Effect of Time Dependent Exposure of Cadmium on Hippocampus Dependent Cognitive Functions and Their Post Exposure Recovery in Mice

Master of Science (MS)

By

Muhammad Dawood Amjad

(00000274678)

Atta ur Rahman School of Applied Biosciences

National University of Sciences and Technology (NUST), Islamabad, Pakistan

2021

Effects of Time Dependent Exposure of Cadmium on Hippocampus Dependent Cognitive Functions and Their Post Exposure Recovery in Mice

A thesis submitted as a final year project in partial fulfillment of the requirement for the degree of Master of Science

In

Healthcare Biotechnology

By

Mr. Muhammad Dawood Amjad

MS HCB-2018

(Registration # 00000274678)

Supervised by: Dr. Touqeer Ahmed

Atta-ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

2021

Thesis Acceptance Certificate

Thesis Acceptance Certificate

THESIS ACCEPTANCE CERTIFICATE

It is certified that the contents of thesis entitled "**Effects of Time Dependent Exposure of Cadmium on Hippocampus Dependent Cognitive Functions and Their Post Exposure Recovery in Mice**" submitted by **Mr. Muhammad Dawood Amjad** has been found satisfactory for the requirement of the degree.

Supervisor: _____

Dr. Touqeer Ahmed

Atta-ur-Rahman School of Applied Biosciences

National University of Science and Technology

Head of Department: _____ 17/9/2021

Dr. Touqeer Ahmed

Atta-ur-Rahman School of Applied Biosciences

National University of Science and Technology

Principal: _____

Dr. Hussnain Janjua

Atta-ur-Rahman School of Applied Biosciences

National University of Science and Technology

FORM TH-4

National University of Sciences & Technology

MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by:

Muhammad Dawood Amjad (00000274678)

Titled: "Effects of time dependent exposure of Cadmium on Hippocampus dependent cognitive functions and their post exposure recovery in mice" be accepted in partial fulfillment of the requirements for the award of **MS in Healthcare Biotechnology** degree with (A grade).

Examination Committee Members

1. Name: Dr. Saadia Zahid Signature:

2. Name: Dr. Maria Shabbir Signature:

3. Name: Dr. Salman Khan Signature:

Supervisor's name: Dr. Touqeer Ahmed Signature:
Date: 30/8/2021

Dr. Touqeer Ahmed
Head of Department (HoD)
Deptt of Healthcare Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Date: 30/8/2021 Head of Department

COUNTERSINGED

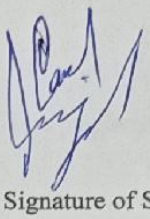
Date:_____

Dr. Hussnain A. Janjua
Principal
Atta-ur-Rahman School of Applied Biosciences (ASAB)
NUST, Islamabad

Dean/Principal

DECLARATION

I certify that this research work entitled "**Effects of Time Dependent Exposure of Cadmium on Hippocampus Dependent Cognitive Functions and Their Post Exposure Recovery in Mice**" is my own work. The work has not been presented elsewhere for assessment. The materials that have been used from other sources has been properly acknowledge/referred.



Signature of Student

Muhammad Dawood Amjad

00000274678

CERTIFICATE FOR PLAGIARISM

It is to confirm that MS thesis entitled "**Effect of Time Dependent Exposure of Cadmium on Hippocampus Dependent Cognitive Functions and Their Post Exposure Recovery in Mice**" of Mr. Muhammad Dawood Amjad Reg No. 00000274678 has been examined by me. I undertake that,

1. Thesis has significant new work/knowledge as compares to already elsewhere. No sentence, table, equation, diagram, paragraph or section has copied verbatim from previous work except when placed under quotation marks and duly referenced.

2. The work presented is original and own work of author i.e. there is no plagiarism. No idea, results or work of others have been presented as author's own work.

3. There is no fabrication of data or results such that the research is not accurately represented in the records. The thesis has been checked using Turnitin, a copy of the originally report attached and focused within the limits as per HEC plagiarism policy and instruction based on time to time.

Dr. Touqeer Ahmed
Head of Department (Acting)
Dept of Healthcare Biotechnology
Atta-ur-Rahman School of Applied
Biosciences (ASAB), NUST, Islamabad

(Supervisor)

Dr. Touqeer Ahmed

Associate Professor

ASAB, NUST

COPYRIGHT STATEMENT

Dedicated to

My beloved parents

Mr. Amjad Bashir

Mrs. Naveeda Amjad

Acknowledgements

All praise and glory be to Allah, the true Master of all seen and unseen knowledge, all aware and all-comprehending, who is the sole provider of all skills and means, and who provided me with the strength and guided me to accomplish this work.

I express my sincere gratitude to my supervisor, Dr. Touqeer Ahmed for his extraordinary supervision and guidance throughout my research year. I would like to thank Principal of ASAB, Dr. Hussnain Janjua and HOD healthcare biotechnology, Dr. Touqeer Ahmed for providing the opportunity to carry out research work in a professional environment and with great facilities. My sincere regards and thanks to my GEC members Dr. Saadia Zahid, Dr. Maria Shabbir and Dr. Salman Khan (external). I would like to thank Ms. Shumaila Khan (Lab technician of Neurobiology Lab) for her full attention and help during my work in lab. Thanks to my Lab seniors, Ms. Amina Liaquat and Ms. Sara Ishaq for their guidance. I would like to thanks my lab colleagues Ms. Alveena Ahad Saddique and Ms. Maham Noreena Nawaz for their support during the lab work. I would also like to thank my friend Anam Rabail Baloch for assisting me in managing my experiments and lab juniors Ms. Nishat Malik, Ms. Sakina, Ms. Natasha Naeem, Ms. Areeba and Mr. Umar Farooq for their support and always cheering me up. Thanks to my friends Mr. Abu Bakar, Mr. Huzaifa, Mr. Mudassir, Mr. Abdullah, Mr. Usama, Ms. Zomia for their support and valuable memories during my MS journey.

Thanks to my parents **Mr. Amjad Bashir** and **Mrs. Naveeda Amjad** and my family members for their inseparable love, support, prayers and their confidence in me that has made me go through the tough times.

Table of Contents

List of Acronyms

Cad	Cadmium
CNS	Central nervous system
hr	Hour
S	Seconds
Kg	Kilogram
M	Molar
µl	Microliter
mg	Milligram
µg	Microgram

List of Figures

List of Tables

Abstract

The Cadmium (Cd) has been identified as the most toxic industrial and environmental pollutant. It disturbs the functioning of several organs (kidneys, lungs) and tissues following either acute or chronic exposure. Cd has a long half-life in the human body (about 10–30 years) so it can induce cell death in different neuronal cells and primary neural stem/progenitor cells. This study was aimed to investigate the toxic effect of Cd on cognitive function in mice. Three groups of 6-8 weeks-old BALB/c male mice was exposed to 900mg/Kg Cd (in the form of $CdCl_2$) through drinking water for 20, 35 and 50 days respectively. The behavior tests were conducted after 15, 30 and 45 days respectively. After the exposure, the recovery of same animals was also checked after 20 days period through same behavior tests. The results of Morris water maze test showed highest impairment in learning and memory in 50 day group and this group also showed least recovery after recovery period. Y-maze results showed highest impairment in 20 day and 50 day group. The 50 day group showed a little recovery but the 20 day group had irreversible damage. While the 35 day group have less impairment and also recover on its own. Social interaction results were also similar with the previous results, as the 50 day group showed highest deficit in novelty preference with no significant recovery. 20 day group also showed impairment but there was some recovery observed in novelty preference. Hole board test to check the working and reference memory error showed highest impairment in 50 day group and then 20 day group as compared to control. While 35 day group show less impairment and better recovery. It can be inferred from the results that both long term exposure with low dose and short term exposure with high dose both cause almost irreversible impairment on hippocampal dependent learning and memory. Future studies can reveal mechanism of temporal exposure of Cadmium suitable treatment depending on Cadmium daily exposure and duration.

CHAPTER 1

INTRODUCTION

CHAPTER 1
INTRODUCTION

1.1 Brain

The brain is the most complex organ in the human body. It produces our every thought, action, memory, feeling and experience of the world. This jelly-like mass of tissue, weighing in at around 1.4 kilograms, contains a staggering one hundred billion nerve cells, or neurons (Solms et al., 2018). The complexity of the connectivity between these cells is mind-boggling. Each neuron can make contact with thousands or even tens of thousands of others, via tiny structures called synapses. Our brains form a million new connections for every second of our lives. The pattern and strength of the connections is constantly changing and no two brains are alike. It is in these changing connections that memories are stored, habits learned and personalities shaped, by reinforcing certain patterns of brain activity, and losing others (Bars et al., 2010).

1.2 Brain regions involved in learning and memory

Formation and storage of learned behaviors and memories are associated with the change in neuronal connections and neuronal plasticity in different regions of brain. Synaptic transmission (functional plasticity) and changes in synaptic connections (structural plasticity) forms the basis of any memory storage that occur (Korte and Schmitz, 2016). Various region of brain is involved directly or indirectly with the function of memory. Some of the major are hippocampus, prefrontal cortex, amygdala and cerebellum.

1.2.1 The Hippocampus

Hippocampus is responsible for converting short-term memory into long term memory. It forms the neural foundation for attainment and packaging of configure association between events. There are two kind of memory process: a simple associative process that does not require hippocampus network and configure associative system that requires hippocampal formation. The configuring associative system creates a unique representation of an elementary stimulus event and builds association between different elementary representations (Sutherland and Rudy., 1989).

