Summary
Chapter 5

SUMMARY

The research database on aluminium is enormous and several biological effects have been reported. However, experiments have been, in general, carried out to answer specific research questions, not to set permissible values for either domestic or occupational human exposure. A detailed study in this regard is needed. Aluminium is a trivalent cation that does not undergo redox changes. It has, nonetheless, been implicated in a variety of neurological disorders that have been associated with an increase in the formation of reactive oxygen species. The exact mechanism of aluminium toxicity is not known. The controversial hypothetical link between aluminum and Alzheimer’s disease has been supported by several epidemiological studies. A detailed study on the events that occur at the cellular and molecular levels in different regions of brain would largely explain the neuropathological and neurophysiological events associated with aluminium exposures. The conjunctive administration of neuroprotectants will further strengthen the mechanisms.

In the present investigation, we have created a model of aluminium neurotoxicity and thereafter studied the various cellular and molecular consequences. Projecting results from animal models to clinical set ups have imposed problems and/or they are usually incomplete because the end points of the two are different. The animal models mainly rely on the histopathological observations whereas the clinicians are more concerned with functional outcome. Nonetheless, the animal models have to be designed so as to evaluate the mechanism of the insult so that appropriate therapeutic interventions can be aimed at. The animal model for aluminium toxicity was created by preliminary studies done at three different doses i.e. 40mg/kg body weight, 100mg/kg body weight and 150mg/kg body weight for a period of 8 weeks in different regions of the rat brain viz cerebral cortex, mid brain and cerebellum. 60% mortality rate was observed in animals receiving dose of 150mg/kg body wt. No evidence for mortality was however seen in animals receiving 40 & 100 mg/kg of body wt dose. Though some neurological defects were evident in 100mg/kg of dose which includes forward head tilt, hemiplegic gait, loss of appetite, splaying of the extremities and paralysis, no such symptoms were observed in 40 mg/kg group animals. Further, the behavioral alterations like short-term memory loss
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and motor dysfunction were more prominent in the animals receiving dose of 100mg/kg of body wt. Significant alterations in oxidative stress parameters and biogenic amines (done with the help of HPLC) were observed in 100mg/kg of body wt group in all the three regions of brain i.e. cerebral cortex, mid brain and cerebellum. A dose of 100mg/kg body weight/day of aluminium chloride administered orally was found to be neurotoxic i.e. sufficient for causing functional changes in brain of treated rats. Henceforth, only the 100mg/kg body weight/day group was used for further investigations. The results of the various investigations obtained during the present study are summarized below:

- Chronic aluminium exposure for eight weeks resulted in a net reduction in the body weight gain and increase in brain weight when compared to the respective normal control rats.
- The activities of acetyl cholinesterase and cytochrome oxidase in the serum and platelets respectively, were decreased, whereas the activity of glucose-6-phosphate dehydrogenase, in the erythrocytes was elevated following chronic aluminium administration.
- There was a significant accumulation of aluminium in all the three regions of the brain following chronic aluminium exposure, the maximum being in the mid brain region (5 fold) followed by cerebral cortex and finally cerebellum.
- Cellular distribution of aluminium in brain revealed maximum localization in neuron followed by glial cells.

Several newer drugs/photochemicals are being tried in the treatment/management of neurodegeneration caused by aluminium toxicity. The curcumin (1,7-bis (4-hydroxy-3-methoxy phenyl)-1, 6-hetadiene-3, 5-dione), a major yellow phenolic active curcuminoid present in turmeric used in the diet, is non-toxic and protective pharmaceutical, nutritaceutical, and phytoceutical agent. It has a plethora of beneficial effects such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-viral, and anti-infectious effects etc. Its neuroprotective role is not widely explored. In this context the present study was designed to delineate the role of curcumin in aluminium induced neurotoxicity.

Further, 21-Aminosteroids or “lazaroids,” a novel series of lipid peroxidation inhibitors, have the ability of localizing in cerebral microvasculature and have been shown to attenuate BBB disruption and protect the neural tissue by inhibiting lipid
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peroxidation. We extend our present study to evaluate the potential therapeutic
efficacy of U74500A following aluminium neurotoxicity.

Therefore, the present study was conducted to assess the precise role of natural
and synthetic antioxidants in conditions where aluminium toxicity leads to a series of
cellular and molecular events that may initiate and promote neurodegeneration. The
modulatory effects of curcumin and U74500As are evaluated for behaviour responses,
neurotransmission, antioxidant defence system, carbohydrate metabolism,
mitochondrial dysfunction, apoptosis, inflammation and histoarchitecture in animals
exposed to aluminium treatment.

