Efficacy of Ginkgo biloba in Aluminium induced neurotoxicity on Rat brain: Magnetization transfer and Diffusion weighted MRI study

S. Srivastava[1], S. Tripathi[2], A. A. Mahdi[2], and R. Roy[1]
[1]Centre of Biomedical Magnetic Resonance, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, [2]Department of Biochemistry, Chatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh, India

Introduction:
Aluminium (Al) is a known neurotoxin which causes alterations in blood-brain barrier, deposition of lipofuscin, demyelination and also promotes the oxidative damage of various bio-molecules. The controversial contribution of this metal ion in the pathogenesis of Alzheimer's disease has attracted numerous researches in the field of neuroscience[1]. Several cellular and ultra-structural studies have confirmed that demyelination and mitochondrial atrophy gets enhanced in the hippocampus of Al-exposed animals, indicating its possible involvement in Alzheimer's disease (AD). Ginkgo biloba (GB) extract (Ginkocer, Ranbaxy) is viewed as a possible therapeutic agent in the treatment of neurodegenerative disease like AD. The day-to-day exposure of aluminium in its various forms to the population and those undergoing dialysis indicate the need for studies which investigate the effectiveness of protective effects of such therapeutic agents against aluminium toxicity. In the present study, we have investigated the neuro-protective efficacy of Ginkgo biloba against aluminium induced neurodegenerative changes with the help of T1-weighted magnetisation transfer imaging (MTI), for identifying the structural status of brain and diffusion weighted imaging (DWI) for observing the functional changes occurring in brain at different time points of Al toxicity.

Material and methods:
The male Wistar rats (n=20) were divided into four groups with six rats in each group, except Al-treated group. 100 mg/kg/day of $AlCl_3$ (n=8) was orally administered to rats for 90 days. Another group of rats was administered only with GB (Ginkocer, Ranbaxy) (n=6; 40mg/kg/day) in order to assess its toxicological effect in rats. The control rats (n=6) were treated with only vehicles (water). DWI and T1 weighted MRI with and without saturation pulse was performed on 0, 30, 60 and 90 days of treatment. For MTI, gaussian saturation pulse was applied at 3000Hz. From day 60 onwards, half of the rats (n=4) of aluminium group were treated with GB only. MRI of rats was performed vertical 9.4-T Bruker Avance wide bore (80 mm) magnet equipped with microimaging acessories. Animals were anesthetized with ketamine (50mg/kg body wt.) and xylazine (16mg/kg body wt.), and were positioned in the vertical magnet bore in a standard orientation relative to gradient coils. ADC in cortex and hippocampal regions were measured with the use of a spin-echo sequence with diffusion weighting, as described by Stejskal and Tanner, along the phase-encoding direction. The diffusion gradient duration was 10 ms, and the diffusion time 50 ms. Five consecutive images with b_{min} value ranging from 29.5 to b_{max}= 1000 s/mm^2 with TR/TE of 3000/82.1 ms were acquired to quantify ADC. The results were statistically analyzed by one-way Anova with a post-hoc Bonferroni test. Values indicated are mean ± SEM.

Results:
Rats treated with aluminium showed significant decrease ($p<0.05$) in MTR values with respect to control group at second month of its oral administration. The variations in MT ratio values over time are described in Table 1 and 2. The ADC values increased significantly in Al-exposed group (Figure 1) while, when the same group of rats was treated with GB after two months of Al administration, the ADC values decreased significantly.

Discussion:
MT imaging can provide information about alterations in microscopic structures of brain reflecting its patho-physiological status. Several studies have correlated decrease in MT ratio with demyelination in multiple sclerosis, edema, infarcts and wallerian degeneration. According to earlier studies, Al is closely related with promotion of AD as it accelerates the accumulation of beta amyloid protein formation, which is neuro-toxic in nature. The higher Al content in brain promotes aggregation of beta-amyloid protein which results in formation of senile plaques and AD. The decreased MT ratio suggests demyelination in hippocampal region which is indicative of degeneration of intra-hippocampal nerve fibers including the preforant pathway, as supported by earlier studies[2]. The improvement in MT ratio in the Al toxicated post-GB treated rats, suggests its effectiveness against toxicity induced by Al-induced neuro-toxic damages. While MTI provided information regarding structural alterations, DWI provides information about the micro-environmental architecture of the brain. Diffusion depends primarily on the presence of micro-structural barriers like myelin sheath, axons and membranes of cell bodies of tissues which effect random motion of water molecules. Significant variations in mean ADC values in hippocampal and cortex region of rats may arise due to Al-induced neurofibrillary conditions in brain which involve synapse and neuronal loss. The protective effect of GB becomes visible after its post-treatment where it shows comparable ADC values as that of control group, indicating its effectiveness in Al induced toxicity and cell-death. On the basis of the result it may be concluded that aluminum alters macromolecular degeneration in the brain and GB is effective and possibly ameliorating the neurotoxicity during prolonged aluminum insult.

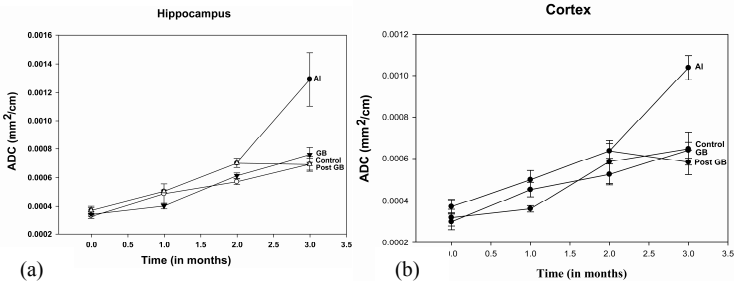

Figure 1: Variation of mean ± SEM ADC values (mm^2/cm) over time in (a) hippocampal region and (b) cortex of all the groups has been depicted, where Al=aluminium treated group, GB= Ginkgo biloba and Post GB= aluminium group treated with GB.

Table 1:MTR mean ± SEM value in hippocampus region of the Brain

	Control	GB	Aluminium	Post GB
1st Month	31.4 ± 3.2	29.7 ± 2.1	21.3 ± 2.2[a]	
2nd Month	27.9 ± 2.7	28.3 ± 2.9	17.5 ± 0.8[ab]	
3rd Month	31.2 ± 0.6	30.8 ± 0.6	14.7 ± 0.8[a]	22.3 ± 3.9[c]

Table 2:MTR mean ± SEM value in cortex region of the Brain

	Control	GB	Aluminium	Post GB
1st Month	23.76 ± 6.9	21.3 ± 1.6	18.33 ± 1.17	
2nd Month	20.4 ± 1.32	19.13 ± 1.14[b]	11.51 ± 1.06[a]	
3rd Month	17.12 ± 2.16	16.69 ± 0.99[b]	8.56 ± 1.02[a]	15.02 ± 1.55[b]

Table (1) and (2): The MT ratio are described in cortex and hippocampus regions of brain. Values are expressed as Mean ± SEM. Superscripts relate significant ($p< 0.05$) comparison with (a) control, (b) GB, (c) Al, (d) Post GB.

References:

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