Chapter I

Introduction

Cadmium (Cd), one of the naturally occurring trace elements present in earth crust is distributed in the environment due to its extensive anthropogenic applications (Méndez-Armenta and Ríos, 2007; Wang and Du, 2013; Gomes de Moura and Ribeiro, 2017). Use of cadmium is common in smelting, mining and electroplating and cadmium salts are frequently used as color pigments in metals and plastics and in the manufacture of batteries and phosphate fertilizers (Järup et al., 1998; Järup and Åkesson, 2009; Satarug et al., 2011). Volcanic eruptions, burning of fossil fuel, incineration of municipal waste, recycling of cadmium plated scrap and electronic waste significantly contribute in the release of cadmium in the environment. Exposure to cadmium could occur through ambient air in urban areas close to industrial settings (Khade and Adholeya, 2009). High cadmium levels in drinking water have also been found in certain regions (Cai et al., 1995; Cerutti et al., 2003). As cadmium has high rate of transfer from soil to plants, vegetables, fruits and cereals have been found to be highly contaminated grown on cadmium contaminated soil. Interestingly, contaminated rice is one of the chief sources of

cadmium exposure in population at large in different regions across the world (dell'Omo et al., 1999). Besides, cigarette smoke is one of the potential sources of cadmium exposure (Wong and Klaassen, 1982; Satarug and Moore, 2004; Piadé et al., 2015). Human exposure to cadmium may therefore occur both in occupational and non-occupational settings and poses a serious risk to health and associated problems.

Ever since *Itai-itai* disease was associated with chronic cadmium poisoning in Japan in 1912 (Murata et al., 1969; Inaba et al., 2005), there has been a great impetus to assess the adverse health effects of cadmium. Cadmium is carcinogenic in nature and has been classified as class IA carcinogen according to IARC (Baan, 2010). Besides carcinogenic potential, it has been found to disrupt the functioning of lungs and liver in exposed individuals (Kolonel, 1976; Huff et al., 2007). Renal dysfunctions and decreased bone mineral density have been reported on exposure to cadmium in non-occupationally exposed individuals in a cross sectional study (Lemen et al., 1976). While monitoring the health outcomes, increased urinary levels of cadmium have been associated with hypertension, diabetes and diabetic nephropathy in different set of population (Bernard, 2008). The biological half life of cadmium in human body is around 18 – 20 years. Due to poor elimination, it is cumulative in nature and distributed in body organs (Méndez-Armenta and Ríos, 2007). A number of autopsy reports exhibit high accumulation of cadmium in body organs including brain (Chang et al., 2012; Hayashi et al., 2012). Cadmium easily crosses the blood brain barrier and thus brain is one of the soft target (Viaene et al., 1999). Toxic effects of cadmium are largely attributed due to its poor elimination from the body and thus high accumulation in tissues including brain (Shukla et al., 1996; Zalups and Ahmad, 2003; Gonçalves et al., 2010). Further, it has been found to affect the functioning of central and peripheral nervous systems adversely (Wong and Klaassen, 1982; Waalkes et al., 1992). Headache, vertigo and other neurological abnormalities including olfactory dysfunctions have been reported in cadmium exposed individuals (Marlowe et al., 1983). Cadmium exposure has been found to cause cognitive dysfunctions and affect the development of visual perception in children (Thatcher et al., 1982). 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. Although blood cadmium is considered to be an index to assess the extent of exposure, levels of cadmium have also been measured in urine, hair and other biological samples to monitor the neurological performance (Pihl and Parkes, 1977; Viaene et al., 2000). Epidemiological studies have found that exposure to cadmium may increase neuropsychological disturbances and memory deficits (Sanders et al., 2015). Children are more vulnerable to adverse health effects of cadmium since a negative correlation has been observed between maternal cadmium levels and performance IQ of children exposed during pregnancy. Risk of Alzheimer's disease has been associated with cadmium exposure as high cadmium levels in plasma, brain and liver were detected in Alzheimer's patients (Basun et al., 1990; Panayi et al., 2002). Involvement of cadmium in the formation of neurofibrillary tangles and amyloid beta peptides in experimental studies has further suggested its role in the pathogenesis of Alzheimer's disease. Numerous experimental studies have found that cadmium may cause brain cholinergic alterations and functional deficits (Carageorgiou et al., 2004). Motor dysfunctions have also been reported in humans on cadmium exposure (Okuda et al., 1997). Chronic exposure to cadmium has been found to cause peripheral neuropathy and affect psychomotor functions in occupational workers. In a cross sectional epidemiological study, cadmium levels in urine were inversely associated with visuomotor functions, peripheral neuropathy and disturbed equilibrium. Further, acute exposure to cadmium has been found to cause Parkinson's disease (Okuda et al., 1997). Interestingly, role of dopaminergic neurotransmission in the regulation of motor and reward system has been demonstrated (Beaulieu and Gainetdinov, 2011) and thus the possibility that cadmium may disrupt the functioning of brain dopaminergic system exists.