Many neuroscientists believe that hippocampus is important in forming new memories as it helps in identification of new stimuli event, experience and places (Cohen and Eichenbaum., 1993). It

is also regarded as a medial temporal lobe, memory system, for declarative memory, memory that can be verbalized such as facts. It also encrypts emotional data from amygdala. Episodic memories and places are connected (Tomer et al., 2014).It also plays role in working memory, spatiotemporal situation tagging, temporal and spatial mapping, anxiety, storage of neocortical cell-assembly addresses, change in irrelevant events, response inhibition, memory-retrieval processes, and associations (Sutherland and Rudy., 1989).

Hippocampus also plays role in spatial memory and navigation by help of place cells. Pyramidal cells are known to show response for place cells. Another important function is approach avoidance conflict. The anterior portion of hippocampus can detect conflicts whereas larger cortical and subcortical makes the decision. It occurs in a decision-making situation that requires a certain decision, either rewarding or punishing, the decision making is influenced by anxiety (O'Neil et al., 2015).

The loss of hippocampal formation results in impaired learning and memory. The bilateral symmetry is important, if one hemisphere gets damaged the other structure and functioning remain unaffected. Severe damage of both hemisphere results in anterograde amnesia, which is described as inability to form new memories and retrograde amnesia, in which the memories before damage are difficult to retrieve (Di Gennaro et al., 2006).

1.2.2 The prefrontal cortex

Prefrontal cortex located in cerebral cortex is important for human memory. Many neurologists and psychologists believe that the functioning of PFC and a person's personality are linked (DeYoung et al., 2010). PFC controls decision making, speech, language, social behavior and complex cognitive behavior (Yang & Raine, 2009). Basic function is the arrangement of thoughts according to person's will (Gabrieli et al., 1998).

Working memory, including all executive functions are controlled by PFC (Miller et al., 2002).Goldman-rakic determined this creates the representational knowledge which then helps in guiding actions, thoughts and emotions(Baddeley et al., 1986). Fuster proposed that PFC allows connection of future and past which is necessary in determining goals. According to dynamic filtering theory PFC directs processing levels such as maintain information, selecting and

retrieving information. It provides guidance to other parts of brain for proper processing of a given task (Miller & Cohen, 2001).

Some region of PFC is involved in generating language, speech and response before speaking. Words and sentences are processed majorly by left ventrolateral PFC. The retrieval of explicit memory is controlled by right prefrontal cortex for use of that memory in speech. The deactivated left is retrieves implicit memory for producing verbs. In amnesic patients there is impairment in nouns recollection (Hoffman., 2019).

Any injury in PFC affects cognitive memory. Such as loss in motor control, difficult to concentrate, loss of creativity and reasoning, short term memory deficits, temporal and source memory problems and difficulty in associative learning (Hoffman, 2019). Amygdala is located as two almond structures in the brain. Its major role is in generating emotions, processing memory and making decision (Amunts et al., 2005). Amygdala projections are extended to many parts of brain such as hypothalamus, thalamic reticular nuclei, facial nerves, to the ventral tegmental area, the lateral dorsally tegmental nucleus and trigeminal nerve nuclei and nucleus accumbens (Taskar et al., 2004). Thus it is involved in receiving information from olfactory bulbs and pheromone processing. It basically forms the connection with different parts of brain and then aids in processing information (Nieh et al., 2013).

1.2.3 The amygdala

Emotional learning is the major role of amygdala. It processes the emotional information and then stores the related memories. Long term potentiation refers to relation between stimuli and unpleasant event, which usually occur during fear conditioning. It is responsible for retrieval of long term fear associated memories (de Calignon et al., 2012). Memories related to emotions are usually stored in synapse all over the brain. Such as memories related to fear are stored in neural connections that extend from lateral nuclei of amygdala to its central nuclei. Nuclei of amygdala also process information from other brain parts that are important in making memory (Lalumiere., 2014).

Amygdala not only creates fear conditioning but it also creates positive (Appetitive) conditioning through distinct nuclei. Different nuclei within amygdala have different role in interpreting appetitive memory. Amygdala also has role in generating reward system. It is influenced by

dopamine, primary pheromones and secondarily attractive odorants (Lalumiere., 2014). Another important function of amygdala is in memory modulation. For any event the long-term memory is formed immediately instead it is solely stored through long term potentiation. And during this process the memory might get modulated. Greater emotional arousal and stress related to event, greater are the chances that the event gets retained in memory as it is. Any damage to amygdala results in loss of long-term potentiation function. It impairs generation of emotional response. The emotional memories are not formed if the neuromodulators in amygdala gets affected in damage (Uematsu et al., 2017).

1.3 Higher Cognitive Functions:

Cognitive functions refer to multidimensional executive and control processes characterized by being voluntary and highly effortful. These functions include the ability to evaluate, organize, and reach goals as well as the capacity to flexibly adapt behavior when confronted with novel problems and situations. Higher cognitive functions are executive function performed by brain comprising of thinking, problem solving, attention and decision making (Nelson et al., 2015). Cognitive functions are modified throughout our life time depending on neuronal plasticity.

Learning can be defined as the attainment of new information/knowledge, or processing the already known information, values or skills, & behavior or preferences (Gross., 2015). It can be the result of single event (e.g. being burned by a hot stove), but repeated experiences may result in accumulation of much skill and knowledge. The changes resulted from learning are usually lifelong, but it's often difficult to discriminate apparently 'lost' learned things from those which are not retrievable (Schacter et al., 2011).Memory is the function of brain, vital to experiences by which information is encoded, stored and retrieved when required to influence future actions (Sherwood., 2015). It can be declarative (data storage and its retrieval from conscious mind) or non-declarative (Jeneson et al., 2012).

When memories are formed they can be long term, short term or working memory. Long term memory is then further divided into two types, one is declarative memory (memory of facts and figures) and other is procedural memory (unconscious memory of a skill like cycling). Declarative memory is referred to those fact and events that can be recalled consciously (recalling answer to the exam question) while procedural memories are related to unconscious working and are improved or enhance by practicing riding a bike (Knudsen et al., 2015).

Working memory is limited capacity information storage while performing a task. Information is retained and manipulated to achieve the goal (Baddeley et al., 1986).

1.4 Cognitive Impairments:

Cognitive impairments (or neurocognitive disorders) are defined as acquired deficits in cognitive ability having underlying brain pathology. According to DSM-5 these cognitive functions include learning, memory, language, motor function, attention and socialization (Yochim et al., 2014).

These cognitive disorders include various medical conditions that affect mental functions like depression, bipolar disorder, attention-deficit hyper-activity disorder, post-traumatic stress disorder, schizophrenia, obsessive compulsive disorder, generalized anxiety disorder, thinking, memory and the reasoning capability, which includes degeneration of frontotemporal lobe, Huntington's disease, lewy body disease, prion disease, traumatic brain injury, Parkinson's disease, dementia, delirium and HIV induced neurocognitive abnormalities. Alzheimer's stands on the top of the list (Millan et al., 2012; Simpson., 2014). Some other disorders like psychosis, anxiety and mood abnormalities may also affect memory and cognition but these are not considered as cognitive deficits, as the causal (primary) symptom is not the loss of cognition (Guerrero et al., 2008). Different disorders have different causes but most of them include damaged brain portions which are responsible for memory formation. Treatments depend on the causes, including medicines and behavioral therapies most commonly. But there are certain types such as amnesia, for which there is currently no cure. Treatment can only suppress the symptoms (Meyer et al., 2005; Torpy et al., 2008;Torpy et al., 2010). Their possible risk factors include oxidative stress, HFD, depression, pollution, genetic disabilities and alcohol consumption (Iqbal & Ahmed., 2019).

1.5 Metals and Health Hazard:

Metals in spite of being unsafe to wellbeing have been utilized by people for a long time in numerous ways. Some of the metals are essentials for human body but some metal like arsenic and lead are lethal. Some essential metals required by body can also become dangerous when their amount exceeds permissible limits like Cadmium (Shekhar et al., 2008). Exhibition to these metals and their health-related issues has been expanding day by day around the world, particularly in developing countries. Various studies and investigations conducted in Pakistan

have clearly depicted hazardous effects of metals in general population. Exposure to these harmful effects is most visible in children and older age people in the form of growth disorder and neurodegenerative diseases respectively.

1.5.1 Metal toxicity in Pakistan

Due to unpredictable and low economic and social conditions, Pakistan is facing environmental challenges. Due to high expansion of population various region are expanded in an unplanned way, which has resulted in haphazard environmental load. Due to high urbanization resources are limited that resulted in poor quality of natural resources like soil water and air (Merolla et al., 2014). Population of Pakistan is at disposal of risky metals through routes like unclean drinking water consumption, air pollution and industrial waste. Hazard index of toxic metal lies high in contaminated water and air. Areas of Pakistan that are at high threat of metal toxicity are Central area of Khyber Pakhtunkhwa, Central and Northern areas Punjab and Southern area of Sindh. Consumption of toxic metals like lead arsenic and Cadmium is highest through drinking water in areas of Khyber Pakhtunkhwa. Through the route of air inhalation metal toxicity is highly concentrated in Punjab region (Shakir et al., 2017).

