International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Role of Apoptosis in Cadmium Induced Dopaminergic Alterations in Rat Brain

Shraddha Prakash[1], Chitranshu Pandey[2]

[1]CSIR (IITR) Indian Institute of Toxicology Research, Lucknow, U.P., India

[2]R &D MRD Life Sciences Pvt. Ltd. Lucknow, U.P., India

Abstract: ***Toxicity of cadmium has been demonstrated in several organs. Cadmium leads to injury of tissue by creating oxidative stress, epigenetic changes in DNA. Among various organs in the body, brain is a soft target of cadmium. Cadmium has a very detrimental effect on the nervous system. The review discusses the current knowledge on Role of Apoptosis in Cadmium Induced Dopaminergic Alterations in Rat Brain.***

Keywords: toxicity, cadmium, apoptosis, Neurotransmitter, Lowry method, (DA-D1 and DA-D2

1. Introduction

Heavy metals are natural components of the Earth's crust which cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Its poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater.

The **term "Heavy Metal"** refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include
cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl) and lead (Pb). Heavy metals tend to accumulate in an organism over time, thus increasing the concentration in the organism. This is called as bioaccumulation.

Cadmium (Cd), one of the toxic heavy metal has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC) (ATSDR1999). It was discovered by German scientist as a by-product of the zinc refining process during the year 1817. Its name has been derived from the Latin word *cadmia* and the Greek word *kadmeia* (Duruibe*et al*, 2007).

Cadmium having atomic no.48, a soft bluish white metal, and chemically similar to in group 12, Zn and Hg. The most common forms of cadmium found in the environment exist in combinations with other elements. Hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and nitric acid (HNO_3) dissolve cadmium by forming cadmium chloride ($CdCl_2$), Cadmium Sulphate ($CdSO_4$) or Cadmium Nitrate ($Cd(NO_3)_2$). Most cadmium used in this country is obtained as a by-product from smelting (melting) zinc, lead, or copper ores.

The cadmium by-product is mostly used in metal plating and to make pigments, batteries, and plastics.

Prolonged exposure to Cd will cause toxic effect due to its accumulation over time in a variety of tissues, including kidneys, liver, and central nervous system (CNS) and peripheral neuronal systems.

2. Materials and Methods

2.1 Animals

Adult male rats of wistar strain (180 ± 20 g) were obtained from animal-breeding colony of CSIR-Indian Institute of Toxicology Research, Lucknow. The animals were housed in plastic polypropylene cages under standard animal house conditions with a 12 hours light/dark cycle at 25 ± 2^{o}C. The animals had access to pellet diet (Ashirwad Industries Pvt. Ltd, India) and water *ad libitum.* All experimental protocols were approved by the Animal Care and Ethics Committee of Indian Institute of Toxicology Research, Lucknow.

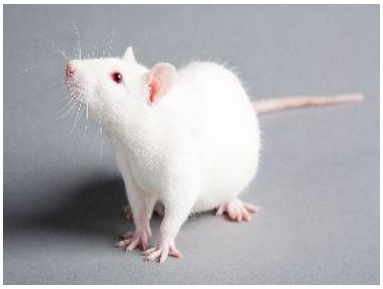


Figure 3.1: Wistar Strain Adult Rat

There were two treatment groups as per following details,
Group I– Rats treated with vehicle (distilled water) daily for 28 days and served as controls
Group II – Rats treated with cadmium as cadmium chloride (dissolved in distilled water, 5 mg/kg body weight, p.o.) daily for 28 days

Rats were sacrificed by cervical decapitation 24 h after the last dose of treatment. Brain part were taken out quickly, washed in ice cold saline and dissected into regions (corpus striatum) following the standard procedure as described by Glowinski and Iversen, (1966)

Instruments Used:
The major instruments used are as fallows-

- Milli-Q water purification assembly (Millipore, USA)
- Millipore filter assembly (Millipore, USA)
- RC-5B sorvall high speed centrifuge (Du-Pont, USA)
- UV-Visible Spectrophotometer (Thermo Spectronic, USA), were the major instruments used in the study
- Biorad western blot assesmbely (Biorad)

2.2 Neurotransmitter Receptor Binding Assay

Assay of dopamine DA-D_2 receptors in corpus striatum were carried out by radioligand receptor binding technique following the standard procedure (Khanna et al. 1994). Methods for preparation of crude synaptic membranes and binding assays are briefly described below.

2.3 Preparation of Crude Synaptic Membrane

Crude synaptic membrane was prepared by homogenizing the brain regions in 19 volumes of Tris-HCl buffer (5mM, pH 7.4) followed by centrifugation at 40,000 x g for 15 min at 4ºC. The sedimented pellet was washed twice by re-suspending in homogenization buffer followed by re-centrifugation at the same speed for 15 min at 4ºC. The pellet was finally suspended in Tris-HCl buffer (40 mM, pH 7.4) and stored at -20ºC.