- Short-term memory as assessed by a retention time was significantly decreased
  (66%) in aluminium treated animals when compared to normal controls.
  Curcumin and U74500A administration in conjunction with aluminium was able
to improve the memory by 131.6% with curcumin and 89% with U74500 as
compared to their respective aluminium exposed animals. Further, the
aluminium exposed animals showed a significant increase in the number of
escape trials (active avoidance test) as compared to the normal control animals.
Curcumin and U74500A co-administration proved beneficial, and the animals
escaped 4.35 trials after curcumin and 6.57 trials after U74500A administration
as compared to 10.64 trials (on average) in the aluminium treated group.

- A significant decrease in the muscular activity [as analysed by rota rod response
  (28%) and total locomotor activity (36%)] was observed after aluminium
treatment as compared to control animals. Whereas, in combined treatment
group with curcumin, 43% improvement in muscle strength and 45%
improvement in total locomotor activity was observed. U74500A co-
administration did not show much improvement in the muscular activity.

- Aluminium had a detrimental effect on the cholinergic system. There was a
decrease in the activity of acetyl cholinesterase in all the regions, following
aluminium exposure. Curcumin co-administration to aluminium treated animals
significantly increased the AChE activity in all the three regions of brain (92%
in cerebral cortex, 50% in mid brain and 70% in cerebellum) and the activity
were restored to normal levels in cerebral cortex. Similar results were obtained
after co-administration of U74500A (111% cerebral cortex, 63% mid brain and
70% cerebellum) with aluminium. The neurochemical impairments following
aluminium neurotoxicity are ultimately reflected in terms of the neurobehavioral
deficits i.e. of memory and motor functions, which were significantly depressed at the completion of the treatment in the chronically treated animals.

- A significant increase in the monoamine oxidase enzyme activity was observed after aluminium exposure. The enzyme activity was decreased in all the three regions of brain following curcumin (39% in cerebral cortex, 38% in mid brain and 59% in cerebellum) treatment. No significant change was observed after U74500A treatment (13% in cerebral cortex, 11% in mid brain and 6% in cerebellum).

- A significant decrease in the serotonin and dopamine levels was observed in all the three regions of brain after aluminium exposure. Curcumin administration to aluminium treated rats proved beneficial and an increase in the serotonin (with maximum in cerebral cortex-75%) and dopamine (with maximum in mid brain-147%) levels were observed in all the three regions and the values were restored to normal levels. U74500A co-administration did not show much significant change in serotonin (27%) and dopamine (22%) levels.

- A statistically significant increase in the levels of lipid peroxidation and reactive oxygen species in all the three regions was observed as compared to normal control animals. Curcumin supplementation to aluminium treated rats was able to reverse the effects and significantly decreased the lipid peroxidation (by 27%) and reactive oxygen species levels (by 65%) in all the three regions of brain. Comparatively U-74500A was more effective in decreasing the lipid peroxidation burdens but total ROS was same. Following U-74500A co-administration was also able to significantly decrease the lipid peroxidation by 47% and reactive oxygen species levels by 65% in all the three regions of brain.

- Significant increase (2 to 2.6 folds) in the activity of SOD was observed in all the three regions of the brain following aluminium exposure. Curcumin administration to aluminium treated animals significantly decreased SOD by 55% and that following the conjunctive administration of U74500A the decrease was to an extent of approx 70%.

- In contrast to SOD levels, the aluminium exposure decreased the catalase activity by 45% in cerebral cortex, 47% in mid brain and 36% in cerebellum. The conjunctive administration of both curcumin and U74500As independently significantly increased catalase activity by ~71%.
The levels of total and oxidized glutathione were significantly decreased in all the three regions of brain in aluminium treated animals in comparison to normal controls. However, GSH contents were found to be significantly increased in cerebellum whereas decrease in GSH content was observed in cerebral cortex and mid brain after aluminium treatment when compared to normal control. Significant decrease in redox ratio was observed in mid brain (54%) and cerebellum (83%) after aluminium exposure. Following Curcumin co-administration along with aluminium, and also in the animals that received conjunctive exposure of U74500A and aluminium the total glutathione level was increased to about 36% but the levels were not normalized. These changes were in turn reflected in the redox ratio, which also improved in conjunctive groups.

Conjunctive treatment of curcumin had a significant effect on GR activity where a two-fold increase was observed which was able to restore the total glutathione content. Simultaneously, the glutathione peroxidase activity also increased by 50% which overall improved the redox ratio as mentioned earlier. A similar increase in GR activity was also observed after U74500A co-administration, which was around 3 folds.

In contrast no significant alterations were seen in the GST activity in any of the treatment groups.