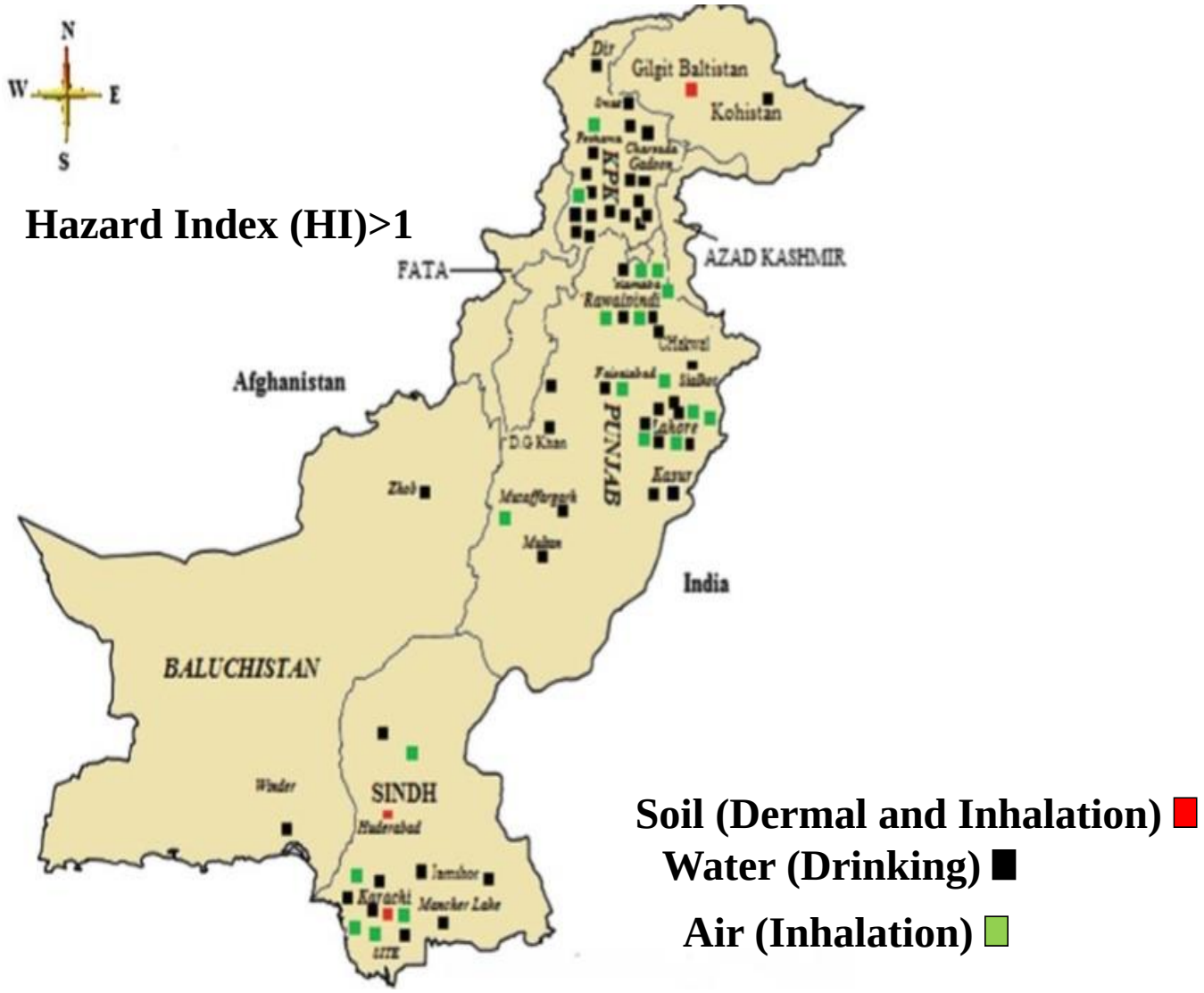


Figure 1. Pakistani map indicating the areas with HI> 1 with heavy metals pollution in water, air, and soil. Only those areas are shown here which pose the higher possible risks of metals contamination (Modified from Shakir et al., 2016).

1.6 Cadmium

Heavy metal cadmium has its concern regarding environment and occupation. Naturally, its average concentration is 0.15mg/kg in earth's crust. As per the ATSDR classification, the seventh toxic heavy metal is cadmium. It is a zinc manufacturing by-product that may be subjected to individuals or livestock at work or in the atmosphere. Once this metal is consumed by animals, it will build up within the body throughout lives.

1.6.1 Exposure and Occurrence

No effective recycling method is available for cadmium although the spectacular manufacturing, usage, and discharge of Cd in the setting are significant globally. Human exposure to Cd compounds could, therefore, lead to a severe health problem (Munisamy et al., 2013).

Japanese endured various rates of emissions after World War II, 1960's and 1970s. One of these circumstances was Itai-Itai disease due to rice fields contaminated with acute cadmium. It was reported that around 400 people were infected by the illness between 1910 and 2007 (Kaji., 2012). Cadmium is present in marine phosphates and sedimentary rocks in an outrageous concentration of 15mg/kg. It is present in batteries, alloys, and pigments (Paschal et al., 2000). About 3/4th of cadmium in the alkaline batteries is used as an electrode; the rest portion is used as a plastic stabilizer in coatings, pigments, and plantings. Human beings may be subjected mainly by inhalation and ingestion to this metal and may die from acute and chronic poisoning. For several centuries, cadmium dispersed in the atmosphere remains in groundwater and sediments. These metals are gradually consumed by plants and concentrated along the food chain, eventually entering the human body.

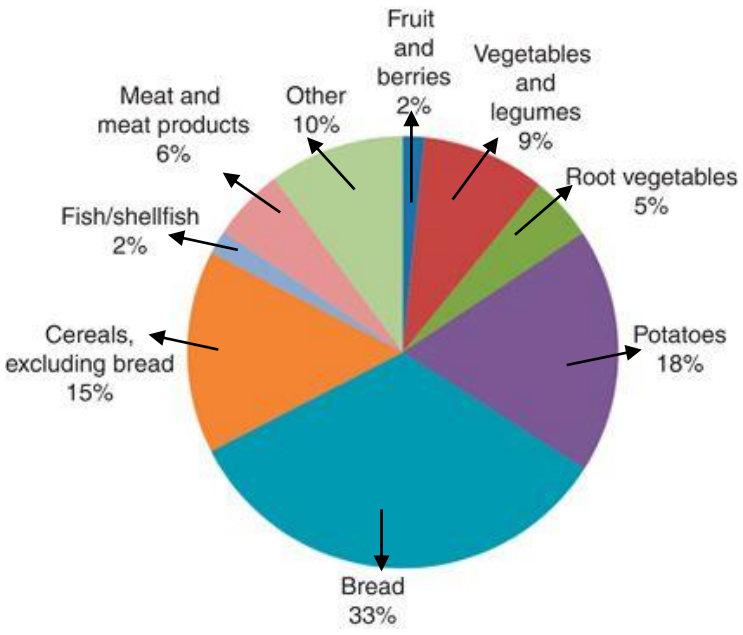


Figure 2. Dietary assessment of cadmium exposure in different foods. High level is present in cereals including wheat and rice (Modified from Julin et al., 2012).

The main exposure sources for human implicates working in metal industries, smoking cigarettes and consuming Cd-contaminated food (Paschal et al., 2000). Cadmium is present in some food in trace amounts including potatoes, leafy vegetables, liver and kidney (Satarug et al., 2003). Cadmium gets disseminated mainly through the bloodstream (Davison et al., 1988). Cadmium causes pulmonary infection, emphysema, and loss of olfactory function on chronic inhalation of cadmium (Mascagni et al., 2003).

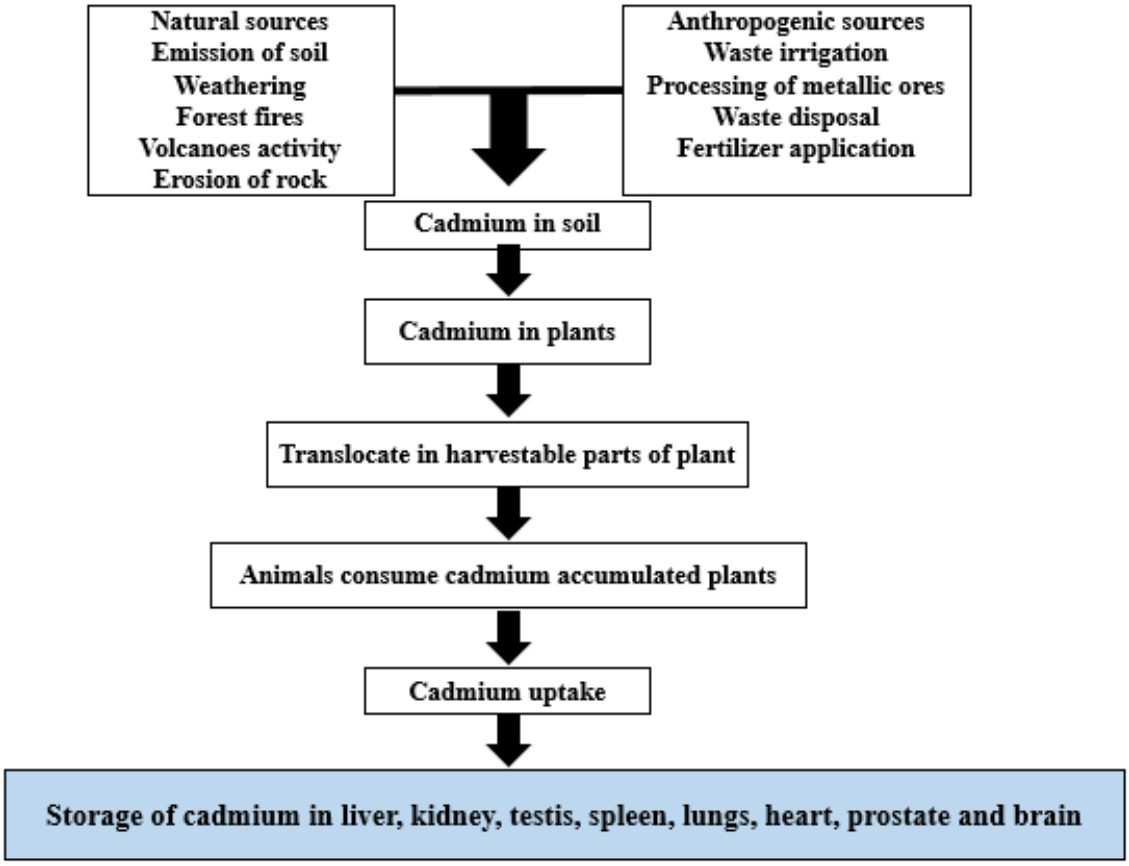


Figure 3. Different sources of cadmium exposure. Route for cadmium transportation and storage in plants and toxic effects on consuming cadmium accumulated plants in animals (Modified from Verma et al., 2017).