2.4 Protein estimation by Lowry method

Protein concentration in tissue homogenates was measured following the method of (Lowry *et al*., 1951) using bovine serum albumin (BSA) as reference. 5 ml of alkaline copper reagent (containing 1 part of 2.68% sodium potassium tartarate, 1 part of 1% copper sulphate and 98ml parts of 2% sodium carbonate in 0.1N NaOH) was added to 980 µl of water and 20 µl of tissue homogenate (protein sample), taken in duplicate, mixed and kept at room temperature for 10 minutes, to digest the protein sample. Adding 0.5 ml of 1N FolinCiocalteu's reagent for the reaction. The intensity of the color developed 30 minutes later, was measured at 660 nm in spectrophotometer against blank samples without protein. The final protein content in the sample was calculated using BSA standard curve drawn.

2.5 Radioligand Receptor Binding Assay

Binding incubations was carried out in triplicate in a final volume of 1 ml. The reaction mixture containing Tris buffer (40 mMTris-HCl, pH 7.4), along with appropriate radioligand and protein was incubated for 15" at 37ºC. The details of radioligands and competitors used for the assay of neurotransmitter receptors are described in Table – 1. Binding tubes was rapidly filtered on glass fiber discs (25 mm diameter, 0.3 µm pore size, Whatman GF/B) soon the incubation is over and washed twice rapidly with 5 ml cold 40 mMTris-HCl buffer to remove unbound radioligand. Filter discs was dried and counted in 5 ml of scintillation mixture (PPO, POPOP, naphthalene, Toluene and methanol) beta-scintillation counter (Packard, USA) at an efficiency of 30-40% in order to determine membrane bound radioactivity. Control incubations were carried out simultaneously containing unlabelled competing ligand to determine the extent of nonspecific binding. Specific binding was calculated by subtracting the nonspecific binding (in the presence of competitor) from the total binding (in the absence of competitor) and has been expressed as pmoles ligand bound/g protein.

Table 1: Details of ligand, competitor to be used for receptor binding assays

Receptor	Brain Region	Radioligand (concentration)	Competitor (concentration)
Dopamine – D_2	Corpus striatum	^{3}H-spiperone ($1x10^{-9}$ M)	Haloperidol ($1X10^{-6}$M)

2.6 Expression of Bax, Bcl2 and caspase3 Protein Involving Western Blotting:

Expression of pro- and anti-apoptotic proteins in corpus striatum brain regions was assessed by Western Blotting following the method of Jamal et al. (2007). Briefly, the protein samples (50 mg protein / lane) were electrophoresed on SDS–PAGE (12%), electro blotted to nitrocellulose membranes, blocked with blocking buffer and incubated with primary antibody Bcl-2 rabbit monoclonal antibody (Cell signalling, 1 : 1000 dilution), Bax rabbit monoclonal (Cell Signaling,1 : 1000 dilution), Caspase-3 rabbit monoclonal (Cell Signaling,1 : 1000 dilution), followed by incubation with horseradish peroxidase-linked secondary antibody (anti-mouse IgG, 1:4000; anti-rabbit IgG 1:4000) at room temperature for 60 min. After the incubation, blots were washed and developed using an immobilon western chemiluminescent HRP substrate (Millipore, USA) following the recommended procedure. Beta Actin was probed as an internal control and used to confirm that an equal amount of protein was loaded in each lane. A digital gel image analysis system (VersaDoc, Model 1000, Bio Rad, and Quantity 1) was used for quantification of protein.

2.7 Expression of dopamine receptor proteins (DA-D1 and DA-D2) involving Western Blotting

Expression of dopamine receptor (DA-D1 and DA-D2) proteins in corpus striatum was assessed by Western Blotting following the method of Jamal et al. (2007). Briefly, the protein samples (50 mg protein / lane) were electrophoreses on SDS–PAGE (12%), electro blotted to nitrocellulose membranes, blocked with blocking buffer and incubated with primary antibody D1rabbit polyclonal (protein tech, 1: 1000 dilution), D2 receptor rabbit polyclonal (protein tech, 1: 1000 dilution), followed by incubation with horseradish peroxidase-linked secondary antibody (anti-rabbit IgG 1:4000) at room temperature for 60 min. After the incubation, blots were washed and developed using an immobilon western chemiluminescent HRP substrate (Millipore, USA) following the recommended procedure. β actin was probed as an internal control and used to confirm that an equal amount of protein was loaded in each lane. A digital gel image analysis system (VersaDoc, Model 1000, Bio Rad, and Quantity 1) was used for semi-quantification of protein.

2.8 Steps involved in western blotting process

2.8.1 Protein sample preparation for western blotting:
Dissect out brain tissue (Corpus Striatum) with clean tools, on ice preferably and as quickly as possible to prevent degradation by protease. Take dissected brain part in eppendroff tube & weigh it. Add Lysis Buffer & Protease inhibitor cocktail in 20% w/v. Homogenize the tissue with the help of Homogenizer. Centrifuge it at 12000g for 15 minute at 4ºC. Collect supernatant and store -20 ºC.

2.8.2 Protein Estimation
Lowry method was used for protein estimation. Method is sensitive as it employs two colour-forming reactions. It involves biuret reaction in which Cu^{2+} (in the absence of base) reacts with the peptide bond to give deep blue colour. In addition Folin-Ciocaltue chemistry, in which complex mixture of inorganic salts reacts with tryptophan and tyrosine residue to give blue-green colour, is also used. The combination of two reaction gives an assay that is more sensitive that either reaction alone. (Lowry et al., 1951)

2.8.3 Protein standard used: Bovine serum albumin (BSA, 1 mg/ ml) was used as a reference standard.

2.8.4 Preparation of Copper Alkaline Reagent (CAR)
a) 2% sodium carbonate and 0.1N of sodium hydroxide was dissolved in 100ml of water to make up a solution.
b) 2ml of this solution was removed and 1ml of Na^{+}/K^{+} (2%) tartarate and 1ml of copper sulphate (1%) was added and mixed thoroughly to get a solution of alkaline copper reagent.

