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Untangling the Role of Metallothionein- III in Cadmium Induced Alteration in Brain Biogenic Amines: Protective Potential of Quercetin

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Abstract

Cadmium, a non-transition heavy metal, is largely responsible for the development of neurological and neurodegenerative disorders. Epidemiological and prevalence studies suggest that high exposure to cadmium and consecutive high cumulation in the human body pose a high risk of various diseases. It has been identified that cadmium-induced motor deficits in rats are due to alterations in canonical and noncanonical Cyclic Adenosine (cAMP)-dependent PKA/DARRP32/PP1α associated with decreased Deficiency of Adenosine Deaminase 2 (DA-D2) receptors in the corpus striatum. Continuing those studies, current studies have been executed to untangle the role of Metallothionein- III in cadmium-induced alterations in biogenic amines in selected brain regions of rats. Treatment of rats with cadmium (5mg/kg, per oral) for 28 days caused a marked decrease in neurotransmitter levels in various brain regions while there was no significant change in DA turnover as compared to controls. Further, there was a striking increase in the levels of Metallothionein (MT-III) and levels of cadmium in these brain regions. *Insilico* studies involving ADMET Regression analysis further confirmed the changes wherein it has been shown that the cadmium easily crosses the blood-brain barrier and accumulates in the brain regions, binds with the Metallothionein and produces neurotoxicity. Interestingly, simultaneous exposure with quercetin (25mg/kg, per oral) was found to protect cadmium-induced alterations. The results exhibit that cadmium-induced alteration in biogenic amines may be associated with increased levels of metallothionein in the brain, predicting the toxicity of cadmium.

Keywords: Cadmium, Metallothionein, Neuroprotection, Neurotransmitter, Quercetin

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1. Introduction

Cadmium, a nonessential transition heavy metal has extensive industrial and other uses[1]. It is one of the most common environmental pollutants and toxic heavy metals. Due to its non-corrosive properties, cadmium has highly been used as an electroplating material in various industries as the other metals[2,3]. The high existence of cadmium in the atmosphere is because of its extensive anthropogenic and other uses, enhancing the probabilities of human exposure more in both non-occupational and occupational settings[4,5]. Other than industrial applications, high levels of cadmium in drinking water, food, beverages and tobacco smoke are also potential sources of exposure to cadmium in the general population[6-9]. Cadmium has also been classified in the category of group Alkali Metals (IA) chemicals and reflected as a potent carcinogen[10,11]. Interestingly, Due to its long half-life in the biological system, it gets cumulated into different body systems and thus gets distributed in the body organs and disrupts the functioning of lungs, liver and kidney in exposed individuals[5,11]. Further, with the significant reporting of peripheral neuropathy cases, cadmium has been reported to disturb the physiology of the central and peripheral nervous systems equally[12-15]. High levels of cadmium in blood and urine are associated with cognitive deficits frequently reported in population studies[2,16]. Epidemiological reports have further revealed that high exposure to cadmium increases the incidences of neurological and neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's diseases[5,17-20].

Metallothionein -3 is shown to be a key contributor to cadmium neurotoxicity, and its role has been widely accepted[1,21,22]. Expression of metallothionein-3, a cysteine-rich protein is enhanced in the brain and other tissues on exposure to cadmium and it may be due to its high affinity[23]. As cadmium is pro-oxidant in nature and impairs the antioxidant defence in body tissues, enhanced oxidative stress and apoptosis have been implicated in the aetiology of cadmium neurotoxicity[24]. The role of biogenic amines in relaying the signals among neurons to undergo signal transduction is well documented[25,26]. Even though biogenic amines are differentially distributed in brain regions, they are distinct to orchestrate functional behaviour including learning, memory, attention and motor activity[27,28]. Fluctuations in their levels either in the pathological state or on exposure to drugs/chemicals may lead to neurobehavioral abnormalities. Behavioural deficits on exposure to cadmium have been associated with a disruption in synaptic signalling due to alterations in the levels of brain biogenic amines[29,30].

With the upsurge of evidence of cadmium-related neurological disorders and neurobehavioral abnormalities, many studies are currently being conducted to identify the potential of various herbal and pharmacological agents in cadmium toxicity. Quercetin, a polyphenolic flavonoid is widely found in vegetables and fruits[31]. Divergent glycosidic forms of quercetin have been found present in plants[32]. The various properties of quercetin as anti-inflammatory, antioxidant and metal chelating effects are well established. It has also been found to be effective in managing neurological and neurodegenerative disorders like Parkinson's, Alzheimer's and Huntington's disease. The protective effect of quercetin in chemical-induced neurotoxicity is largely due to its antioxidant and free radical scavenging properties. In a recent study, quercetin was found to protect cadmium-induced brain cholinergic deficits by modulating mitochondrial functions and Mitogen-Activated Protein (MAP) -kinase signalling[33]. Interestingly, quercetin was found to attenuate cadmium-induced DA-D2 receptor-mediated motor deficits by modulating the post-synaptic signaling[34]. Continuing these leads, the present study has been carried out to assess the impact of cadmium on the integrity of biogenic amines in selected brain regions of rats and investigate the protective potential of quercetin. Further, the effect on the integrity of metallothionein has also been investigated to understand its involvement in cadmium-induced alterations in brain biogenic amines and the protective effect of quercetin assessed simultaneously.

2. Material and Methods

2.1 *In Vivo* Studies

2.1.1 *Experimental Animals and Housing Conditions*

Adult male rats (180 ± 20g) of Wistar strain obtained from the central animal breeding colony of Council of Scientific and Industrial Research (CSIR) -Indian Institute of Toxicology Research (CSIR-IITR), Lucknow were acclimatised in an experimental facility 7 days before the start of the experiment. The experimental facility was hygienic and had controlled temperature (25 ± 2 °C) and illumination (12-h each light/dark cycle). Pellet diets were arranged through the national suppliers

and water was available *ad libitum*. Due care was taken to maintain the animals and the study was approved by the institutional animal ethics committee of CSIR-IITR, Lucknow (IITR/IAEC/50/13). Further, all the protocols for the experiments conformed to the guidelines framed by the Committee for the Control and Supervision of Experiments on Animals, Ministry of Environment and Forests (Government of India), New Delhi, India.

2.1.2 Experimental Design and Treatment Procedure

The rats of the Wistar strain were randomly alienated into four different treatment groups and exposed to cadmium and quercetin alone or in combination as per the following details,

Group I – The rats were treated with cadmium as cadmium chloride (dissolved in distilled water, 5mg/kg body weight, p.o., once daily for 28 days).

Group II – The rats were treated with quercetin (suspended in 0.1% tween 20, 25mg/kg body weight, p.o., once daily for 28 days).

Group III – The rats were treated with cadmium and quercetin simultaneously as in Groups I and II respectively.