The kidney, cardiovascular, liver and reproductive system may be affected by cadmium intoxication and it can trigger kidney damage, osteomalacia, and lung cancer. Long-term cadmium exposure causes Itai-Itai illness in females primarily and is associated with severe glomerular and tubular structure and generalized osteoporosis and osteomalacia. This is the reason that sudden infant mortality, ear disorders, asthma, respiratory diseases, lung cancer, and cardiac diseases are at risk in passive smokers (Verma et al., 2017).

1.6.2 Epidemiology of Cadmium Toxicity in Pakistan

In Pakistan, the most frequently discovered heavy metals in surface water as well as in groundwater are the arsenic, the cadmium, the chromium, the copper, the nickel and the zinc,

which all contribute to safety risk and the atmosphere (Jaishankar et al., 2014; M. R. Singh, 2007).

Drinking water is a fundamental requirement for human survival. Today, there are more than one billion individuals in the globe as a whole. Most of this one billion inhabitants live in urban regions. According to the 1998 World Health Report, water supply has varied extensively for distinct regions and nations. Still, most people in emerging nations, particularly, are vulnerable to terrible conditions as the drinking water carrying heavy metals that are not safe to drink (Baig et al., 2012).

Cd was discovered both in the crust of earth and in the seawater in Pakistan. Ocean water has an average Cd of less than 5-110 ng / L, according to IARC (Leung& Chen., 2012)Cd, which is regarded to be allowable for drinking water, is 0.003 mg / L according to the WHO (M. R. Singh., 2007).

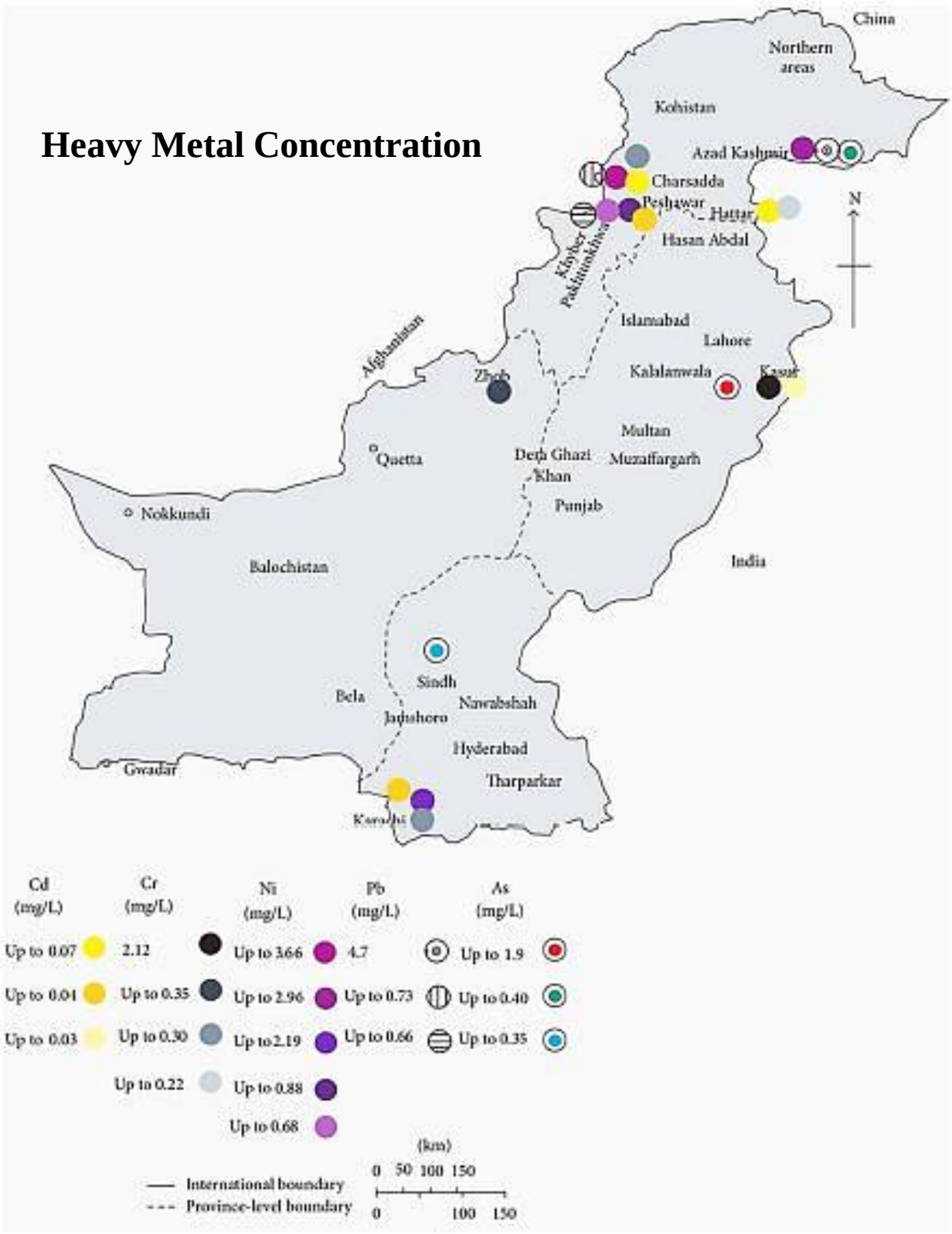

Heavy Metal Concentration
China
Northern areas
Kohistan
Azad Kashmir
Charsadda
Peshawar
Hattar
Hasan Abdal
N
Khyber Pakhtunkhwa
Afghanistan
Islamabad
Lahore
Kalalanwala
Kasur
Zhob
Multan
Qaetta
Dera Ghazi Khan
Muzaffargarh
Punjab
Nokkundi
Balochistan
India
Sindh
Bela
Nawabshah
Jadshoro
Hyderabad
Gwadar
Tharparkar
Karachi
Cd (mg/L)
Up to 0.07
Up to 0.01
Up to 0.03
Cr (mg/L)
2.12
Up to 0.35
Up to 0.30
Up to 0.22
Ni (mg/L)
Up to 3.66
Up to 2.96
Up to 2.19
Up to 0.88
Up to 0.68
Pb (mg/L)
4.7
Up to 0.73
Up to 0.66
As (mg/L)
Up to 1.9
Up to 0.40
Up to 0.35
(km)
0 50 100 150
0 100 150
International boundary
Province-level boundary

Figure 4. Pakistan map displaying Cr, Pb, Cd, Ni and as groundwater quantity (average numbers; the largest numbers are used where the average value is not accessible). This figure shows Pakistan provinces with the most concentrated heavy metal regions in colored dots, whereas light colors show upper and lower colors with lower density ranges for heavy metals (Modified from Rehman et al., 2018).

A study to evaluate amount of heavy metal in drinking water was performed in Pakistan. According to the study, the Cd level in samples obtained at various areas of Pakistan was discovered to be 0.001-0.21 mg. The largest Cd values of approx. 0.02 mg / L were thus discovered in water specimens obtained from Hayatabad Industrial Estate tube well water sources in Khyber Pakhtunkhwa in Pakistan state (Manzoor et al., 2006).

Table 1. Allowable constraints in drinking water for heavy metals consumption. Standard threshold values for cadmium consumption set by worldwide research institutes (Rehman et al., 2018)

Standards	Cadmium (µg/L)
IARC	**Group 1**
US-EPA	5
WHO	**3**
NSDWQ-PAK	10
EU	**5**

1.6.3 Worldwide Epidemiology of Cadmium Toxicity

An evaluation of the quality of groundwater in South-Western Nigeria indicates that Cd and certain other heavy metals were surpassed by the concentrations of WHO in potable water (Adekunle et al., 2007). In Nigeria, Cd-contaminated drinking water was discovered to be 0.06-1.1 µg / L (Asubiojo et al., 1997). Likewise, a drinking water survey in Sweden has resulted in Cd concentrations of up to 5 µg / L (Friberg et al., 1979). The average Cd concentration in Saudi Arabia from private wells was 1-26 µg / L (Mustafa et al., 1988).

This demonstrates that drinking water is contaminated with Cd in a worldwide manner which may harm people. In the Process readings, we will discuss the main safety issues linked to exposure of Cd.

1.6.4 Cadmium Entry into the Food Chain

The prevalent origin of Cd toxicity are polluted cereal crops, drinking water and food irrigated with polluted water in non-smokers and non-professionally exposed populations (A. Singh et al., 2010). Irrigated cereals with wastewater have reduced concentrations of heavy metals in cereals, compared to vegetables.