2.8.5 Folin- ciocalteu Reagent (FR)

Diluted with equal volume of water to prepare the desired volume

Procedure

Table 2: Protein Estimation Assay

Assay	Distilled water (µl)	Protein standard (BSA) (µl)	Sample (Membrane) (µl)	Alkaline copper reagent (ml)	Folin-Ciocalteu reagent (ml)
Blank	1000	__	__	5	0.5
Standard	900	100	__	5	0.5
Samples	980	__	20	5	0.5

a) Sample and standard were added to make the volume up to 1ml, and two tubes were also kept parallel as blank containing water only.
b) 5ml of alkaline copper reagent was added in each tube and mixed thoroughly.
c) Tubes were incubated at room temperature for 10 min.
d) 0.5 ml of diluted Folin-ciocalteu reagent was added in each tube and vertexed immediately.
e) Incubated at room temperature for 30 min.

A. The tubes were vertexes and zero was adjusted in spectrophotometer with blank and took the absorbance at 660 nm.
B. Total protein was calculated using following formula:

$$\frac{100 \times \text{Mean of the sample}}{1000 \times \text{Volume of the sample} \times \text{Mean of the standard}}$$

2.8.6 Gel Electrophoresis
The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. By far the most common type of gel electrophoresis employs polyacrylamide gels and buffers loaded with sodium dodecyl sulphate (SDS). SDS-PAGE polyacrylamide gel nets to remove secondary and tertiary structure (e.g. S-S disulfide bonds to SH and SH) and thus allows separation of proteins by their molecular weight. Sampled proteins become covered in the negatively charged SDS and move to the positively charged electrode through the electrophoresis maintains polypeptides in a denatured state once they have been treated with strong reducing age acrylamide mesh of the gel. Smaller proteins migrate faster through this mesh and the proteins are thus separated according to size (usually measured in kilo Daltons, kD). The concentration of acrylamide determines the resolution of the gel - the greater the acrylamide concentration the better the resolution of lower molecular weight proteins. The lower the acrylamide concentration the better the resolution of higher molecular weight proteins. Proteins travel only in one dimension along the gel for most blots. Samples are loaded into wells in the gel. One lane is usually reserved for a marker or ladder, a commercially available mixture of proteins having defined molecular weights, typically stained so as to form visible, coloured bands When voltage is applied along the gel, proteins migrate into it at different speeds. These different rates of advancement separate into bands within each lane.

2.8.7 Purpose of stacking gel
Stacking gel is used to concentrate the protein sample into a sharp band before it enters the resolving gel. This is achieved by utilizing the differences in ionic strength and pH between the electrophoresis buffer and the stacking gel, which involves a phenomenon known as isotachophoresis. Stacking gel has larger pore size, which allows the protein samples to concentrate and move freely under the effect of electric field. Band sharpening is attained by the difference in the electrophoresis mobility of glycinate ions, protein-SDS complex and chloride ions in the loading buffer. For having a steady electric circuit all the ionic species have to migrate at the same speed under the influence of the applied field. Field strength is inversely proportional to conductivity that is proportional to concentration.
[Cl-] > [protein-SDS] > [Glycinate]
Due to lower concentration of protein-SDS complex they concentrate in a very tight band between glycinate and chloride ion boundaries. Once glycinate ions reach the resolving gel due to the higher pH environment they get easily ionized and their mobility increases.

2.8.9 Electrophoresis
After protein estimation equal amount of sample mixed with 1x lamelli buffer then boil at 100 °C for 5 to 8 minute. Equal amount of protein is loaded in to each well of gel. Gel was run at 25Volts through the stacking part of the gel. Turn the volts up to 50V after the proteins have gone through the stack and are migrating through the resolving gel. Allowed migration to continue until the blue dye front reached the end of the glass plates, but does not migrate off the gel.

Transfer

Transfer of the protein samples was done using following method:

a) The pads, filter paper were presoaked in chilled transfer buffer.
b) A sandwich of gel and Nitrocellulose membrane was compressed in a cassette between the presoaked pads and the filter papers and immersed in a buffer between a pair of electrodes.
c) Precautions were taken to avoid any air bubbles between the gel and the membrane.
d) A constant current (150mA for 110 minute) was passed at right angles to the gel, which caused the separation of proteins to electrophorese out of the gel and into the Nitrocellulose membrane.