Group IV – The rats were treated with vehicle (0.1% tween 20, body weight, p.o., once daily for 28 days) for the duration of the treatment and served as controls.

The rats from each treatment group were sacrificed by cervical dislocation 24 hours after the last treatment. Brains were isolated carefully and the frontal cortex, hippocampus and corpus striatum were dissected following the standard procedure[35]. The selection of doses was done based on a literature review and an already-established dose from the laboratory.

2.1.3 Neurochemical Studies

2.1.3.1 Estimation of Neurotransmitter Levels and their Metabolites in Brain Regions

Reversed-phase high-performance liquid chromatography was employed to determine the levels of neurotransmitters (dopamine, norepinephrine, epinephrine and serotonin) and metabolites of dopamine (3,4-dihydroxyphenylacetic acid and homovanillic acid) in brain regions (frontal cortex, corpus striatum and hippocampus) of rats following exposure to cadmium, quercetin alone and their simultaneous treatment for 28 days. The HPLC system (Waters, Melford USA) contained an isocratic pump (515 HPLC pump), sample injector and column (C-18, 250 x 4mm) and was connected with the electrochemical detector fitted with glassy carbon and silver nitrate electrode. Further, the method of Kim *et al.*,[36] was followed with minor modifications. In short, brain regions were homogenised in perchloric acid (0.1M) individually and 3,4 – dihydroxybenzylamine (~25ng/ml) was added to each sample. The homogenate was centrifuged (36,000×g*)* for 10 minutes at 4°C. The supernatant was carefully separated and filtered through nylon filters (0.25µm) and used for the determination of neurotransmitter levels. The column was conditioned with the mobile phase (pH 4.2) that contained sodium dihydrogen phosphate (0.15 M), ethylenediaminetetraacetic acid (0.25mM), sodium octyl sulphate (1.75mM) and methanol (4%). Soon after the conditioning of the column, a sample (20µl) was injected and run with the same mobile phase (flow rate 1.5ml/min) to separate the peaks. The electrochemical detector was set at a potential of +0.800V with a sensitivity range of 2nA at ambient temperature with λ ex/λ em (493/513nm). The data was recorded and analysed by the software (Empower2) and results have been expressed as ng/g tissue weight.

2.1.3.2 Expression of Metallothionein in Brain Regions

The expression of metallothionein in brain regions (frontal cortex, hippocampus and corpus striatum) was assessed by Western blotting following the standard method[37]. Briefly, an equal amount of protein samples (~50mg protein/lane) were electrophoresed on SDS–PAGE (12-15 %) and electroblotted onto nitrocellulose membranes. The membranes were blocked using a blocking buffer (containing 5% BSA) soon after and incubated overnight at 4°C with primary MT III rabbit monoclonal antibody (Abcam, USA; 1:1000 dilution). β-actin rabbit monoclonal antibody (Cell Signalling, USA; 1:1000 dilution) was used as an internal control to verify that an equal amount of protein was loaded. The membranes were washed with TBST buffer and incubated with HRP-linked secondary antibody (anti-mouse IgG, CST, USA; 1:4000 dilution) at room temperature for one hour. The blots were washed and developed using an immobilon western chemiluminescent HRP substrate (Millipore, USA) following the recommended procedure. A digital gel image analysis system ((Image Quant LAS 500) was used for the semi-quantification of protein.

2.1.3.3 Estimation of Cadmium Levels in Brain Regions

Levels of cadmium in brain regions (frontal cortex, hippocampus and corpus striatum) were estimated involving an Atomic Absorption Spectrophotometer and following the method as described earlier[38]. Briefly, samples were prepared

by digesting the tissue in the acid mixture (concentrated nitric acid and perchloric acid in the ratio of 4:1) at 130°C for 3 hours. The samples were left overnight in a sand bath to ensure that the solution became yellow. More acid mixture was added and the process of oxidation was repeated in case the colour of the digested tissue was brown. After the process of mineralisation, samples were diluted with nitric oxide (0.1mol/L) and used for the estimation of cadmium levels by Atomic Absorption Spectroscopy. The detection limit of detection of the instrument was 1ppb. Further, the standard of cadmium was also run in parallel to construct the curve.

2.1.3.4 Protein Estimation

Protein content in the samples was determined following the method of Lowry *et al.*,[39] and using bovine serum albumin as a reference standard.

2.1.4 Statistical Analysis

The data was analysed by one-way Analysis of Variance (ANOVA) using the GraphPad Prism software. Further, the Newman–Keuls test was employed to assess the level of significance. The results were expressed as mean ± SEM and values up to $p < 0.05$ are considered to be significant.

2.2 *In Silico* Studies

2.2.1 ADMET Analysis

Prediction of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of Cadmium was performed by the admet System Activity Report (SAR) sever. This is a chemoinformatics-based web server which integrates 50 high-quality QSAR models for chemical ADMET profiling like Blood blood-brain barrier, Coco-2 permeability, Cytochrome P-450 (CYP) metabolism, carcinogenicity, AMES test and Human Intestinal absorption etc. ADMET structure-activity relationship database, abbreviated as admetSAR. In addition, the database includes 22 qualitative classifications and 5 quantitative regression models with highly predictive accuracy, allowing to estimate ecological/mammalian ADMET properties for novel chemicals. AdmetSAR is accessible at http://lmmd.ecust.edu.cn:8000/predict/.

2.2.2 Preparation of Ligand and Protein Structures

Structural coordinates or SMILE ID of ligand files of Cadmium Chloride were obtained from PubChem (CID: 24947) and a 3D model file was generated using CORINA3D online server. The 3D structure of macromolecule Protein Metallothionein-3/MT III (Uniprot ID: P37361), was not available in the Research Collaboratory for Structural Bioinformatics (RCSB) protein bank, so this protein was modelled using I-Tasser Server[40]. The Chimera was cast off for the minimisation of the energy and also for the removal of steric collision 1,000, steepest descent size 0.02 A˚, conjugated gradient steps 1,000 and the conjugate gradient step size 0.02 ˚A for the conjugate gradient minimisation[41].

2.2.3 Molecular Docking Studies

The Molecular docking studies were performed by Autodock version 4.0 suite Morris *et al.*,[42] was used. The macromolecule was added with the Kollman united charges and salvation parameters and the Grid box was set to cover the maximum part of the macromolecule. Lamarckian Genetic Algorithm (LGA) was used for MT III and ligand docking calculations. The LGA parameters like population size (ga pop size), energy evaluations (ga num generation), mutation rate, crossover rate and step size were set to 150, 2,500,000, 27,000, 0.02, 0.8, and 0.2 A˚, respectively. The LGA runs were set to 10 runs. All 10 conformations of MT III and ligand complex obtained were analysed for the interactions and binding energy of the docked structure using Discovery Studio Visualiser version 3.1 and Pymol.

