Preface

After the discovery of cadmium as an element in 1817, it attracted the attention for industrial use because of its non-corrosive nature. It has wide occurrence in the environment due to extensive anthropogenic uses and thus enhances the risk of human exposure in occupational settings. Besides, general population may also be exposed in non-occupational settings by consuming contaminated food and through air, soil and cigarette smoke. Although the first report of damaged lungs in cadmium exposed workers appeared in 1938, later health effects associated with *Itai-itai* disease aroused a great concern among the scientists. A number of epidemiological and experimental studies have been undertaken since then to understand the impact of cadmium on body organs and etiology associated in cadmium induced toxicity.

Although cadmium has been found to be carcinogenic and classified in the category of group IA chemicals according to IARC, disruption in the functioning of lungs, liver and kidney have also been reported extensively in cadmium exposed individuals. Due to long half life in the biological system (~ 18 - 20 years), it is cumulative in nature and

distributed in body organs. While peripheral neuropathy is widely recognized in cadmium exposed population, it has been found that cadmium easily crosses the blood brain barrier and affects the functioning of central nervous system. Risk of Alzheimer's disease has been associated with cadmium exposure as high cadmium levels in plasma, brain and liver have been detected in Alzheimer's patients. Acute exposure to cadmium has also been found to cause Parkinsonism. Increasing incidences of neurological and psychiatric disturbances associated with cognitive deficits on cadmium exposure in recent years is a cause of concern and reflect the vulnerability of brain while the exact mechanism is not known.

In view of increasing risk of cadmium induced neurotoxicity, a number of experimental studies have been carried out involving pharmacological agents and natural extracts to assess their protective potential. Quercetin, a polyphenolic flavonoid has wide occurrence in vegetables, fruits and many other dietary items. Although anti-carcinogenic, anti-inflammatory and vasodilating effects of quercetin are well documented, it has also been found to be effective in the management of nervous system disorders including Parkinson's and Huntington's disease. Further, protective effects of quercetin in chemical induced neurotoxicity are largely due to its antioxidant and free radical scavenging properties.

In view of this, the present study has been aimed to carry out *in vivo*, *in vitro* and *in silico* studies in an attempt to

i). unravel the molecular mechanisms associated with cadmium neurotoxicity focusing at the expression of the selected proteins involved in the process of synaptic transmission, oxidative stress and neuronal signaling and asses protective potential of quercetin, a flavonoid in rat brain

ii). screen the prophylactic, protective and therapeutic effect of quercetin in cadmium induced neurotoxicity in PC12 cells and

iii). elucidate the bimolecular targets involving docking studies

Experimental studies undertaken to decipher the molecular mechanisms associated with cadmium induced cognitive deficits exhibited decrease in the binding of cholinergic-muscarinic receptors and mRNA expression of cholinergic-receptor genes (M1, M2, M4) in frontal cortex and hippocampus on exposure of rats to cadmium (5.0 mg/kg body weight, p.o.) for 28 days as compared to controls. Cadmium exposure decreased mRNA and protein expression of ChAT and AChE and enhanced ROS generation associated with mitochondrial dysfunctions. Ultrastructural changes exhibiting mitochondrial damage both in frontal cortex and hippocampus assessed by transmission electron microscope and learning deficits monitored by Y-maze and passive avoidance response were also distinct in cadmium exposed rats. Enhanced apoptosis as evident by alterations in key proteins involved in pro and anti-apoptotic pathway and MAPkinase signaling was evident on cadmium exposure. Simultaneous treatment with quercetin (25 mg/kg body weight, p.o.) protects cadmium induced alterations in cholinergic-muscarinic receptors, mRNA expression of genes (M1, M2 and M4) and expression of ChAT and AChE. The protective effect on brain cholinergic targets was attributed to antioxidant potential of quercetin which reduced ROS generation and protected mitochondrial integrity by modulating proteins involved in apoptosis and MAPkinase signaling.

While investigating the molecular mechanisms associated with cadmium induced motor dysfunctions in an attempt to identify targets that govern dopaminergic signaling in brain involving *in vivo, in vitro* and *in silico* approaches, selective decrease in dopamine (DA)-D2 receptors was evident in corpus striatum of rats exposed to cadmium (5.0 mg/kg body weight, p.o.) for 28 days. There was no change in the expression of DA-D1 receptors in corpus striatum. Interestingly, cadmium induced decrease in DA-D2 receptors affected the post-synaptic PKA/DARPP32/PP1α and β-arrestin/Akt/Gsk-3β signaling concurrently in rat corpus striatum and PC12 cells. Pharmacological inhibition of PKA and Akt *in vitro* exhibit that both pathways are independently modulated by DA-D2 receptors and associated with deficits in motor activity. Ultrastructural changes in corpus striatum were distinct, exhibiting neuronal degeneration on cadmium exposure. Molecular docking studies *in silico* provided interesting evidence that decrease in DA-D2 receptors could be due to direct binding of cadmium at the competitive or non-competitive sites of dopamine

on DA-D2 receptors. Simultaneous exposure with quercetin (25 mg/kg body weight, p.o.) resulted to protect cadmium induced behavioral and neurochemical alterations. DFT studies suggest that quercetin has the tendency to form complex with cadmium and may be attributed to the metal chelating property of quercetin and thus reduced toxicity of cadmium. While investigating the prophylactic, protective and therapeutic effect of quercetin in cadmium induced neurotoxicity, studies were undertaken on differentiated PC12 cells *in vitro*. Protective and therapeutic effect of nano-quercetin was clearly evident. Interestingly, nano-quercetin was found to be more effective as compared to bulk quercetin in protecting cadmium induced dopaminergic alterations in PC12 cells. More interestingly, cadmium induced alterations in biogenic amines and increased expression of metallothionein (MT3) in brain regions were also found to be protected on simultaneous treatment with quercetin.

As neuroinflammation and autophagy have emerged as important mechanisms in chemical induced neurotoxicity and also in the progression of neurodegenerative diseases, effect of cadmium on the key targets involved in these processes and protective efficacy of quercetin were assessed. Increased expression of IBA1 and GFAP in hippocampus, frontal cortex and corpus striatum was evident on exposure of rats to cadmium exhibiting activation of glial cells. Further, activation of proinflammatory cytokines (TNFα, IL-1β and IL-6) associated with increased levels of NO and expression of both iNOS and nNOS was evident in all the three brain regions on cadmium exposure. Consequently, increase levels of NO and ROS may lead to the activation in the expression of MAPK and CamkIIα as observed on cadmium exposure may cause neuronal death. Cadmium exposure altered the expression of LC3II, p62 and Atg, marker proteins associated with the integrity of autophagy in rat hippocampus, frontal cortex and corpus striatum in *vivo* and also in SHYSY5Y cells *in vitro*. Using the specific pharmacological inhibitor (3MA) and activator (rapamycin) of autophagy, it was found that cadmium increased the autophagic flux and thus impaired the autophagic pathway that may lead to neurotoxicity. Interestingly, simultaneous exposure with quercetin was found to protect these changes.

The present study provides new information on understanding the cellular and molecular mechanisms associated with cadmium induced neurotoxicity. More interestingly, the potential of quercetin in protecting cadmium induced neurobehavioral alterations by modulating the signaling targets appear to be promising and provide a lead to further explore its role as a protective and therapeutic agent.