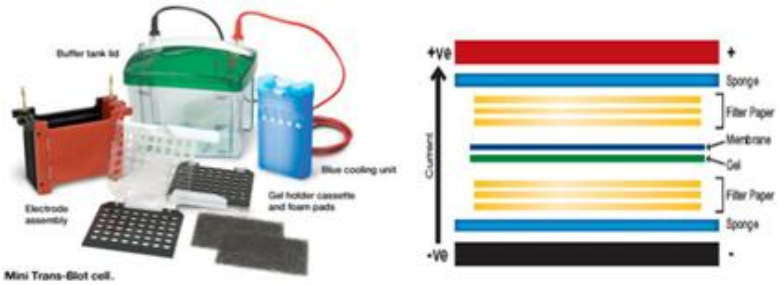


Fig 1:- Process of Transfer in Western Blotting
(source:http://www.bio-rad.com/)

2.8.10 Blocking

Blocking is a very important step in the immunodetection phase of Western blotting because it prevents non-specific binding of antibody to the blotting membrane. The open sites on the membrane were blocked with 5% BSA by incubating the membrane in it for 40 min at room temperature with intermittent rocking.

2.8.11 Incubation with Primary Antibody

The Nitrocellulose membrane was incubated with the primary antibody at 4^0C temperature over night hour with intermittent shaking. The antibody was prepared in 0.5 BSA at a titter of 1:1000.

2.8.12 Washing

This step is to remove excess primary antibody (unbound). Washing solution was prepared by adding 0.1% of tween-20 in 1liter PBS. For washing, the membrane was incubated in washing solution for 1hr with changing washing solution at every 10 minute.

2.8.13 Incubation with Secondary Antibody

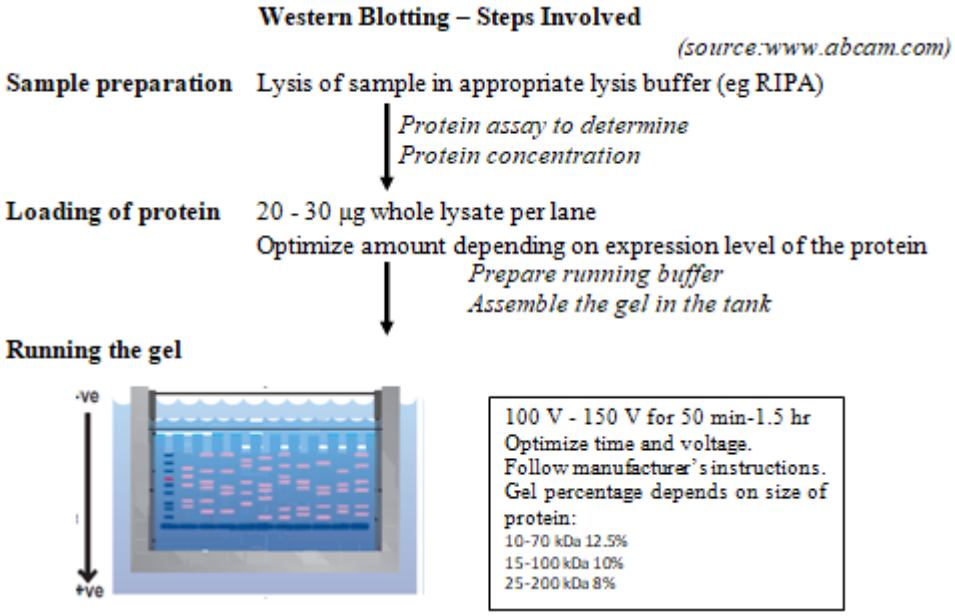
The membrane was incubated for 90 min with Anti-rabbit HRP conjugate secondary antibody, diluted in the ratio of 1:2000 in blocking buffer.

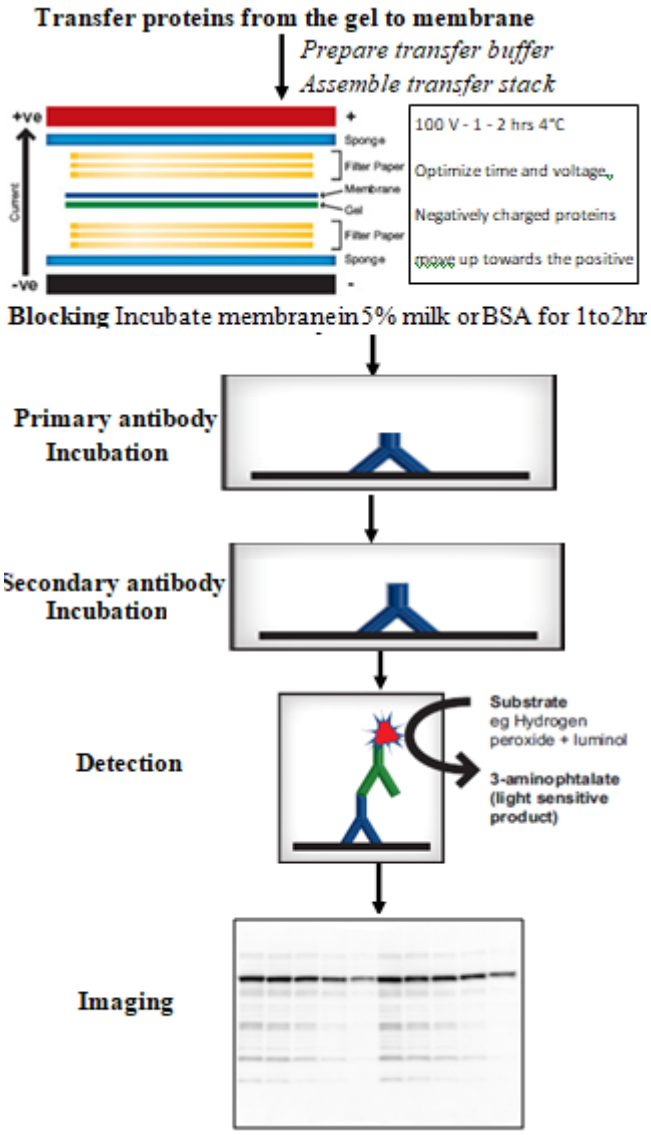
2.8.14 Washing

washing is to remove unbound secondary antibody that are not bound to primary antibody.