3. Results

3.1 *In Vivo* Studies

3.1.1 Effect on the Level of Biogenic Amines and their Metabolites in Brain Regions Following Exposure of Rats to Cadmium and Quercetin Alone and their Simultaneous Exposure for 28 Days

Exposure of rats to cadmium caused a significant decrease in the levels of Dopamine (DA) ($F_{(3, 16)}$ = 5.726, 40% $p<0.001$), HVA ($F_{(3, 16)}$ = 4.232, 29.4% $p<0.05$), DOPAC ($F_{(3, 16)}$ = 3.669, 31.5%, $p<0.05$), NE ($F_{(3, 16)}$ = 9.164, 52% $p<0.01$) and 5HT ($F_{(3, 16)}$ = 4.345, 29%, $p<0.05$) in corpus striatum as compared to controls (Figure 1). Cadmium exposure however was found to increase the levels of EPN ($F_{(3, 16)}$ = 2.114, 42%, $p>0.05$) in corpus striatum. Further, an increase in dopamine turnover was also found in rats exposed to cadmium. Simultaneous exposure of rats to

cadmium and quercetin increased the levels of NE ($F_{(3, 16)}$ = 9.164, 34.6%, p<0.05), DA ($F_{(3, 16)}$ = 5.726, 46%, p<0.05), HVA ($F_{(3, 16)}$ = 4.232, 31.4%, p<0.05) and DOPAC ($F_{(3, 16)}$ = 3.669, 10.5%, P>0.05) and decreased the levels of EPN ($F_{(3, 16)}$ = 2.114, 19%, p>0.05) as compared to rats treated with cadmium alone (Figure 1). A significant increase in the levels of 5-HT ($F_{(3, 16)}$ = 4.345, 28.2%, p>0.05) was also evident in rats simultaneously exposed to cadmium and quercetin in corpus striatum as compared to those treated with cadmium alone. No significant change in the levels of any of these biogenic amines was observed in rats treated with quercetin in comparison to rats in the control group (Figure 1).

A decrease in the levels of DA ($F_{(3, 16)}$ = 11.00, 34%, p<0.01), DOPAC ($F_{(3, 16)}$ = 4.357, 41%, p<0.05), HVA ($F_{(3, 16)}$ = 4.520, 32%, p<0.05) and NE ($F_{(3, 16)}$ = 15.57, 43%,

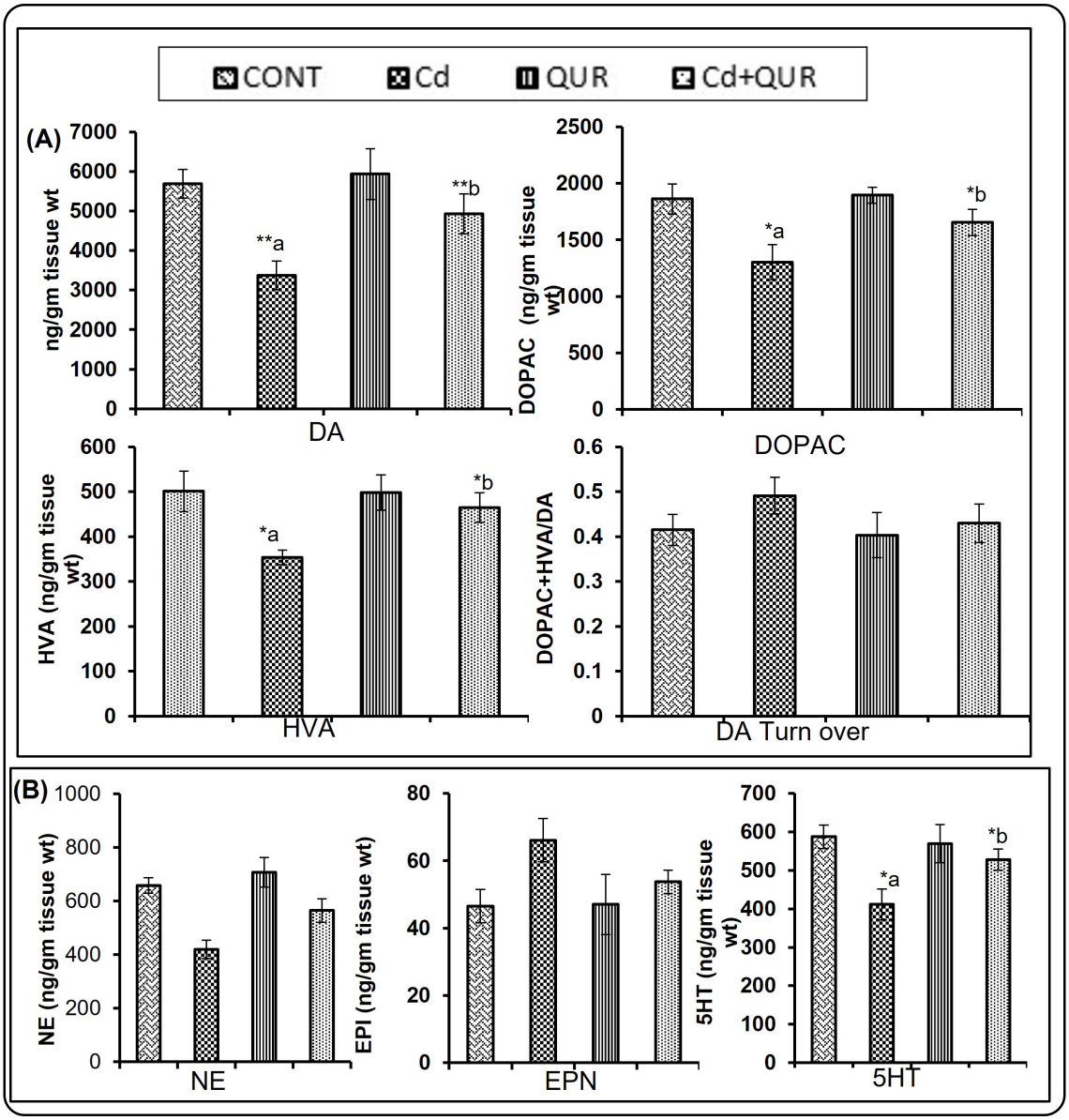


Figure 1. Effect on the level of biogenic amines **(A)** dopamine and its metabolites and turnover **(B)** nor-epinephrine, epinephrine and serotonin in corpus striatum following exposure of rats to cadmium or quercetin alone or their simultaneous exposure for 28 days.

