al treated, Group III as Spirulina control and Group IV as Spirulina + Al treated group. Treated groups were administered 78.4mg/kg body weight of Al for 7 days.

Autopsy

All animals of a group were sacrificed by cervical dislocation. Their brain was dissected out carefully and examined for gross abnormality, then cleaned with normal saline (0.9% NaCl solution), weighed and quickly fixed in Bouin’s solution for histopathological studies.

Statistics

Data expressed as Mean ± SEM were statistically evaluated using ANOVA. A significance level of P < 0.05 was accepted.

RESULTS

General health

Dullness, dyspnea, blackening of nails and tail, relatively less hairy fur becoming fragile were the visible symptoms of aluminum toxicity in group-II & IV whereas mortality (20%) was observed in only group II. Before death, body of animals swelled increasing their body size by 20-30%. When dead mice were dissected, ballooning of their alimentary canals and lungs were noted. In contrast, control mice (group I and III) were healthy and active having better fur quality, more particularly in Spirulina group.

Histopathological studies

In group I (Fig. A,B,C,D), III (Fig. E,F,G,H) and IV (Fig.I), the cortex region of cerebellum had three deeply stained layers namely molecular, granular and purkinje layer. Layers found closely attached to each other in controls (group I & IV) were hyperchromatic (group III) but were lying apart in group II due to large gaps in between molecular and granular layers (Fig. J). Necrosis of nerve cells was pronounced in the molecular layer of group II and IV (Table 1) and was marked by the occurrence of dead shrunken cell mass (Fig. J, K, L, M, N, O). Granular cell counts decreased (22% and 12%, significant at P<0.01%) in group II and IV in comparison to their controls (Table 1). The lysis of purkinje cells disrupted continuity of purkinje layer in Al treatments (group II and
Micro-photographs of T.S. of cerebellum in control mice: Fig. A. Closely placed all three layers of cerebellar cortex (100X). B. C. Normal structure of cerebellar cortex and Nerve fiber layer of medulla (400X). D. Normal structure of Purkinje Cell Layer; Granular layer and Molecular layer of cortex (1000X)
ML = Molecular Layer; PCL = Purkinje Cell Layer; NFL = Nerve Fiber Layer; GL = Granular layer

IV) (Fig. J,K,L,M,N,O). Other abnormal features noted in purkinje cells were alteration in their size (both increase (balloon cell) (Fig. J,K,L) and reduction in size), vacuolization, enucleated condition (Fig. J,K,L,M,N,O) and reduction in number (19-30.5%, Table 1).

The medullary nerve fibers present in between granular layers were compact, distinct and form a clear network in controls but these were loose and hazy in Al treatments (Fig. J, K, L, M, N, O). Their thickness also increased (5.3-8.8%) in treated groups compared to their controls (Table 1).
Microphotomicrographs of T.S. of Cerebellum of *Spirulina* supplemented Control mice: **Fig. E.** Closely placed all three layers of cerebellar cortex (100X). **F.** Normal structure of cerebellar cortex and NF of medulla (400X). **G, H.** Normal structure of PCL; GL; ML of cerebellum cortex (1000X). **ML = Molecular Layer; PCL = Purkinje Cell Layer; NFL = Nerve Fiber Layer; GL = Granular layer.**
Microphotographs of T.S. of Cerebellum of Spirulina supplemented aluminum treated mice: **Fig. I.** Closely placed all three layers of cerebellar cortex (100X)

**Microphotomicrographs of T.S. of cerebellum in aluminum treated mice:** **Fig. J.** PCL and GL lying apart to each other (100X), **K.** Necrosis in ML; damaged PCL; shrunken hyperchromatic Purkinje cell; loose and hazy NF; lysis in nerve fibers (400X), **L.** Vacuolization in Purkinje cell; aggregated Granular cells; depletion in GL; necrosis and lysis in ML (1000X)

ML = Molecular Layer; PCL = Purkinje Cell Layer; NFL = Nerve Fiber Layer; GL = Granular layer; Shrunken hyperchromatic Purkinje cell; Lysis (□); Vacuolization (★)

Microphotographs of T.S. of Cerebellum of Spirulina supplemented aluminum treated mice