2.8.15 Detection:

After the unbound probes are washed away, then next step is detection of probes that are labeled and bound to protein of interest. Substrate was prepared by the mixing of 1ml of luminal substrate with 1ml of Enhancer solution in the dark. The substrates were added and the membrane was observed for Chemiluminescencein VersaDoc Image analyzer (Bio-Rad) for development of bands that conforms the presence of the interested protein against which the antibody was used. This reaction was carried out at dark, since substrate used is light sensitive compound. Densitometry was done by the software provided by Alpha innotech Image analyzer (USA).





3. Results

3.1 Effect of cadmium exposure on the expression of DA-D1and DA- D2 receptors in rat corpus striatum

The results of present study demonstrate that exposure to cadmium caused an increase in the expression of DA-D1 receptor (1.13 Fold; $p>0.05$) and a significant decrease in the expression of DA-D2 receptor (1.64 Fold; $p<0.01$) in corpus striatum of rat as compared to controls (Figure 3.1.1).

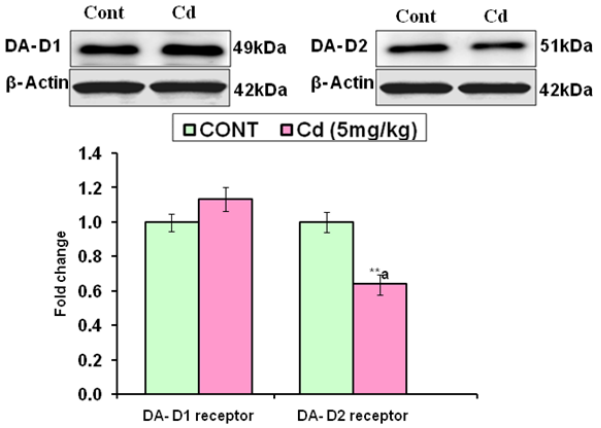


Figure 3.1.1: Effect of cadmium exposure on the expression of DA-D1 and DA-D1 receptor in corpus striatum of rates for 28 days

3.2 Effect of cadmium exposure on the binding of 3H-spiperone to rat straital membranes

Exposure to cadmium caused a significant decrease in the binding of 3H-spiperone (51%; $p<0.001$) in corpus striatum of rats as compared to controls shown in Figure 3.2.1.

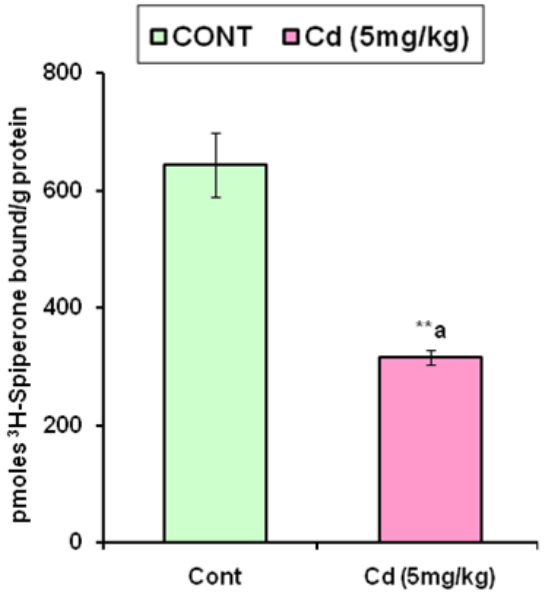


Figure 3.2.1: Effects of cadmium exposure on the binding of 3H- spiperone to rat striatal membrane for 28 days.

3.3 Effect of cadmium on the expression of selected proteins associated with apoptosis

3.3.1Effect of cadmium exposure on the expression of Bax in rat corpus striatum:

Exposure to cadmium caused a significant increase in the expression of pro-apoptotic protein Bax (2.1 Fold; $p<0.001$) in corpus striatum of rats as compared to controls (Figure 3.3.1.1).

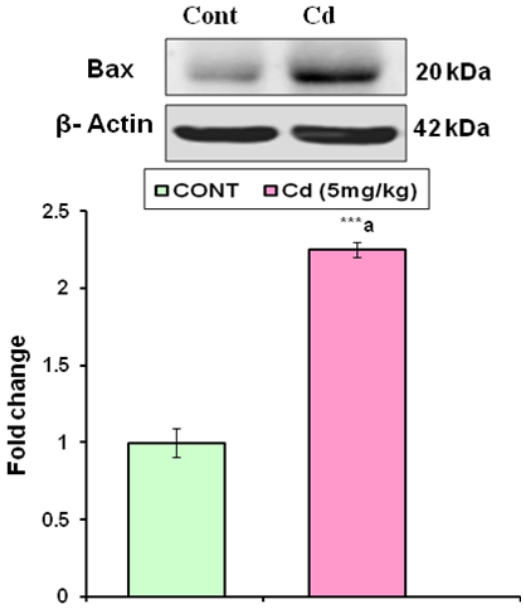


Figure 3.3.1.1: Effect of cadmium exposure on the expression of bax, pro apoptotic protein in corpus striatum of rates for 28 days

3.3.2 Effect of cadmium exposure on the expression of Bcl2 in rat corpus striatum:

The members of the Bcl2 family are important in determining the extent of a cell to apoptosis. Exposure to cadmium caused a significant decrease in the expression of Bcl2, an anti-apoptotic protein (1.74 Fold; $p<0.01$) in corpus striatum of rats as compared to controls (Figure 3.3.2.1).