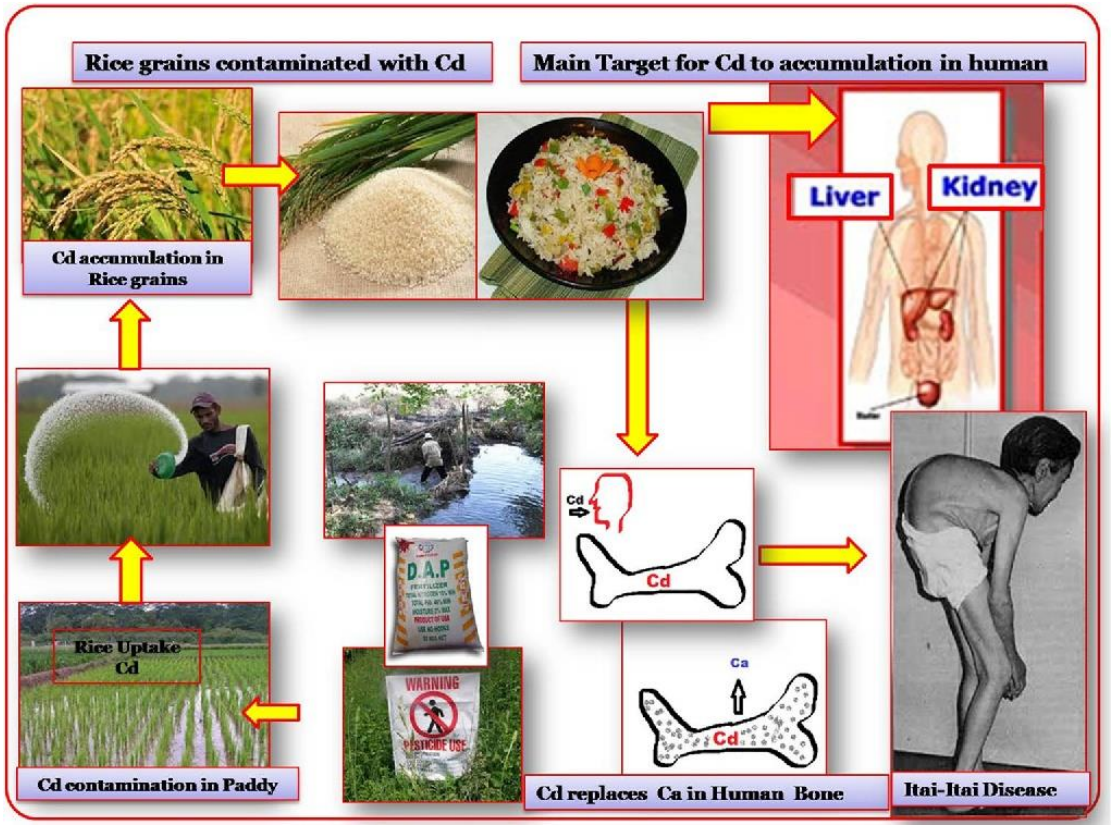


Figure 5. Cadmium entry in food chain. Route by which cadmium transported from rice crops to humans. Chronic absorption of cadmium and replaces calcium in bones causing itai-itai disease (Modified from Manohar et al., 2012)

1.6.5 Absorption of Cd in Body

By various mechanisms Cd may enter the human body. Cd particles (Cd oxides or Cd dichloride) are carried along the main olfactory neurons and accumulated without further migration into the brain in the olfactory bulb. Alternatively, Cd accumulates in the lungs after inhalation and enters

blood circulation through the alveolar cells. The other significant mechanism for Cd entry is Cd uptake through ingestion of food or water containing Cd. The proton-metal cotransporter (DMT1) is transporting Cd at the enterocytes apical membrane. Cd exports via the basolateral membrane also involve transporters such as calcium-ATPases and zinc transporters (Branca et al., 2018).

When cadmium taken up in the blood, it is mostly transferred to proteins like metallothionein and albumin. Following the intake of the GI blood, the initial organ is the liver. Cadmium here induces metallothionein's manufacturing. Cd-Metallothionine complexes are washed in sinusoidal blood following subsequent hepatocyte necrosis and apoptosis. Part of the absorbed cadmium enter here as cadmium-glutathione conjugates in the bile duct, via secretion, through the entire hepatic cycle. Cadmium reenters the small intestines, which is enzymatically broken down into cadmium cysteine composites in the bile tree

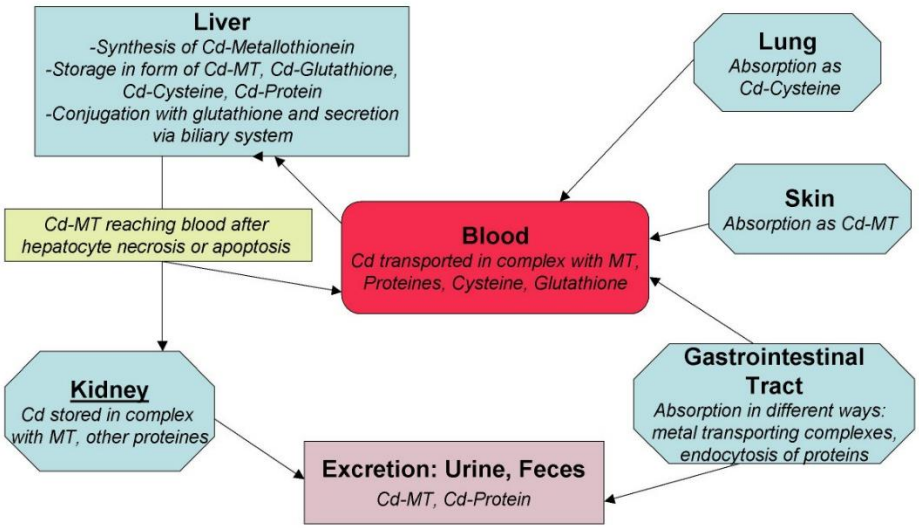


Figure 6. Cadmium handling in human body. Transport of cadmium though blood to different organs and excretion out of the body. Effects produce by cadmium in different organs (Modified from Godt et al., 2006).

1.6.6 Impacts on Health

For a variety of metabolic mechanisms such as peptide hormones synthesis and their action, enzyme metabolism, bio membranes, essential heavy metals are needed. The availability of prerequisite cations for body development is reduced among people at contaminated locations, where there is an increased probability of cadmium absorption, leading to effective bio augmentation and bioaccumulation in the human body, resulting in detrimental physiological action. Once consumed, cadmium is maintained effectively in the human body and builds up over life (Bernard., 2008b). Cadmium is the highly poisonous pollutant and neurodegenerative diseases are caused by its exposure (Chen et al., 2011). Kidneys, particularly proximal tube cells is the principal location for long-term build-up (Bernard, 2008a).

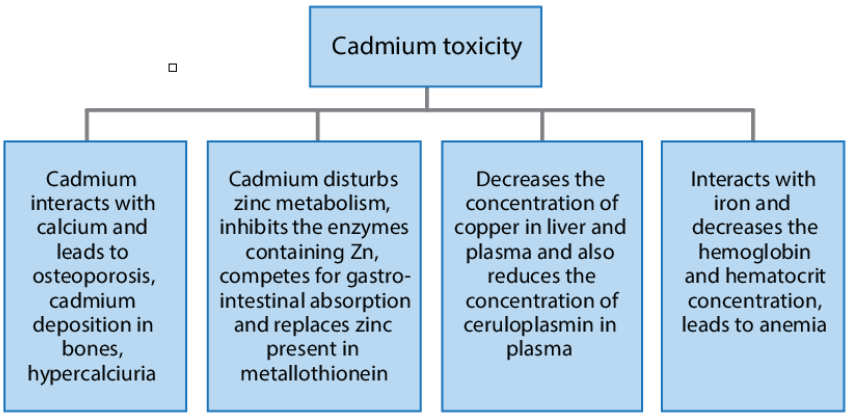


Figure 7. Cadmium-induced pathologies in bones, GI tract, liver and kidney. Interaction of Cd with calcium, zinc, copper and iron and the related effects produced by it in respective tissues (Modified Flora et al., 2008)

1.6.7 Mechanism for Toxicity

In the human body, toxic metals are metabolized in a similar way as essential elements. The increased consumption of cadmium in the body may be due to a number of reasons such as low intake of Ca, vitamin D, and zinc. The reason for its higher compensating consumption could be it's specific, be relevant molecular resemblance of Cd with Zn and Ca(Godt et al., 2006)Cd being non redox active metal and unable to participate in Fenton & Haber reaction, it doesn't produce free radicals (Prasad., 2008).

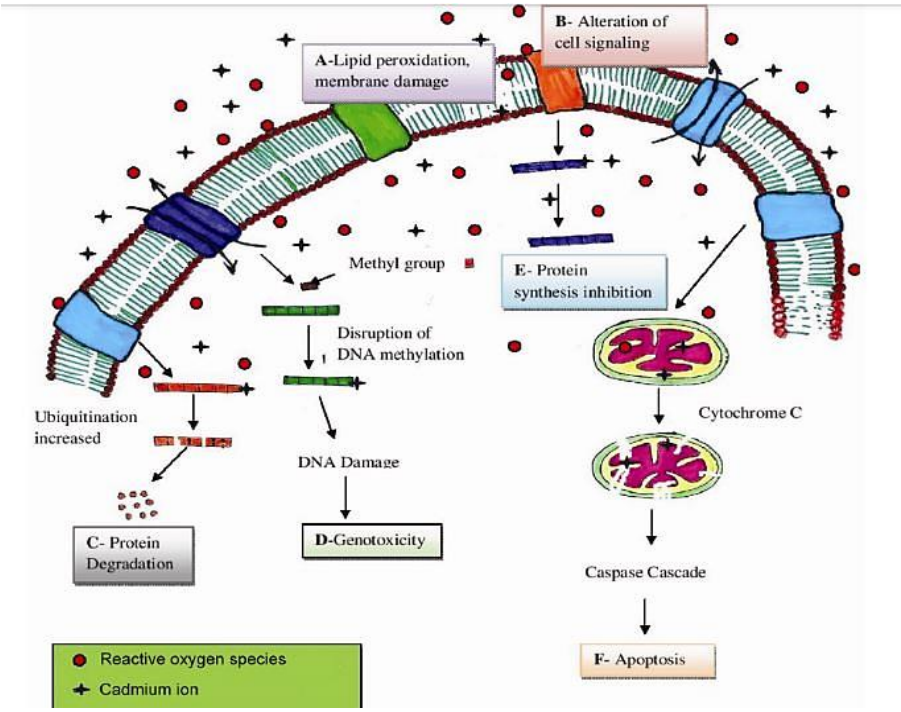


Figure 8. Cadmium toxicity mechanisms at the cellular level. (A) Lipid peroxidation (B) Effects on signaling pathway (C) Effects on protein (D) Effects on DNA (E) Effect on synthesis of proteins (Modified from Verma et al., 2017)

Oxidative stress symptoms such as LPO are the result of (GSH) reduction, as cadmium is linked to GSH and protein chelators derived from GSH (PC) formation. Cadmium affects progression, proliferation, differentiation and apoptotic pathways of the cell cycle. It prohibits replication and repair of DNA. The transporter (DMT1) is a non-specific metal transporter capable of carrying not only iron but also step in intestinal uptake of Cd(Park et al., 2002). After Cd entering in the body, the activity of several antioxidant enzymes either reduces or increases (Prasad, 2008).