**M.** Vacuolization in PCs, hyper chromatic PCs; degeneration and aggregation in NF (400X); **N, O.** Initiation of nuclear vacuolization in PC; depletion and clumping in GL; necrosis and lysis in ML (1000X); ML = Molecular Layer; PCs = Purkinje Cells; GL = Granular layer; NF = Nerve Fiber; degeneration (←→); nuclear vacuolization (★); necrosis (★); lysis (□); NFL = Nerve Fiber Layer
Table 1. Quantification of selective cellular structures and layers in the brain of control and aluminum treated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of necrosed cells in molecular layer per mm²</th>
<th>No. of granular cells per mm²</th>
<th>No. of Purkinje cells per mm²</th>
<th>Gaps b/w granular and molecular layer(μm)</th>
<th>Thickness of nerve fibers(μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet : Standard Feed</td>
<td>124±23</td>
<td>31667±728</td>
<td>103±6</td>
<td>14.5±2.5</td>
<td>5.7±0.3</td>
</tr>
<tr>
<td>Control</td>
<td>(−166.7%)</td>
<td>(−22.1%)</td>
<td>(−19.0%)</td>
<td>(+90.1%)</td>
<td>(+8.8%)</td>
</tr>
<tr>
<td>Al Treatment</td>
<td>331±40***</td>
<td>24667±898***</td>
<td>83±4*</td>
<td>37.6±48**</td>
<td>6.2±0.4</td>
</tr>
<tr>
<td></td>
<td>(+166.7%)</td>
<td>(+22.1%)</td>
<td>(+19.0%)</td>
<td>(+90.1%)</td>
<td>(+8.8%)</td>
</tr>
<tr>
<td>Diet : Standard Feed + Spirulina</td>
<td>145±20</td>
<td>28227±649</td>
<td>113±5</td>
<td>10.6±1.8</td>
<td>5.7±0.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al Treatment</td>
<td>231±26*</td>
<td>24827±598**</td>
<td>79±5***</td>
<td>24.3±4.1**</td>
<td>6.0±0.3*</td>
</tr>
<tr>
<td></td>
<td>(+59.5%)</td>
<td>(+30.5%)</td>
<td>(+129.8)</td>
<td>(129.8)</td>
<td>(+5.3%)</td>
</tr>
</tbody>
</table>

*Significance at 5%, ** 1% and *** 0.1% probability, data in parenthesis indicate percent change in values in comparison to control

**DISCUSSION**

Histopathological studies of lower brain i.e. cerebellum revealed disorganisation and degeneration of neuronal layers and increase in gaps between them. This might be due to ‘Al’ promotes biological oxidation both in vitro and in vivo. The reactive oxygen species (ROS) interact with all biological macromolecules, including lipids, proteins, nucleic acids and carbohydrates. The resulting oxidative stress increases neuronal death, which contributes to the neuropathy associated with several diseases (Baydas et al. 2003). Bhalla and Dhawan (2009) reported disorganization in layers of cerebrum and vacuolar spaces due in Al treatment indicating structural damage.

During present study, lysis and necrosis were noticed in molecular layer of both control and treated mice. Lysis was however, prominent and quantitatively number of necrosed cells were higher in Al treated mice.

The irregular distribution of granular cells, depletion in granular layer and number of granular cells in aluminum intoxicated mice was possibly on account of lysis or apoptosis. Besides, instead of complete lysis, only dendrites and axons were lost that led to aggregation of granular cells. Tripathi and Pandey (2007) reported degenerated granular layer in ‘Al’ phosphide exposed human brain. The subcortical zone of brain showed degeneration of nerve fibers and the appearance of necrotic patches.

Purkinje neurons displayed several irregularities in their structures and distribution in aluminum treated mice. Purkinje cells were shrunken, ballooned, kidney shaped, round and hyperchromatic. Vacuolization in cells and enucleated cells were also noticed in treated mice. In disrupted Purkinje layer, cells were absent at some places might be due to cell death by lysis or autolysis, necrosis or by apoptosis.

Scherini et al. (1981) documented the presence of dark purkinje neurons, which exhibit strongly electron dense cytoplasm with many free ribosomes, swollen mitochondria, dilated cisterons of rough and smooth endoplasmic reticula and hypertrophy of golgi complexes with many multivesicular bodies. They interpreted these features as signs of degeneration. Cummings and Lahunta (1988) reported shrunken purkinje neurons and also vacuolated purkinje cells in canine pups inheriting cerebellar degeneration. Rani et al. (2001) reported dark purkinje cells or hyperchromatic neurons under stressed conditions generated by immobilization of rats. Weijung et al. (2003) reported that long term nicotine exposure reduces Purkinje cell number in the adult rat cerebellar vermis. Purkinje neuron displayed many irregularities in their structures and distribution compared to control in fluoride treatment (Shashi 2003).

Changes in purkinje and granular cells, cellular disruption and degenerative changes may adversely affect coordination and control of voluntary movement in body. Dietary supplements like blueberry, spinach and Spirulina have neuroprotective effect and antioxidant enriched dietary pretreatment may induce protection through an anti-apoptotic mechanism (Wang et al. 2005).

During present study, Spirulina protected organization of tissue and layers in cortex region of cerebellum. It also protected molecular layer and nerve fibers. This protection may be due to its antioxidant properties.
Thus the results of present study have established toxic nature of aluminum to animals. These toxic effects may be reduced by supplementing their diet with Spirulina.

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