Values are mean ± SEM of five rats in each group; Values are expressed as ng/g tissue weight; Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001); a-compared to control group, b-compared to cadmium exposed group. DA - Dopamine, DOPAC - 3,4-Dihydroxyphenylacetic acid, HVA - Homovanillic acid, NE - Norepinephrine, EPI- Epinephrine, 5HT- Serotonin.

p<0.001) was observed in hippocampus of rats exposed to cadmium for 28 days as compared to controls. However, there was an increase in the levels of EPN ($F_{(3, 16)}$ = 3.121, 27%) in the hippocampus of cadmium-exposed rats (Figure 2). Levels of 5-HT ($F_{(3, 16)}$ = 5.171, 34%, p>0.05) were found to decrease in the hippocampus of cadmium-treated rats. Simultaneous exposure with quercetin in cadmium treated rats caused an increase in the levels of DA ($F_{(3, 16)}$ = 11.00, 33%, p<0.05), HVA ($F_{(3, 16)}$ = 4.520, 37%, p<0.05), DOPAC ($F_{(3, 16)}$ = 4.357, 27%, p<0.05) and NE ($F_{(3, 16)}$ = 15.57, 44%, p<0.01) and decrease the levels of EPN ($F_{(3, 16)}$ = 3.121, 15%, p>0.05) in comparison to rats treated with cadmium alone. These rats also exhibited a significant increase in the levels of 5-HT ($F_{(3, 16)}$ = 5.171, 28.8%, p<0.05) in the hippocampus as compared to those treated with cadmium alone. No significant effect on the levels of DA, NE, EPN, 5-HT, DOPAC and HVA was observed in the hippocampus of rats treated with

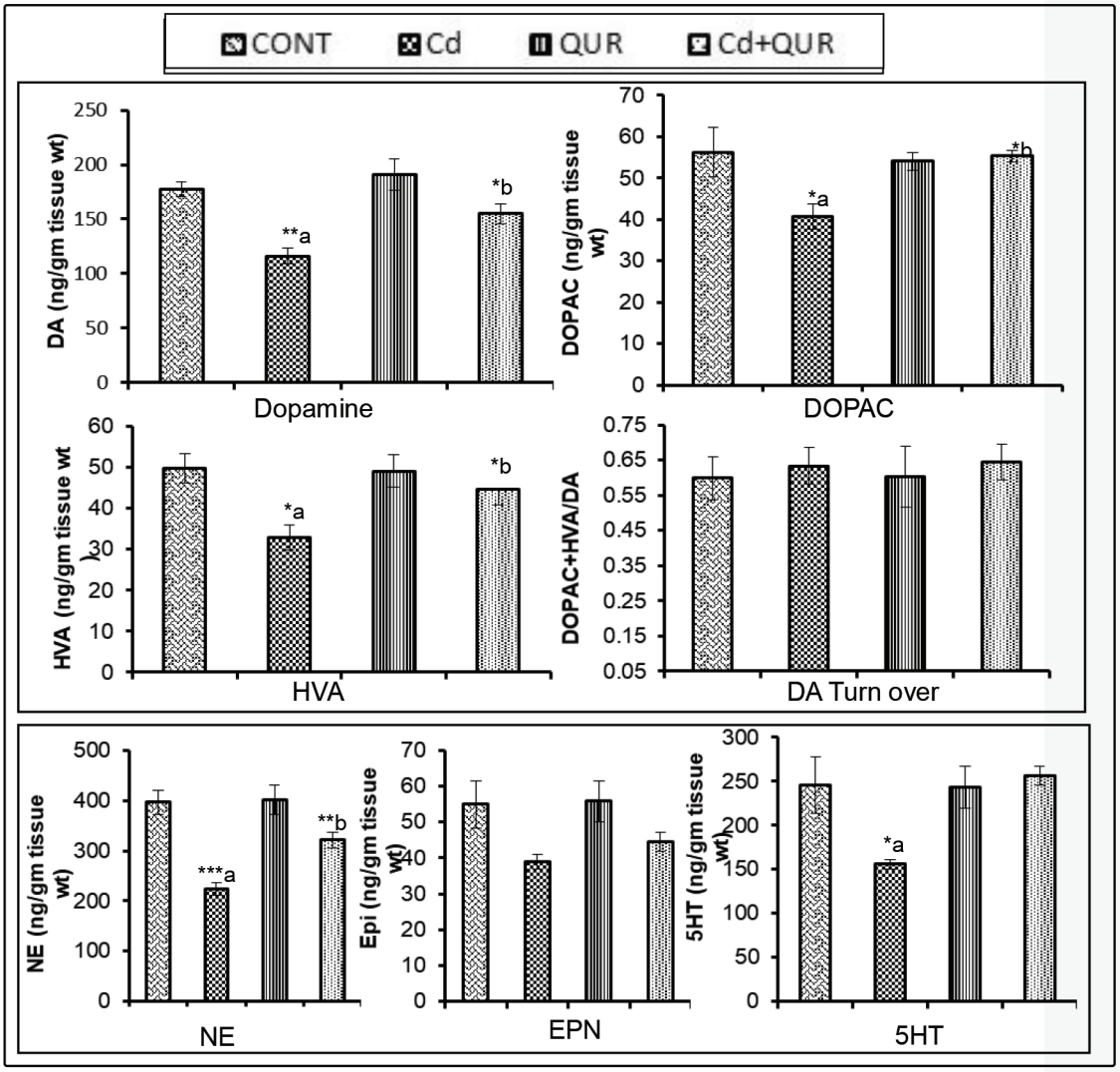


Figure 2. Effect on the level of biogenic amines **(A)** dopamine and its metabolites and turnover **(B)** nor-epinephrine, epinephrine and serotonin in hippocampus following exposure of rats to cadmium or quercetin alone or their simultaneous exposure for 28 days.

Values are mean ± SEM of five rats in each group; Values are expressed as ng/g tissue weight; Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001); a-compared to control group, b-compared to cadmium exposed group; DA - Dopamine, DOPAC - 3,4-Dihydroxyphenylacetic acid, HVA - Homovanillic acid, NE - Norepinephrine, EPI- Epinephrine, 5HT- Serotonin

quercetin as compared to rats in the control group (Figure 2).