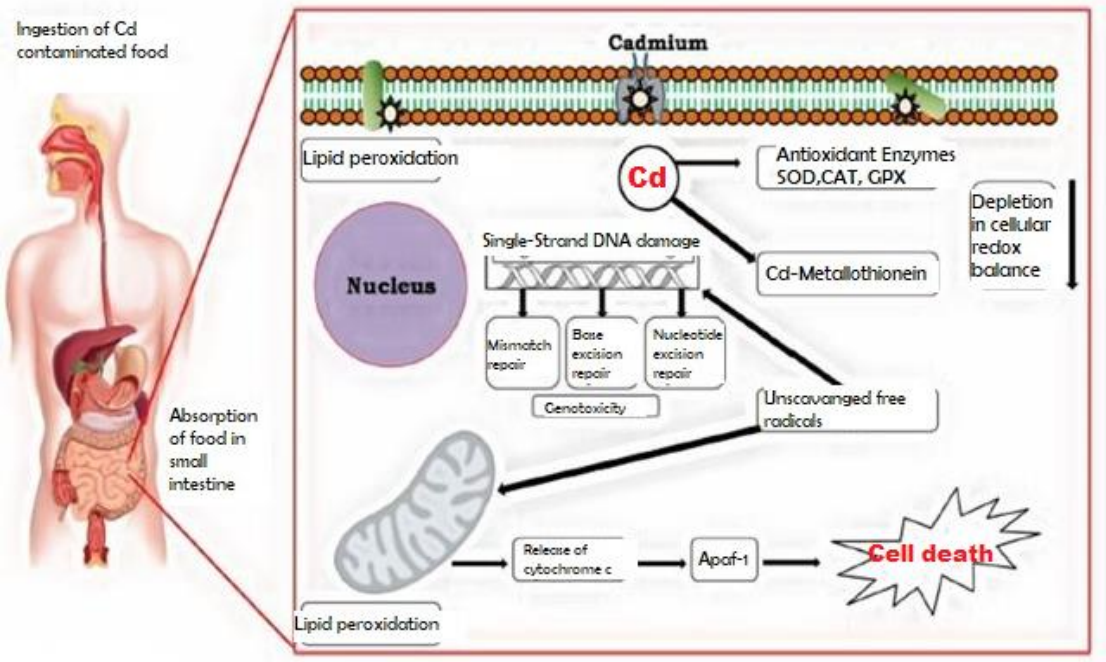


Figure 9. Mode of toxicity mechanism of Cd in humans. Intracellular changes caused by chronic exposure of cadmium. Depletion in balance of cellular redox status and cell death by Cd (Modified from Kumar et al., 2018).

In addition, cadmium causes intracellular ATP concentrations to decline and the mitochondrial membrane eventually breaks down.Ca and cytochrome C concentrations subsequently boost in the matrix of cell and stimulate the triggering of various caspases and induce apoptosis (Méndez-Armenta & Ríos, 2007).

Cadmium activates the pathway of (MAPK), through the formation of (ROS), which not only activates the Upstream, Erk1/2 and c-Jun N-terminal kinase (JNK), but also impedes the negative regulatory system, PP2A and PP5 (Protein Phosphate 5) leading to caspase-dependent &-independent neuronal cells apoptosis (Shanker, 2008).

It also activates the (mTOR) through a ROS induction which is associated with the upregulation of NADPH oxidase (NOX2) expression and its regulatory proteins (Chen et al., 2011).

1.6.8 Neurotoxicity

Cadmium causes neurotoxicity leading to neurodegenerative diseases provided proof that CdCl2 increases nerve cell oxidative stress, a consequence that is entirely hindered by exogenous neurotrophic factors. These activities require inhibition of Janus kinases, Jak1 and Jak2 trans phosphorylation of the receptors. Jak kinases neuronal inhibition, selectively activated by increasing intracellular oxidative stress levels in neurons, offers a new way of implementing heavy metals that have their neurotoxic effects. Cd also enhances free radical production in adult rat brains and impedes with the antioxidant defense system that causes Cd-induced modification of lipid structure and also harms membrane-bound enzymes such as Na+ or K+ATPase (Shukla et al., 1996). The CNS injury generated by Cd seems to be due to a large loss of oxidative phosphorylation along with a range of circumstances causing CNS injury following inhibition of oxidative phosphorylation, all of which deteriorate the white matter selectively in the brain (Fern et al., 1996).

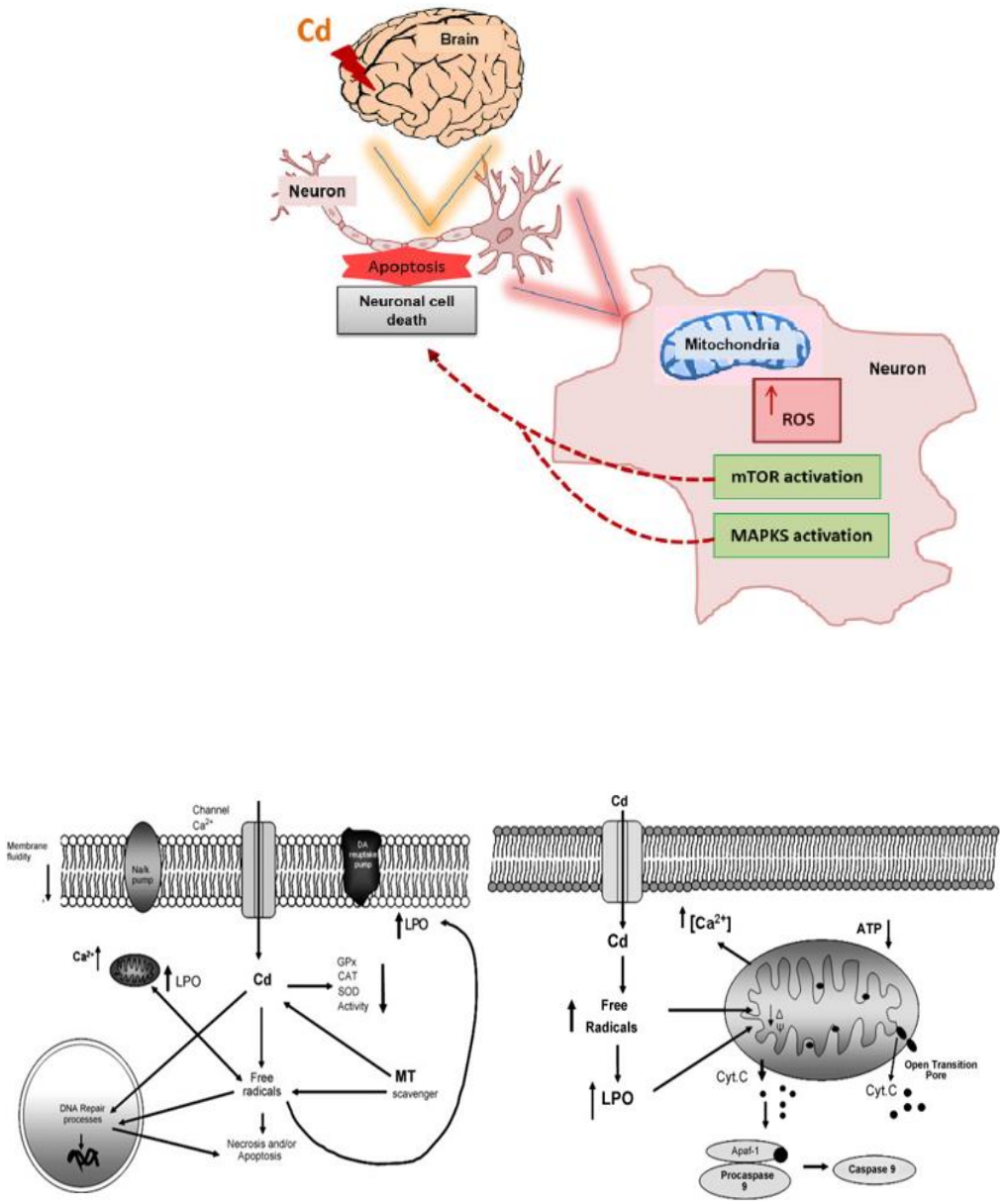


Figure 10. Figure depicting neuronal apoptotic cell death following exposure to cadmium. Role of Cd in increasing ROS and activation of MAPK &mTOR pathways leading to neuronal cell death Mechanism of cadmium neurotoxicity. Two pathways in which role of cadmium in causing malfunctioning in nucleus and mitochondria is shown. In both of these pathways Cd takes entry through calcium voltage gated channels and induces apoptosis (Méndez-Armenta & Ríos, 2007; Rehman et al., 2018)

Cadmium invades neuron through voltage-gated calcium channels (Usai, Barberis, Moccagatta, & Marchetti, 2008). Cd ion enters through Ca2+ channels within the neurons. When Cd is inside the cells GPx, CAT and SOD activity significantly reduces hence cause a rise in free radicals. This free production of radicals can produce and enhance lipid peroxidation with cellular membrane disorder and cause apoptosis or necrosis. In addition, Cd and free radical caused harm to DNA, whereas MT is primarily triggered as free radicals and Cd scavengers (Méndez-Armenta & Ríos, 2007). These symptoms include neurological disturbances, olfactory dysfunction, learning disabilities and mental retardation (Branca et al., 2018). The integral part of the brain is hippocampus and it attaches to the limbic system. It is involved in playing its part in spatial navigation. It is present in the medial lobe of the brain. In the case of Alzheimer's disease, hippocampus gets severely damaged (Kanter, Unsal, Aktas, & Erboga, 2016). Hippocampus and frontal cortex brain areas are very much involved in memory and learning procedures. In daily memories of facts and habits, these areas play a key role. After prolonged cadmium exposure, altered AChE activities have been previously reported in adult Wistar rat in hippocampus, brain cortex, cerebrum, striatum, and hypothalamus (Batool et al., 2017).