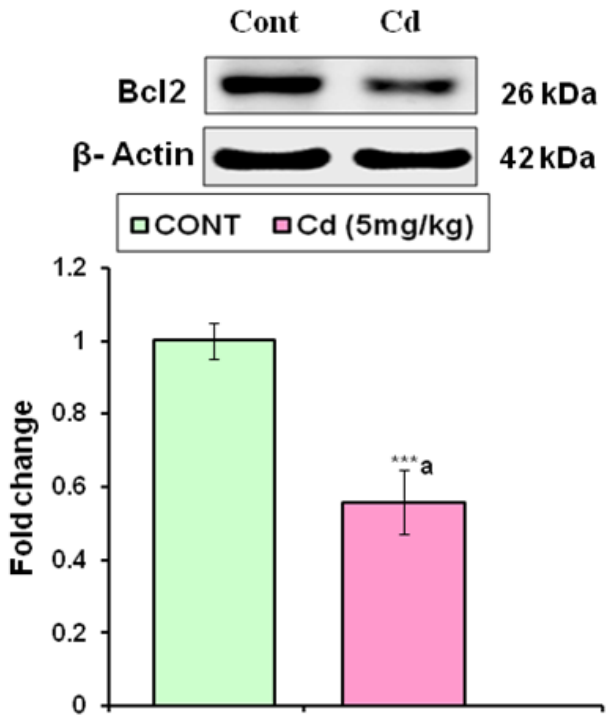


Figure 3.3.2.1-Effect of cadmium exposure on the expression of bcl2, antiapoptotic protein in corpus striatum of rates for 28 days

3.3.3 Effect of cadmium exposure on the expression of Caspase-3 in rat corpus striatum:

Caspases, are a family of cysteine proteases that play essential roles in apoptosis. The caspases that get activated via recruitment to signaling complexes are known as the initiator and executer caspases, as they provide a link between cell signaling and apoptotic execution. As evident from Figure 3.3.3.1, exposure to cadmium caused a significant increase in the expression of executer protein caspase-3 (1.95 Fold; $p<0.01$) in corpus striatum as compared to controls.

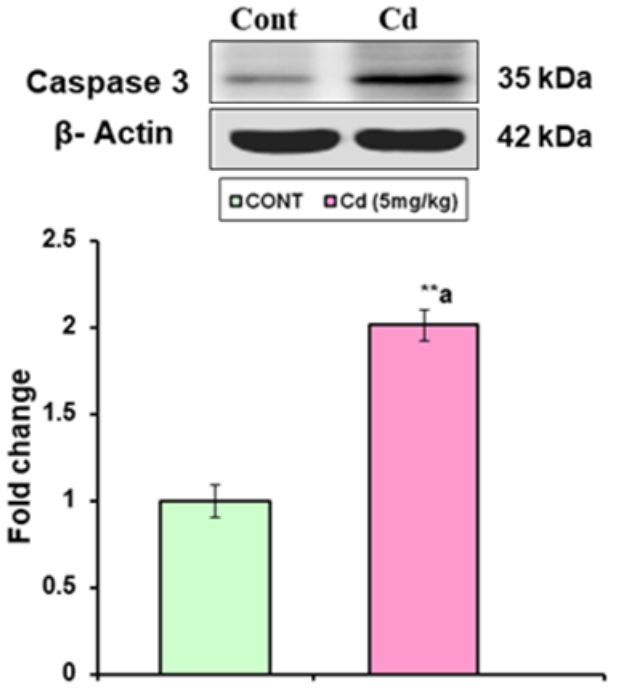


Figure 3.3.3.1: Effects of cadmium exposure on expression of executer- protein (caspase3) in corpus striatum of rats for 28 days.

4. Conclusion

The results of the present study indicates that exposure to cadmium caused a significant decrease in DA-D2 receptor as no significant increase in the expression of DA-D1 receptors were observed in corpus striatum. Further, exposure of rats to cadmium resulted to increase the expression of Bax, a pro-apoptotic protein and decrease the expression of Bcl2, an anti- apoptotic protein, enhanced expression of Caspase - 3, an executer protein in corpus striatum was also distinct in rats exposed to cadmium. The result of the present study clearly indicates that exposure to cadmium could cause brain dopaminergic alterations in rats and these changes could be associated with enhanced apoptosis.

5. Future Prospects

Decrease in the expression of DA-D2 receptor associated with the decrease binding of DA-D2 receptor provide an important lead to unravel the molecular mechanism involved in dopaminergic dysfunction. The study will help to identify molecular targets involved in cadmium induced dopaminergic signaling and associated functional deficits.