Cadmium exposure in rats for 28 days caused a significant decrease in the levels of DA ($F_{(3, 16)}$ = 7.653, 33% p<0.01), HVA ($F_{(3, 16)}$ = 6.229, 35% p<0.001), DOPAC ($F_{(3, 16)}$ = 2.748, 29%, p<0.05) and NE ($F_{(3, 16)}$ = 3.154, 35%, p>0.05) and increase in the levels of EPN ($F_{(3, 16)}$ = 1.569, 73%, p>0.05) in frontal cortex as compared to controls (Figure - 3). Levels of 5-HT ($F_{(3, 16)}$ = 9.683, 32%, p<0.01) in the frontal cortex were found to decrease in cadmium-treated rats. Interestingly, simultaneous exposure of rats to cadmium and quercetin resulted to cause an increase in the levels of DA ($F_{(3, 16)}$ = 7.653, 33%, p<0.05), HVA ($F_{(3, 16)}$ = 6.229, 34%, p<0.05), DOPAC ($F_{(3, 16)}$ = 2.748,

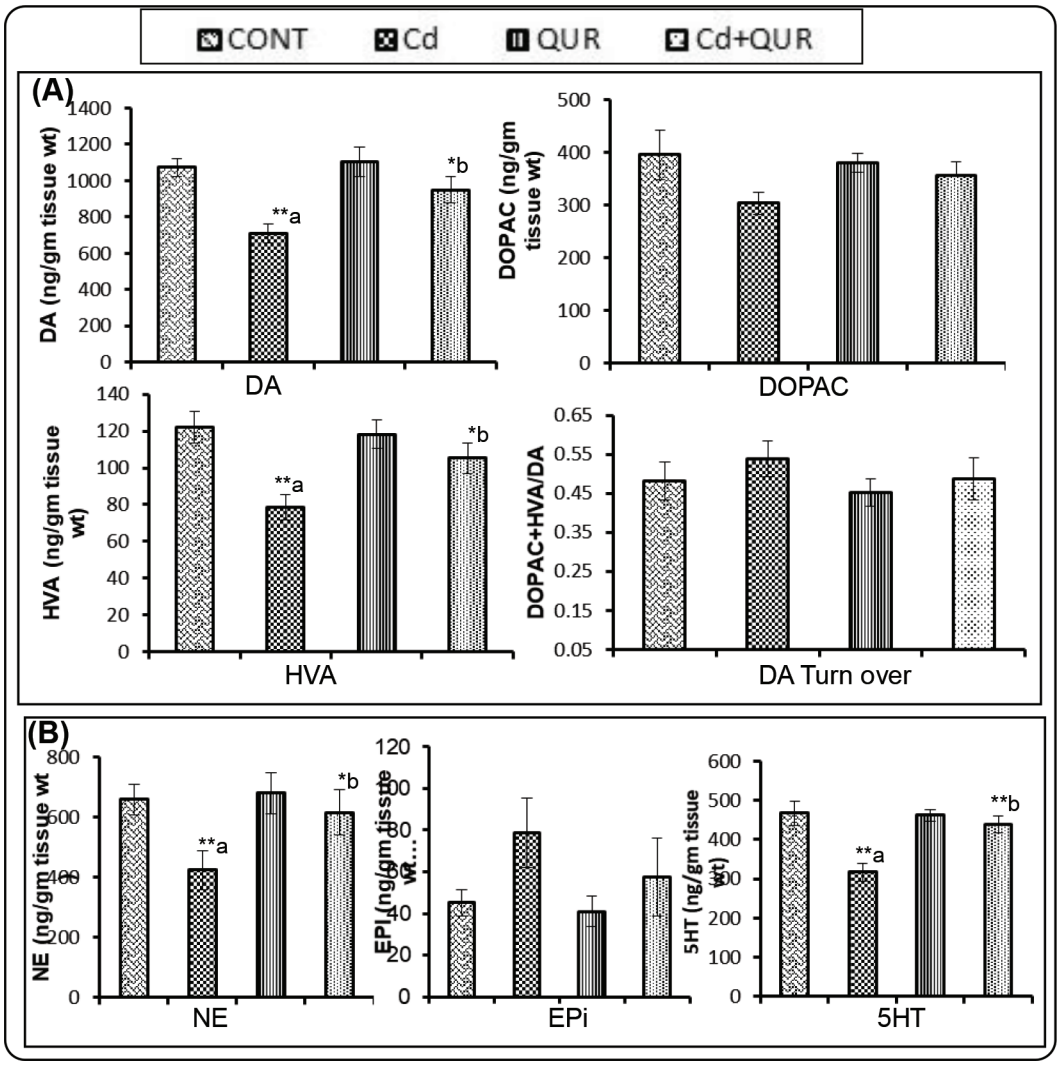


Figure 3. Effect on the level of biogenic amines **(A)** dopamine and its metabolites and turnover **(B)** nor-epinephrine, epinephrine and serotonin in frontal cortex following exposure of rats to cadmium or quercetin alone or their simultaneous exposure for 28 days.

Values are mean ± SEM of five rats in each group; Values are expressed as ng/g tissue weight; Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001); a-compared to control group, b-compared to cadmium exposed group; DA - Dopamine, DOPAC - 3,4-Dihydroxyphenylacetic acid, HVA - Homovanillic acid, NE - Norepinephrine, EPI- Epinephrine, 5HT- Serotonin

Values are mean ± SEM of five rats in each group; Values are expressed as ng/g tissue weight; Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001); a-compared to control group, b-compared to cadmium exposed group; DA - Dopamine, DOPAC - 3,4-Dihydroxyphenylacetic acid, HVA - Homovanillic acid, NE - Norepinephrine, EPI- Epinephrine, 5HT- Serotonin

Values are mean ± SEM of five rats in each group; Values are expressed as ng/g tissue weight; Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001); a-compared to control group, b-compared to cadmium exposed group; DA - Dopamine, DOPAC - 3,4-Dihydroxyphenylacetic acid, HVA - Homovanillic acid, NE - Norepinephrine, EPI- Epinephrine, 5HT- Serotonin

22%, p>0.05) and NE ($F_{(3, 16)}$ = 3.154, 45%, p>0.05) and decrease the levels of EPN ($F_{(3, 16)}$ = 1.569, 26%, P>0.05) in the frontal cortex. A significant increase in the levels of 5-HT ($F_{(3, 16)}$ = 9.683, 38% p<0.05) in the frontal cortex was also evident in rats simultaneously treated with cadmium and quercetin as compared to those exposed to cadmium alone. No significant effect on the levels of DA, NE, EPN, 5-HT, DOPAC and HVA was observed in the frontal cortex of rats exposed to quercetin alone as compared to controls (Figure 3).

3.1.2 *Effect on the Expression of MT-III in Brain Regions Following Exposure of Rats to Cadmium or Quercetin Alone or their Simultaneous Exposure for 28 Days*

To understand the involvement of MT-III cadmium-induced toxicity, the expression of MT-III was assessed in brain regions of rats exposed to cadmium for 28 days. Exposure to cadmium was found to increase the expression of MT-III in the corpus striatum ($F_{(3,8)}$ = 5.123, 2.8 Fold, p<0.0001), hippocampus ($F_{(3,8)}$ = 4.132, 2.4 Fold, p<0.01) and frontal cortex ($F_{(3,8)}$ = 4.23, 2.1 Fold, p<0.05) as compared to controls (Figure 4). Simultaneous exposure with quercetin was found to decrease cadmium-induced changes in the expression of MT-III in the corpus striatum ($F_{(3,8)}$ = 5.265, 1.6 Fold, p<0.01), hippocampus ($F_{(3,8)}$ = 3.567, 1.57 Fold) and frontal cortex ($F_{(3,8)}$ = 3.879, 1.45 Fold, p<0.05) as compared to rats treated with cadmium alone. No significant change in the expression of MT-III was evident in any of the brain regions of rats exposed to quercetin alone as compared to controls (Figure 4).