CHAPTER 2

MATERIALS AND METHODS

CHAPTER 2
MATERIALS AND METHODS

2.1 Ethical Statement and Letter of Permission:
Atta Ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST) laboratory animal house maintained the animal under standard environment. All the testing protocols and procedures of the study were approved by Institution Review Board at Atta Ur Rahman School of Applied Biosciences, NUST (IRB# 135). All the test and procedures were performed under the regulations by the Institute of Laboratory Animal Research, Division on Earth and Life Sciences, National Institute of Health, USA.

2.2 Animal Model:
Study is carried out on male Balb/c mice, 80 provided by Laboratory Animal House, ASAB, NUST and 40 purchased from National Institute of Health (NIH), Islamabad. Animal were kept under standard condition in 14 plastic cages of size (40cm×25cm×15cm). 5 Animal were kept in each cage with soft wood shavings as bedding. Housing conditions were maintained at temperature of 22±2°C and 12-hour light/dark cycle with standard feed and water provision.

2.3 Chemical and Reagents:
Cadmium salt in this study used is Cadmium Chloride by Sigma Aldrich. Solutions were made using standard distilled water.

2.4 Study Design:

2.4.1 Animal Groups:

The animals were separated arbitrarily into the following groups:

Table 2. Animal groups employed in study

Group No.	Description	No. of Mice
1	Control	30
2	20 days group	30
3	35 days group	30
4	50 days group	30
Total No. of mice	**120**	

Control group was sustained at normal water. 20 day group was provided with Cadmium salt solution in distilled water at a dose of 45 (mg/kg/day). 35 day group was given Cadmium salt in distilled water at a dose of 25.71 (mg/kg/day). And 50 day group was sustained at Cadmium salt in distilled water at a dose of 18 (mg/kg/day). Thus total dose for all the exposure groups were 900 (mg/kg). All the groups were given standard feed.

2.4.2 Methodology layout:

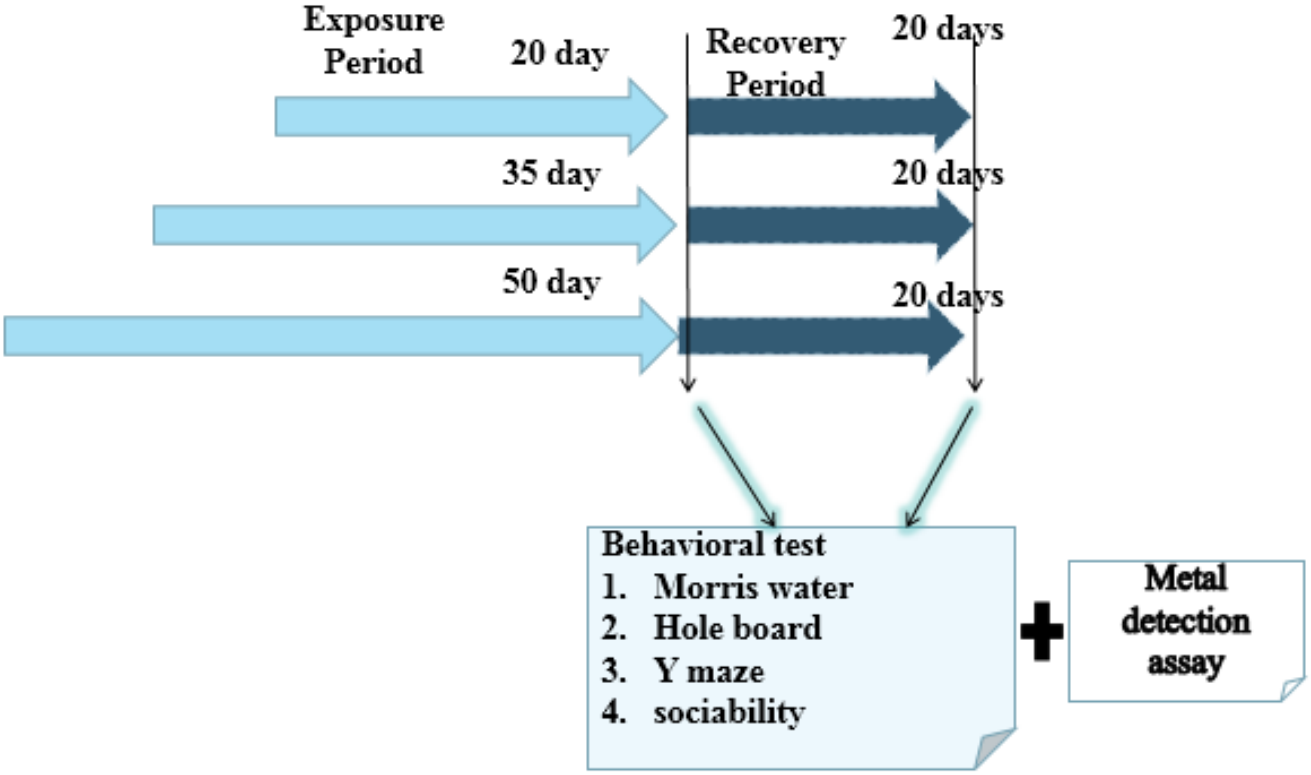


Figure 11. All the exposure groups were given Cadmium dosage according to the days specified. i.e. 20 days, 35 days and 50 days. After the completion of exposure time, a recovery period of 20 days was provided to each group. In recovery group animals were given normal water and feed.

Group Name	Days of exposure	Daily dose of cadmium mg/Kg/Day	Total cadmium exposure mg/Kg	Number of mice
Group 20	**20**	**45**	**900**	**30**
Group 35	**35**	**25.71**	**900**	**30**
Group 50	**50**	**18**	**900**	**30**
Group Control	**20, 35, 50**	**0**	**0**	**60**

2.5 Behavioral Tests:

Behavioral tests were performed on all the animals under standard environmental condition. All the animals were habituated in a different room. And tests were carried out in mice day cycle to prevent variations in the performance due to circadian rhythm disturbance. Inter test interval was kept 45 minutes minimum. Temperature of both the habituation room and testing room was maintained at 22±2°C. Behavioral test performed are mentioned in Table 5.

Table 3. Behavioral test used and their association with learning and memory.

Test	Brain region involved	Behavior
Y maze test	Hippocampus prefrontal cortex	spatial learning and memory
Morris water maze	Hippocampus	spatial learning and memory
Hole board test	Hippocampus Amygdala	exploratory behavior/anxiety
Three chamber assay	prefrontal cortex	Sociability

2.5.1. Morris water maze test (MWM Test)

Purpose:

Morris water maze test is one of the most reliable test to assess hippocampal synaptic plasticity. Many type of Morris water maze have been developed but the best one is originated by Richard G. M. This test is used to determine spatial learning and memory through spatial cues provided to animal in its surrounding environment. Spatial learning is based on time taken by animal to reach submerged hidden platform in repeated trials. Reference memory is analyzed by animal tendency toward platform area when the platform is removed(Vorhees& Williams, 2006).

Apparatus:

Morris water maze test consist of a circular tank filled with water that is made opaque. A platform is placed in one of the quadrant of tank and is camouflaged due to the opacity of water.

The level of water in the tank is such that the platform is just submerged in it. Temperature is maintained at 23±2°C throughout the experiment.

Procedure:

Morris water maze test has a protocol of 6 days. Training trials are conducted for 5 days then probe trial is performed.

Training period

Training period comprised of 5 days in which 5 trials are conducted each day with platform submerged in the water. Animal were dropped in the tank from different directions in each trial. Directions for each day were determined according to the table 4. Each trial is of 90 seconds in which animal is allowed to find the platform by observing cues. If the animal finds platform before 90 seconds and sits on it for a minimum of 5 seconds, then the trial was stopped and time was noted. If animal is unable to find platform in 90 seconds it will be placed on the platform manually for at least 20 seconds. It is then removed from tank and placed in its cage. Inter trial gap of 10 minute was maintained in training session. Time recorded in training session is then used to analyze escape latency of mice.

Table 4. Training sessions for Morris water maze test.

No. Of Days	Direction of Release				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
1	West	South	North	East	South
2	North	West	East	West	South
3	North	East	West	South	North
4	East	South	West	East	North
5	West	South	North	East	South
6 (Probe Trial)	Single probe trial without platform. Direction of release: West				

Probe trial

After the successful completion of training period probe trial was conducted on next day. Platform was removed from the tank. Animal was dropped from the south direction in the tank and allowed to explore and search for platform for 90 seconds. Video was recorded through camera above the tank. Video will then be analyzed for following parameters.

1. Number of entries in target quadrant
2. Time spent in target quadrant
3. Number of platform crossings

2.5.2 Y-Maze Test

Purpose of test

Y maze test is a behavioral test based on hippocampus dependent spatial learning and memory. It is used to analyze the performance of working memory as well as recognition memory. This test is based on rodent natural curiosity to explore their environment. Spontaneous alternations and exploration of novel arm instead of visiting the already explored arm is the basis of this test. Various regions of brain are involved which are directly related to spatial learning and memory. Main regions involved are hippocampus, prefrontal cortex, basal forebrain and septum.