Based on this, suitable strategies which could protect/prevent cadmium induced dopaminergic dysfunction could be developed.

References

[1] Agency for the toxic substance and disease registry (ATSDR). 2011
[2] Ahmed Hasbi, Brian F O'Dowd and Susan R George (2011).Dopamine D1-D2 receptor heteromer signalling pathway in the brain: emerging physiological relevance. *Hasbi et al. Molecular Brain, 4:26*

[3] Allan I. Levey, Steven M. Hersch, David B. Rye, Roger K. Sunaharat, Hyman B. Niznikt, Cheryl A. Kitt, Donald L. Price, Roberto Maggio, Mark R. Brann, and Brian J. Ciliax (1993). Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proc. Natl. Acad. Sci. USA Vol. 90, pp. 8861-8865, Neurobiology*

[4] Ana Paula Rigon, Fabiano M. Cordova, Camila S. Oliveira, Thaı´s Posser, Ana Paula Costa, Ilza G. Silva, Daiane A. Santos, Francesco M. Rossi, Joa˜o Batista T. Rocha, Rodrigo B. Leal (2008). Neurotoxicity of cadmium on immature hippocampus and a neuroprotective role for p38MAPK. *Science Direct NeuroToxicology ,727–734*

[5] Angshuman Sarkar1, Geethanjali Ravindran1 &Vishnuvardhan Krishnamurthy (2013). A Brief Review On The Effect Of Cadmium Toxicity: From Cellular To Organ Level. *International Journal of Bio-Technology and Research (IJBTR) Vol. 3, Issue 1 ISSN 2249-6858*.

[6] Antonio M.T, López N, Leret M.L.,(2002). Pb and Cd poisoning during development alters cerebellar and striatal function in rats. *Toxicology 176, 59–66.*

[7] ATSDR (2004). Guidance Manual for the Joint Toxic *Action of Chemical Mixtures*, http://www.atsdr.cdc.gov/interactionprofiles/ ipga.html.

[8] ATSDR (2004). Guidance Manual for the Joint Toxic Action of Chemical Mixtures, *http://www.atsdr.cdc.gov/interactionprofiles/ ipga.html*.

[9] Bernard (2008). Cadmium & its adverse effects on human health. *Indian J Med Res 128, pp 557-564*

[10] BernhoftA.Robin,(2013). Cadmium Toxicity and Treatment. *Hindawi Publishing Corporation The Scientific World Journal Volume 2013, Article ID 394652,7pages*

[11] Duruibe J. O , Ogwuegbu, M. O, *et.al.*, (2007)**.** Heavy metalpollution and human biotoxic effects. *Int. J.Phys. Sci., 2(5): 112-118.*

[12] E. LoApez, S. Figueroa, M.J. Oset-Gasque& M.P. GonzaAlez (2003). Apoptosis and necrosis: two distinct events induced by cadmium incortical neurons in culture. *British Journal of Pharmacology 138, 901 – 911*

[13] Eidl SE, Potashkin JA. (2011). the promise of neuroprotective agents in Parkinson's

[14] Emine Er, Lisa Oliver, Pierre-FrancoisCartron, Philippe Juin, Stephen Manon, Francois M. Vallette (2006). Mitochondria as the target of the pro-apoptotic protein Bax. *Science Direct Biochimica et BiophysicaActa 1757, 1301–1311*

[15] Fahaid Al-Hashem, Mohammad Dallak, Nabil Bashir, Mohammad Abbas, Riyadh Elessa, Mohammad Khalil and Mahmoud Al-Khateeb (2009). Camel's Milk Protects Against Cadmium Chloride Induced Toxicity in White Albino Rats. *American Journal of Pharmacology and Toxicology 4 (3):107-117*

[16] Gehan, Emery A E, *et.al.*, (2010). Cadmium-ginger two way antagonistic relationship . *Arab J. Biotech., Vol. 13, No. (1) : 115-124.*

[17] Gerhards son L, Englyst V, Lundstrom N G, Sandberg S, Nordberg G,(2002). Cadmium, copper and zinc in tissues of deceased copper smelter workers. *J. Trace. Elem. Med. Biol. 16;261 – 266.*

[18] Glowinski J, Iversen LL. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. *J. Neurochem. 13 (8), 655–659*

[19] Glowinski J, Iversen LL. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. *J. Neurochem. 13 (8), 655–659*

[20] Hellström J L, Alfvén T, *et al*.,(2000)Low level exposure to cadmium and early kidney damage: the OSCAR study. *Occup Environ Med; 57: 668-72.*

[21] Hengartner, O.M., (2000). The biochemistry of apoptosis. *Nature 407, 770–776*

[22] http://www.biomedcentral.com/1471-2121/14/4l

[23] http://www.molecularbrain.com/content/4/1/26

[24] Jamal M, Ameno, K., Ameno, S., Morishita, J., Wang, W., Kumihashi, M., et al., (2007). Changes in cholinergic function in the frontal cortex and hippocampus of rat exposed to ethanol and acetaldehyde. *Neuroscience 144, 232–238*

[25] Järup L, Akesson A (2009). Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.; 238, 201–208.*

[26] Järup L, Akesson A (2009). Current status of cadmium as an environmental health problem. *Toxicol. Appl. Pharmacol.; 238, 201–208.*