3.1.3 *Effect on Cadmium Levels in Brain Regions of Rats Exposed to Cadmium or Quercetin Alone or their Simultaneous Exposure for 28 Days*

There was a striking increase in cadmium levels in the corpus striatum (F (3, 16) = 25.99, 4.8 Fold, p<0.001), hippocampus (F (3, 16) = 11.37, 3.7 Fold, p<0.001) and frontal cortex (F (3, 16) = 7.637, 1.9 Fold, p<0.01) on exposure of rats to cadmium as compared to controls. Interestingly, simultaneous exposure of rats to cadmium and quercetin caused a significant decrease in cadmium levels in corpus striatum (F (3, 16) = 25.99, 1.32 Fold, p<0.001), hippocampus (F (3, 16) = 11.37, 1.52 Fold,

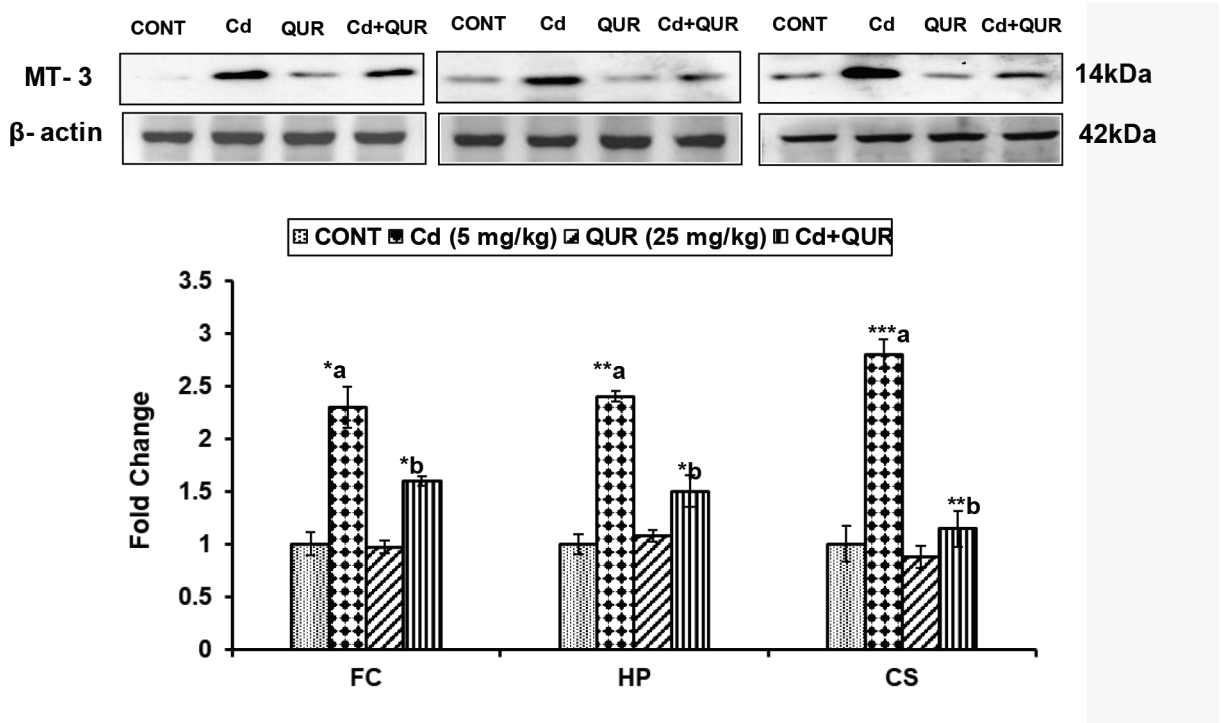


Figure 4. Effect on the expression of MT-III in brain regions following exposure of rats to cadmium or quercetin or their simultaneous exposure for 28 days.

Values are mean ± SEM of three rats in each group; Significantly differs (*p < 0.05, **p < 0.01); a-compared to control group, b-compared to cadmium exposed group; CONT – Control, Cd – Cadmium, QUR – Quercetin, FC- Frontal cortex, HP – Hippocampus and CS – Corpus striatum.

p<0.01) and frontal cortex (F (3, 16) = 7.637, 1.6 Fold, p<0.05) as compared to those treated with cadmium alone. However, there was no significant effect on cadmium levels in any of the brain regions of rats exposed to quercetin alone as compared to controls (Table 1).

3.2 *In Silico* Studies

Prediction of ADMET parameters of Cadmium Chloride (CdCl2) was performed by the admet SAR server and presented in Table 2. The data exhibits that CdCl2 crosses the Blood Brain Barrier (BBB+ve) with a probability of 0.9786 and also predicts acute oral toxicity and carcinogenic potential. According to the derived docking model, CdCl2 binds with MT-III protein. The comparative optimisation was carried out to find the best-docked pose and the energy of CdCl2 with MT-III. The best 10 docked poses were identified and analysed and the figure was generated using AutoDock visualiser. Figure 5 illustrates the best-docked pose of CdCl2 with MT-III with ΔG of -2.14 Kcal/Mol and Ki of 26.88mM. This pose identified binding residues - YS65, CYS42, PRO39, CYS38 and exhibited interactions with the CdC.

Toxicokinetic profiling of cadmium was carried out in silico involving ADMET Predicted Profile Regression.

4. Discussion

The role of neurotransmitters in the aetiology of neurological and psychiatric disorders is well-accepted[43]. The involvement of dopamine in Parkinson's disease and schizophrenia, acetylcholine in cognitive functions and serotonin in depression and anxiety have been quite convincing and well-characterised. While these neurotransmitters are differentially distributed in

Table 1. Effect on cadmium levels in brain regions following exposure of rats to cadmium or quercetin alone or their simultaneous exposure for 28 days

Brain region	Treatment Groups			
	Control	Cadmium (5mg/kg)	Quercetin (25mg/kg)	Cadmium + Quercetin
Frontal cortex	73±9.5	137±11**[a]	55±14	81±15**[b]
Hippocampus	36±10	133±21***[a]	34±11	73±8.2**[b]
Corpus striatum	63±11	308±10***[a]	55±8.4	100±33***[b]

Values are mean ± SEM of five rats in each group; Values are expressed as ng/g wet tissue weight; Significantly differs (**p < 0.01, ***p < 0.001); a-compared to control group, b-compared to cadmium exposed group.