Cadmium may disrupt the metabolism of trace elements specially copper and zinc which play important role in the metabolism and kinetics (Antonio et al., 1999). It has been found that cadmium enhances the expression of metallothionein, a cysteine rich protein due to its high affinity in different organs including brain (Choudhuri et al., 1996; Hidalgo et al., 2001). A number of studies found that cadmium may decrease the calcium influx and inhibit the activity of Na^+, K^+-ATPase in the brain and thus affect the process of neurotransmission and energy metabolism respectively (Abdalla et al., 2014).

Alteration in synaptic transmission due to changes in the levels of brain biogenic amines and their metabolites on exposure to cadmium have been reported in experimental studies (Hastings et al., 1978; Lafuente et al., 2001). Interestingly, these changes were associated with neurobehavioral abnormalities (Andersson et al., 1997; Lafuente et al., 2001; Minami et al., 2001; Lafuente et al., 2003; Ashok et al., 2014). It has been found cadmium easily targets mitochondria in brain and other tissues and affects its functional integrity (Beal, 1996; Wang et al., 2004; Chang et al., 2013). Increased oxidative stress associated with enhanced apoptosis are potential mechanisms, largely accepted in cadmium induced neurotoxicity (Shaikh et al., 1999; Nemmiche et al., 2007; Liu et al., 2009). Further, a line of evidences suggest inflammation as a crucial mechanism in neurodegenerative diseases including Parkinson's and Alzheimer's disease and in chemical induced neurotoxicity (Liu et al., 2003; Griffin, 2006; Rogers et al., 2007; Hirsch and Hunot, 2009; Amor et al., 2010; Chen et al., 2016) (Mosley et al., 2006). Activation of microglia enhances production of inflammatory cytokines which in turn disrupt the signaling cascade and affect the neuronal integrity (Liu and Hong, 2003; Frank-Cannon et al., 2009; Lehnardt, 2010). Although cadmium has been found to affect the glial architecture in brain (Streit et al., 2004; Vargas et al., 2005; Qin et al., 2007; Tilleux and Hermans, 2007), the mechanism and targets associated with cadmium mediated neuroinflammation are not known. Besides inflammation, alteration in basal autophagy may also contribute in the pathogenesis of neurodegenerative diseases (Zhou et al., 2011b; Hensley and Harris-White, 2015). As autophagy and apoptosis work in a coordinated manner to regulate the cell survival and death (Maiuri et al., 2007; Eisenberg-Lerner et al., 2009; Kang et al., 2011; Zhou et al., 2011a; Gordy and He, 2012), any toxic insult or stress lead to the activation and accumulation of damaged proteins inside the cell and contribute towards the autophagy mediated cell death.

In view of increasing risk of cadmium induced neurotoxicity, a number of experimental studies have been carried out involving pharmacological and natural extracts to assess their protective potential. Flavonoids present in plants as glycosides have attracted much attention in recent years due to their high bioactivity, largely associated with their anti-oxidant potential (Unsal et al., 2013). Among flavonoids, quercetin, a class of flavonol is

widely present in vegetables, fruits, tea and many other foods (Formica and Regelson, 1995; Murota and Terao, 2003). The content of quercetin however varies in different dietary products (Wach et al., 2007). High levels of quercetin are also present in medicinal plants - *Ginkgo biloba* and *Hypericum perforatum* (Sultana and Anwar, 2008). High antioxidant capacity of quercetin has made it popular over other established antioxidants - vitamin C, vitamin E and β-carotene. Potential of quercetin to chelate transition metals has been found to protect iron induced Fenton's reaction (Cheng and Breen, 2000; Leopoldini et al., 2006). Although anti-carcinogenic, anti-inflammatory and vasodilating effects of quercetin are well documented, it has been found effective in the management of nervous system disorders (Chopra et al. 2000; Pereira et al. 1996; (Ho et al., 2013). Protective role of quercetin in cognitive deficits has been demonstrated in several experimental studies (Kumar et al. 2008; Liu et al. 2006; Singh et al. 2003; Yao et al. 2010). Interestingly, dopaminergic dysfunctions in animal models of Parkinson's disease and Huntington's disease have been found to be protected by quercetin (Sandhir and Mehrotra, 2013; Chakraborty et al., 2014). Quercetin has also been found effective to protect chemical induced neurotoxicity (Sharma et al., 2013; Chander et al., 2014). Further, promising effects of quercetin to modulate physiological functions in preclinical studies have strengthened its use in clinical situations in the management and treatment of many diseases (Linde et al., 1996; Okamoto, 2005).

Despite considerable investigations on cadmium neurotoxicity and moreover enhanced risk to develop Alzheimer's and Parkinson's disease, the exact mechanism by which it causes brain cholinergic and dopaminergic alterations and affect associated functions is not well characterized. Also, the exact mechanism and targets associated with the protective potential of quercetin in modulating cadmium induced neurotoxicity are not understood. The present study has there 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

iii. elucidate the bimolecular targets involving docking studies.