[27] Jung P D, Liu Y, Curtis K D (2001) Protective effect of metallothionein against the toxicity of cadmium and other metals. *Toxicology 163, 93–100*

[28] Khanna VK, Husain R, Seth PK. (1994). Effect of protein malnutrition on the neurobehavioral toxicity of styrene in young rats. *J. Appl. Toxicol. 14, 351–356.*

[29] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem. 192, 265–275.*

[30] Magnus Akerstrom, Lars Barregard, Thomas Lundh, GerdSallsten (2013). The relationship between cadmium in kidney and cadmium in urine and blood in an environmentally exposed population. *Toxicology and Applied Pharmacology 268 (2013) 286–293.*

[31] Mehrdad M., Messripour M,*et.al.*,(2007). The effect of ginger extract on blood urea nitrogen and creatinine in mice. *Pak J Biol Sci., 10(17):2968-71.*

[32] Méndez-Armenta M., Ríos C., (2007). Cadmium neurotoxicity. Environ. *Toxicol. Appl.*

[33] Nishimura Y, Yamaguchi JY, Kanada A,(2006) Increase in intracellular Cd2+ concentration of rat cerebellar granule neurons incubated with cadmium chloride : cadmium cytotoxicity under external Ca2+-free condition. *ToxicolIn Vitro, 20(2), 211-6.*

[34] Nordic Council of Ministers Cadmium Review (2003)

[35] OSPAR (2002). *Cadmium***.** Hazardous Substances Series. *OSPAR Commission*

[36] P.DAS, S. Samantaray, G.R. Rout (1998). Studies of cadmium toxicity in plants: A review. *Environmental Pollution, Vol.98, No.1, pp 29-36*

[37] Raj Kumar, Ashok K. Agarwal, Prahlad K. Seth (1996). Oxidative stress-mediated neurotoxicity of cadmium. *Toxicology Letters 89, 65-69*

[38] Satarug S, Scott H, Garrett S, and Donald A (2010). Cadmium, Enviromental Exposure, and Health outcomes. *Env.Health. Pers.; vol 118*
[39] Satarug S, Scott H, Garrett S, and Donald A sens. (2010). Cadmium, Enviromental Exposure, and Health outcomes. *Env.Health. Pers.; vol 118*
[40] Seungwoo Kim, Hyo-Soon Cheon, So-Young Kim, Yong-Sung Juhnn and Young-Youl Kim, (2013). Cadmium induces neuronal cell death through reactive oxygen species activated by GADD153. *Kim et al. BMC Cell Biology, 14:4*
[41] Tellez-Plaza Maria & R. Jones Miranda & Dominguez-Lucas Alejandro &GuallarEliseo&Navas-Acien Ana (2013). Cadmium Exposure and Clinical Cardiovascular Disease:A Systematic Review. *CurrAtheroscler*
[42] Ting-Jun FAN, Li-Hui HAN, Ri-Shan CONG, and Jin LIANG(2005). Caspase Family Proteases and Apoptosis .*ActaBiochimica et BiophysicaSinica, 37(11): 719–727*
[43] Tritsch N X and Sabatini B.L (2012). Dopaminergic Modulation of Synaptic Transmission in Cortex and Striatum. *Neuron 76 Elsevier Inc.*
[44] Viaene M.K., Masschelein R., Leeders J., De Groof M., Swerts, L.J.V.C., Roels, H.A., (2000). Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. *Occup. Environ. Med. 57, 19–27.*
[45] Viaene M.K., Masschelein R., Leeders J., De Groof M., Swerts, L.J.V.C., Roels, H.A (2000). Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. *Occup. Environ. Med. 57, 19–27.*
[46] Viaene M.K., Masschelein R., Leeders J., Groof M D, Swerts, L.J.V.C., Roels, H.A (2000). Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. *Occup. Environ. Med. 57, 19–27.*
[47] Wang WO, Yanli Du, (2013). Cadmium and Its Neurotoxic Effects, Oxidative
[48] Wang ZB, Liu YQ, et.al., (2005). Pathways to caspase activation. Cell BiolInt , 29: 489−496
[49] World Health Organisation (WHO) (2011). Exposure to Cadmium: A major public concern, Environmental Health Criteria. *International Program on Chemical Safety, Geneva.*
[50] World Health Organization (WHO) (2011). Exposure to Cadmium: Amajor public concern, Environmental Health Criteria. *International Program on Chemical Safety, Geneva.*
[51] Yi-Sook Jung, Euy-MyoungJeong , Eun Kyung Park , You-Mie Kim , SeonghyangSohn, Soo Hwan Lee, EunJooBaik, Chang-Hyun Moon, Yi-Sook Jung, Euy-MyoungJeong, Eun Kyung Park, You-Mie Kim, SeonghyangSohn, Soo Hwan Lee, EunJooBaik, Chang-Hyun Moon(2008). Cadmium induces apoptotic cell death through p38 MAPK in brain microvessel endothelial cells. *European Journal of Pharmacology 578, 11–18*
[52] Zhou, W.L, and Antic, S.D (2012). Rapid dopaminergic and GABAergic modulation of calcium and voltage transients in dendrites of prefrontal cortex pyramidal neurons. *J. Physiol. 590, 3891–3911.*