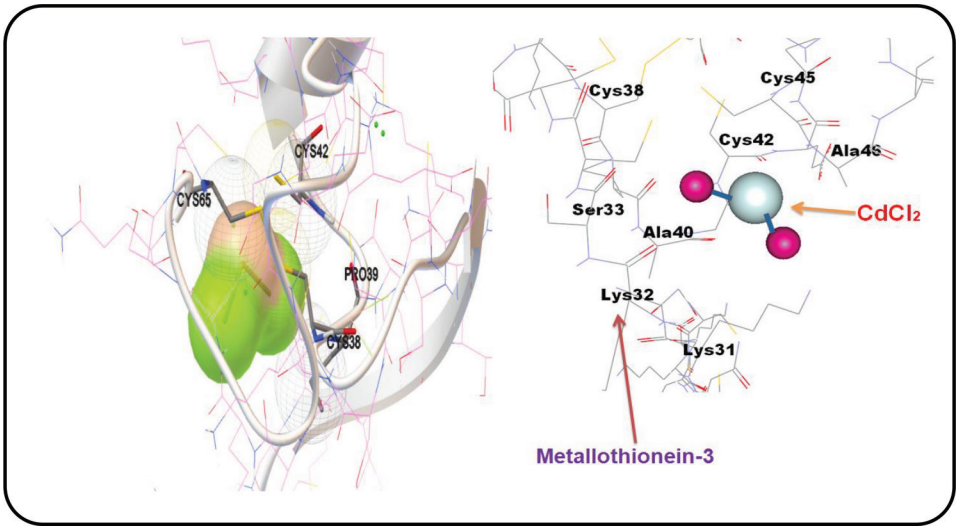


Figure 5. Interaction of cadmium chloride with metallothionein III.
Cadmium interacts with metallothionein III protein on residues CYS65, CYS42, PRO39, CYS38; Possible Binding energy (Cdcl2- MT III complex) -2.55 kcal/mol and Inhibition constant (ki)- 13.49mM

Table 2. Toxicokinetics profiling of cadmium

Model	Results	Probability
Absorption		
Blood-Brain Barrier	BBB+	0.9786
Human Intestinal Absorption	HIA+	0.9935
Caco-2 Permeability	Caco2+	0.6190
P-glycoprotein Substrate	Non-substrate	0.9022
P-glycoprotein Inhibitor	Non-inhibitor	0.9785
Renal Organic Cation Transporter	Non-inhibitor	0.9223
Distribution/Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.8005
CYP450 2D6 Substrate	Non-substrate	0.7503
CYP450 3A4 Substrate	Non-substrate	0.7412
CYP450 1A2 Inhibitor	Non-inhibitor	0.5695
CYP450 2C9 Inhibitor	Non-inhibitor	0.7345
CYP450 2D6 Inhibitor	Non-inhibitor	0.8774
CYP450 2C19 Inhibitor	Non-inhibitor	0.7109
CYP450 3A4 Inhibitor	Non-inhibitor	0.9378
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9075
Excretion		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9547
	Non-inhibitor	0.9746
AMES Toxicity	Non AMES toxic	0.9633
Carcinogens	Carcinogens	0.6621
Biodegradation	Ready biodegradable	0.7326
Acute Oral Toxicity	III	0.5846
Carcinogenicity (Three-class)	Warning	0.4769
Toxicity		
Rat Acute Toxicity	2.0135	LD50, mol/kg

different brain regions, selective loss or increase of neurotransmitters in any brain region may affect the associated functions. Further, multiple neurotransmitters in selected or different brain regions could also be affected by the progression and severity of the disease[27,28]. As biogenic amines are considered to be primary messengers, fluctuation in their levels may disrupt further signalling through ionotropic or metabotropic receptors and affect synaptic transmission.

As these neurotransmitters also modulate behavioural functions, there has been a lot of interest in understanding the effect of chemical exposure on the integrity of neurotransmitters in brain regions. Disturbance in the levels of biogenic amines and their metabolites has also been reported in brain/ brain regions on the exposure to environmental chemicals including lead, arsenic and manganese[44-46]. Cadmium has been found to affect neuroendocrine activity by disrupting the functioning of the hypothalamus in a series of studies[29,47]. It was found that exposure to cadmium may alter the metabolism of biogenic amines including dopamine and serotonin in the hypothalamus and affect prolactin secretion in male rats[47]. Studies also suggested that the hypothalamus is vulnerable to the toxic effects of cadmium and it may impair the endogenous mechanism. Decreases in NE, DA and 5-HT levels were found in the hypothalamus and hypothalamic regions - anterior, mediobasal and posterior hypothalamus[29]. Alteration in the levels of neurotransmitters has been observed in developing rats on prenatal exposure to cadmium[48,49]. Release and turnover of striatal DA increased in nursing rats treated with cadmium for 5 days[50]. These changes were associated with decreased TH activity in the striatum on cadmium exposure[50]. Neurotoxic effects of cadmium

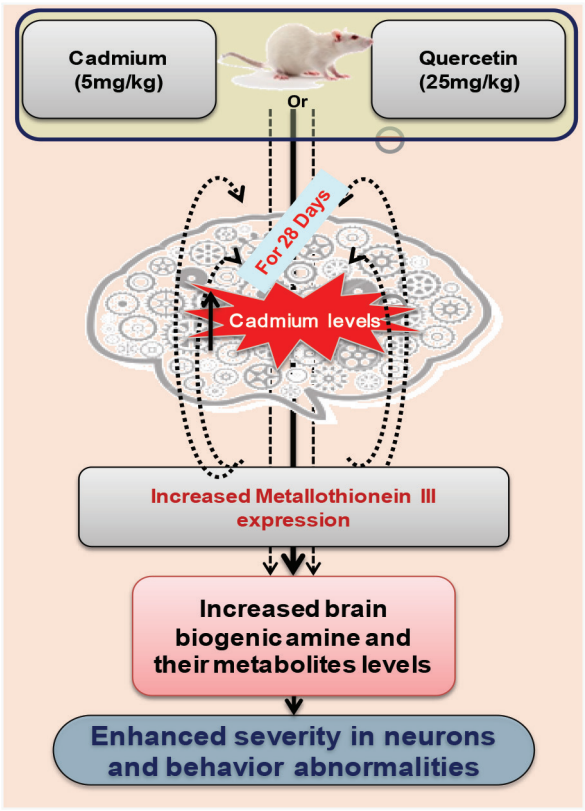


Figure 6. Schematic diagram illustrating the mechanism of cadmium-induced alteration in brain Metallothionein III and brain biogenic levels and potential of quercetin in attenuating such changes (graphical abstract).