The NOS enzyme activity and the levels of L-citrulline were found to be significantly increased during aluminium exposure. Curcumin supplementation to aluminium treated animals significantly decreased the enzyme activity with maximum decrease in cerebral cortex (60%) as well as the L-citrulline levels (~26%). No changes were observed in NO and L-citrulline levels following the co-administration of U74500A and aluminium.

Aluminium exposure brought a significant decrease in the activities of glycolytic enzymes viz hexokinase, SDH, dehydrogenase, phospho glucose isomerase but the activity of lactate dehydrogenase was increased. These alterations in the glycolytic enzyme activities were reversed significantly during conjunctive exposures of curcumin but not significantly by U-74500A.

Similarly the co-treatment groups of both curcumin and U74500A were able to restore alterations in the ATP synthesis and hydrolysis, which were significantly, impaired following aluminium exposure. Further, the components
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of the electron transport chain, i.e. mitochondrial complexes and also the cytochromes depicted similar trends.

➢ To further characterise the mechanisms of cell deaths in the present study, induction of apoptosis, the cellular self-destruction process was evaluated. Decreased expression of Bcl-2 was observed after aluminium exposure, which further leads to the opening of MPTP pore. Cytochrome c was released from mitochondria to cytosol which further activates caspase-3 causing apoptosis and hence DNA fragmentation. The DNA damage caused in the cells as a result of aluminium treatment was examined by single cell gel electrophoresis as well as DNA fragmentation studies. 72% of DNA damage was observed in comparison to 26% in normal control rats. Treatment with curcumin inhibits the cleavage of pro-caspase-3 to the active caspase-3 p17. Curcumin prevented aluminium-induced apoptosis through Bcl-2-mitochondria-ROS pathway. Curcumin induced the over-expression of Bcl-2, which is in contrast to decreased level of Bcl-2 expression during aluminium exposure. This suggests that Bcl-2 could play an important role in mediating the neuro-protective properties of curcumin. DNA damage was reduced to 44% in conjunctive group as compared to 72% after aluminium exposure. Co-administration of U-74500A significantly inhibited caspase-3 levels in the cytoplasmic fraction of mid brain homogenate. No DNA fragmentation was observed after U-74500A co-treatment and also following curcumin and aluminium exposures.

➢ Chronic administration of aluminium resulted in significant increase in levels of TNF-α to an extent of 45%. TNF-α further induced the activation of NF-κB and hence increases in caspase-3 activity. Co-administration of U-74500A significantly inhibited NF-κB p65 unit levels in the nuclear fraction of rat brain homogenate and hence decreases caspase-3 activity, which prevents apoptosis.

➢ Histologically while the normal brain depicted the presence of well organised cortical layer, ventricular spaces with choroids plexus, ependymal lining, necklace of neurons in the ammicon region of the hippocampus, basal ganglion etc.

➢ The cerebral region of aluminium treated group depicted evidences of hypoxia in the cortical neurons and corpus callosum. Loosening of fibers in corpus callosum was also evident. There were significant evidences of perineuronal edema. The cerebellar region also depicted the presence of focal perivascular
inflammation. The sections also depicted paucity of neurons both in the cortical and in the hippocampal regions. No evidences of plaque formation were seen both by congo red and H&E.

Marked improvement was observed in the conjunctive groups of both curcumin and U74500A, which included decrease in the hypoxic state of the neurons, and better organization of cortical layers, reduced edema as well as inflammatory responses which however were totally absent in the curcumin treated groups. Significant improvement in neuronal density in various regions was observed following the conjunctive administration of both curcumin and U74500As independently.

The cerebellar regions of all the treatment groups were well preserved whereby the folium depicted the presence of well-organised molecular purkinje cell and granular layer.

In conclusion, the results of the present study demonstrate the deleterious effects of aluminium administration in terms of altered energy metabolism, generation of oxidative stress, mitochondrial dysfunction and altered neurotransmission. The state of oxidative stress generated by perturbed electron transport chain of mitochondria was further observed by increased lipid peroxidation. Aluminium seemed to be cytotoxic and the specific effects induced by aluminium may initiate or mediate the cellular destruction as well. This study therefore unravels the biochemical mode of action of this environmental toxicant and more systematic studies are required in this direction for the development of an antidote and a better therapeutic regimen.

Further, in the search of neuro-protective effect of antioxidants the present work demonstrated the neuro-protective effect of curcumin and U-74500A in preventing the development of aluminium induced neurodegeneration. However the improvement to normal conditions was more significant after curcumin co-administration, which was projected to be a multi targeted approach as compared to U-74500A, which was more effective only in improving the consequences caused due to peroxidation of membrane lipids.