on the integrity of brain biogenic amines have also been investigated on co-exposure with lead. Antonio *et al*,[49] found an increase in DOPAC, 5-HT, and 5-HIAA levels in the cerebellum and not in the striatum of developing rats co-exposed with cadmium and lead prenatally. While there was no change in DA levels both in the cerebellum and striatum an increase in DOPAC/DA turnover was evident in the cerebellum. Interestingly, these changes in biogenic amines on co-exposure to cadmium and lead were found consistent with those observed on exposure to cadmium earlier[51]. Perinatal exposure to lead however caused a decrease in the levels of biogenic amines in developing rats on PD0, PD5 and PD12. These changes were attributed to the difference in the bioavailability of both the metals, cadmium being taken up in higher amounts during lactation as compared to lead available readily during the prenatal period. A decrease in 5-HT and NE levels in the hypothalamus was found in rats exposed to lead or cadmium alone or in combination in another study[52]. Further, a decrease in DA levels in the hypothalamus was evident only in rats exposed to cadmium and not in those exposed to lead alone or those coexposed to cadmium and lead. Alteration in the levels of biogenic amines was associated with the accumulation of metals while the effects were not additive[52]. Changes in the levels of biogenic amines however are not consistent and have been found to vary with age, duration and timing of exposure. Further, the dose and route of chemical exposure may also be associated with variability in the levels. In the present study, exposure to cadmium resulted in altered levels of dopamine and its metabolites, HVA and DOPAC in the corpus striatum, frontal cortex and hippocampus. Further, dopamine turnover was also found to be affected by exposure to cadmium. Alteration in the levels of norepinephrine and serotonin in the corpus striatum, frontal cortex and hippocampus was also evident while no significant effect on the levels of epinephrine was observed. The decrease in DA levels in the hypothalamus was attributed to inhibition in the synthesis of biogenic amines. A decrease in NE levels may be associated with reduced DA levels since DA is the precursor for the synthesis of NE. Recently, we found that cadmium-induced motor deficits in rats are due to alterations both in canonical cAMP-dependent PKA/DARRP32/PP1α and non-canonical β-arrestin/AKT/GSK3β signalling associated with a decrease in DA-D2 receptors in the corpus striatum. It was further suggested that a decrease in the expression of TH and DAT on cadmium exposure may reduce DA levels and cause these changes[34].

Cadmium easily crosses the blood-brain barrier and is distributed in different brain regions. Toxicokinetic profiling of cadmium involving *in silico* studies in the present study revealed that cadmium has good absorption due to high BBB permeability. Neurotoxic effects of cadmium are cumulative as it has low efflux and a long biological half-life[2]. High levels of cadmium in the frontal cortex, hippocampus and corpus striatum as observed in the present study are consistent with earlier reports[53]. As cadmium like other metals has the potential to form quinones due to oxidation of catecholamines, enhanced oxidative stress associated with mitochondrial dysfunctions may contribute to decreased DA levels. Further, accumulation of cadmium in brain regions may trigger an alteration in the levels of neurotransmitter levels.

Due to the potential of quercetin to counteract free radicals and enhance the capacity of antioxidant

enzymes in the biological system, it has been found effective in modulating oxidative stress in chemical-induced neurotoxicity. Quercetin has been found to protect against neurotoxicity in experimental models of Alzheimer's, Parkinson's and Huntington's disease by inhibiting oxidative stress[54-56]. Neurological impairments in experimental models of traumatic brain injury and stroke were also found to be attenuated by quercetin by decreasing the load of oxidative stress[57]. The protective effect of quercetin in cadmium-induced neuronal damage associated with enhanced oxidative stress and apoptosis has also been observed[58]. The ameliorative effect of methanolic extract of Physalis in cadmium-induced neurotoxicity has been reported[59]. Prior treatment with methanolic extract of Physalis was found to modulate and protect cadmium-induced alterations in DA, 5-HT and 5-HIAA in the brain. Interestingly, the protective effect was associated with the antioxidant potential of Physalis. As the methanolic extract of Physalis is a mixture of flavonoids and withanolide glycosides, it is difficult to comment on the bioactivity of the exact compound. Quercetin has been found effective in reducing the load of metals in body tissues including the brain due to its metal-chelating properties[60]. Simultaneous treatment with quercetin was found to decrease aluminium levels in the brain and attenuate aluminium-induced neurobehavioral toxicity by decreasing oxidative stress in an earlier study[61]. A decrease in cadmium levels in the frontal cortex, hippocampus and corpus striatum on simultaneous treatment with quercetin as observed in the present study may be attributed to its chelating property by inhibiting the accumulation of cadmium and thus reducing the oxidative stress.

The protective nature of Metallothionein, a cysteine-containing protein in cadmium toxicity has been reported[62]. Lu *et al.*[63] suggested that Metallothionein has an important role in the detoxification of cadmium and may be used as a biomarker of cadmium toxicity. While investigating the effect of cadmium on renal dysfunctions in a population study, induction of metallothionein mRNA levels in peripheral lymphocytes was suggested to be a marker of susceptibility[63]. Out of different isoforms of MT, MTIII is present specifically in the brain both in neuronal and glial cells[64]. The role of Metallothionein in modulating zinc homeostasis in neurons has also been suggested[64]. It has been found that cadmium induces the synthesis of metallothionein in the liver, forming a Cd-MT complex which reduces the toxicity of cadmium[65]. *In silico* modeling further exhibited that cadmium interacts with metallothionein III on different residues suggesting that cadmium has high affinity with metallothionein III. Interestingly, MT null mice exhibited high toxicity towards cadmium as compared to control mice. Protection of cerebellar neurons from glutamate and nitric oxide toxicity by MTIII has also been observed[66]. In an interesting report, Gutierrez-Reyes *et al.*,[50] found that dexamethasone-induced Metallothionein may prevent a cadmium-induced increase in striatal dopamine levels. The increase in the expression of MTIII in the frontal cortex, hippocampus and corpus striatum on exposure to cadmium in the present study reflects cadmium neurotoxicity. However, cadmium-induced expression of MTIII was found reduced in all these brain regions on simultaneous treatment with quercetin.

The results exhibit that cadmium-induced changes in biogenic amines governing the motor dysfunctions may be associated with altered levels of Metallothionein in the brain and quercetin has the potential to counteract these changes. *In silico* studies carried out suggesting the strong interactions of cadmium with metallothionein suggesting the potent neurotoxicity of cadmium. ADMET analysis was carried out confirming the high blood-brain permeability leading to altered neurotransmitter and Metallothionein levels. Further, the protective effect of quercetin appears to be attributed to its antioxidant and metal-chelating properties. As quercetin is present in dietary products, its supplementation may be a good strategy to protect cadmium-induced dopaminergic alterations. (Figure 6).

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6. Author Contributions

All authors contributed to the study's conception and design. The experiment was designed by Richa Gupta. The study was performed by Richa Gupta and Lalit P. Chandrvanshi. Data

collection and result analysis were done by Richa Gupta and Rajendra K. Shukla. The first draft of the manuscript was written by Richa Gupta and all authors commented and suggested updated manuscript versions.

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