Apparatus:

The Y-maze is made up of 3 rectangular arms with a dimension of 50 x 16 x 32 cm. These three arms are unified at angle of 120º. Arms are named as “Start arm”, in which animal is placed when trial is started. Second is “Other arm” that is kept unblocked during habituation. And the third is “Novel arm”. The ends of these three arms are labeled with different white and black pattern to provide spatial cues.

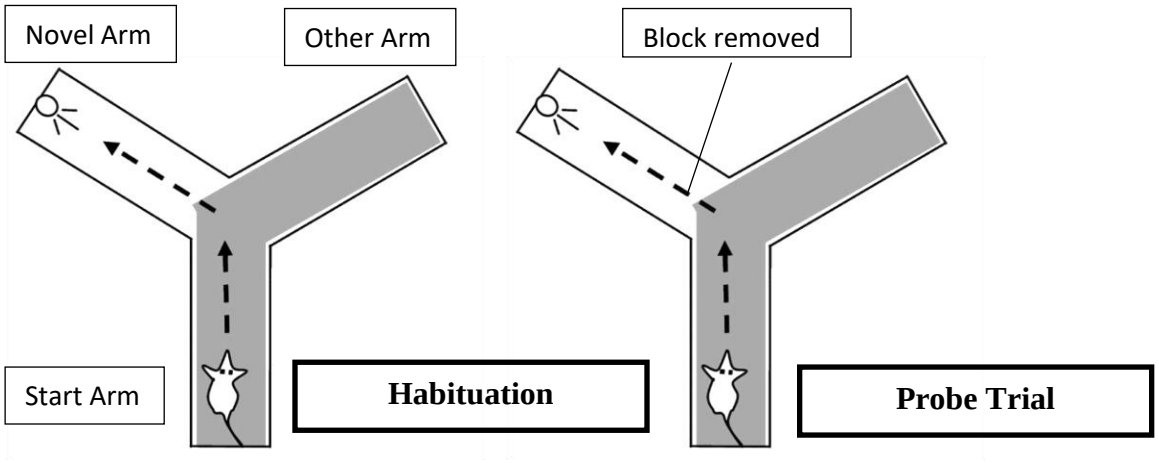


Figure 10. Diagrammatic representation of Y maze test

The rodent enters the maze in the arm labeled "Start arm", facing away from the center. The rodent is then allowed to explore the two arms, in the training session, while in the probe trial, the "novel arm" is opened and the rodent is allowed explore all three arms freely. The rodent is expected to show a tendency towards exploring a less recently visited arm. Total number of arm entries, total time spent in each arm, and the numbers of triads are recorded to determine the spontaneous alternations. An entry is recorded when all four limbs are inside the arm.

Procedure

Y maze test was performed in two sessions. First is the habituation or training period and second is probe trial that is recorded by camera to analyze animal performance in the test. The experiment was executed with a little alteration to the y-maze protocol by Conrad et al., 1996.

Habituation:

In habituation period animal was given free access to two of the arms that are start arm and other arm. The novel arm was kept blocked by a removable wooden block. Session started when the animal was dropped in the start arm of the y maze with its face toward the wall of start arm. Habituation is carried out for 15 minutes in which animal explored its environment. After the end of habituation time animal was removed from the maze and placed back in its cage to provide inter trial time of 30 minutes between habituation and probe trial.

Probe trial

In probe trial the wooden blocked used to block novel arm was removed. Trial was started by dropping animal in the start arm with its head facing toward the center of the maze. Animal is then allowed to explore the maze for 5 minutes. The trial is recorded by a camera above the

maze. Maze was thoroughly cleaned and wiped with 70% ethanol in between habituation, probe trial and next animal session to prevent any olfactory cues to the animal. Recorded videos will be used to assess following parameters:

1. Number of entries in each arm
2. Time spent in each arm
3. Spontaneous alternations
4. Alternate arm repeats and
5. Same arm repeats.

2.5.3 Social Preference and Novelty Test:

Purpose

This test is employed to assess animal general social interaction and preference for novelty object. Performance of an animal can be used to determine sociability and novelty deficit. It can also be used to check cognition by analyzing animal remembrance of familiar and unfamiliar novel mouse.

Apparatus

Three Chamber Assay is made up of glass rectangular box with three compartments in it. Compartments are separated by a glass wall with a door like hole in them so that animal can freely move between three chambers. 2 metal wire cages are placed in left and right chamber named S1 and S2 in which unfamiliar mice will be placed.

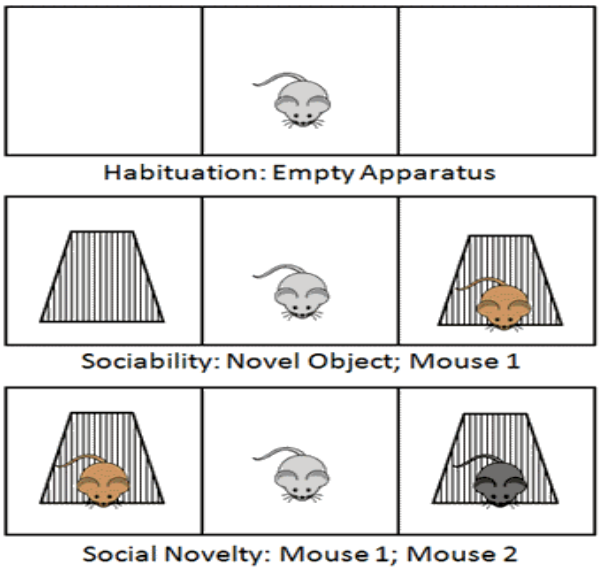


Figure 11. Representation of Social Preference and Novelty Test

Procedure:

Habituation

In habituation period empty cages were placed in chamber. Animal was dropped in the central chamber and was allowed to explore freely for 5 minutes. After habituation time animal was removed and placed back in its cage for 30 minutes before the start of session 1.

Session 1

In this session 1 wire cage was provided with a stranger mouse(S1) while other wire cage was left empty. Animal was dropped in the center compartment and was allowed to move and interact freely for ten minutes. Video was recorded to assess following parameters

1. Time spent in each chamber
2. Interaction time with empty cage and S1 cage.

Session 2

Session 2 will be carried out after 20 minutes of session 1. In this session S1 cage was provided with already familiar mouse of session 1 and a new non familiar stranger mouse in S2 cage. Animal was dropped in center compartment and allowed to move and interact freely for 10

minutes. In both sessions apparatus was thoroughly cleaned with 70% Ethanol. Videos of session was recorded to analyze following parameters

1. Time spent in each chamber.
2. Interaction time with S1 and S2(novelty).
3. Percentage discrimination index.

2.5.4 Hole Board Test:

Purpose:

Hole board test was developed in 1970s to overcome the flaws of open field test. Modified form of hole board test used by (Li et al., 2009). Modified hole board test can be employed to assess working memory and reference memory (spatial reference memory). Working memory is analyzed by observing the recurrent visit to wrong choices (empty hole). If in a trial animal visits a wrong hole (un baited hole) and in the same trial visits that hole again then it has committed a working memory error. Reference memory is based on long term and associated with spatial cues in surrounding. All the visits to wrong holes (unbaited holes) will be considered as reference memory errors (Li et al., 2009). Anxiety of animal can also be assessed by hole board test through latency to reach the first hole.

Apparatus:

Hole board apparatus is a square box made up of sheen wood or board. The box is open from top to record the videos. The bottom of box contains 16 holes in it. These holes will be baited with feed or left un baited during trials. Different spatial cues were pasted on the walls of the hole board to assess reference memory.

Procedure

Pre habituation:

Animals were deprived of feed 24 hours before the start of habituation period to increase their quest for food. Water was provided normally.

Habituation:

Animal were taken to the testing room prior day to acclimatize them to environment. All holes were baited with 100 mg of feed. 2 sessions of habituation were carried out. Animal was dropped in the center of apparatus for 15 minutes and was allowed to freely explore the box. After 15 minutes animal was removed and placed back into its cage. After an inter session interval of 3 hours habituation session was repeated again with the same protocol

Trials session

It was performed on the next day of habituation and continued for 4 days. Each session per day composed of 4 to 5 trials in which animal was dropped in the apparatus from different directions. Trial is of 3 minutes and in all the trial same 4 holes were kept baited with 300gm of feed. After each session and in between trials apparatus was thoroughly cleaned with 70% ethanol. Trials were repeated with same protocols.

Table 5. Parameters assessed in Hole board test.

MEMORY	PARAMETER	NARRATIVE	PURPOSE
Working memory	Working memory error	Any revisit to un baited hole in the same trial. (Animal nose should be below the rim of board floor)	To assess grade of hippocampal damage and effect on learning and memory.
Reference memory	Reference memory error	Any visit to wrong hole (un baited) in a trial. (Animal nose should be below the rim of board floor)	
Latency	Time taken to visit first hole	It is measured as the time required by an animal to visit the first baited or un baited hole when the trial starts.	To assess anxiety of an animal.
Activity	Nose pokes/ Head dips	No. of head dips or nose pokes in a minute	To assess locomotor activity and exploration rate.

CHAPTER 3

RESULTS

Chapter 3
RESULTS

3.1 Morris Water Maze Test

Morris water maze test was employed to assess the effect of temporal exposure of Cadmium (900 mg/kg) on long term memory and learning. Memory deficit caused by Al in various temporal groups was analyzed through escape latency parameter. On day first all the exposure groups have shown deficit in spatial learning as compared to Control group (58.49 ± 3.43). Highest deficit was shown in 50 days exposure group (73.76 ± 4.34) and 20 days exposure group (68.95 ± 4.43). Control group (10.88 ± 2.05), 20 day exposure group (21.50 ± 1.52) and 35 day exposure group (14.58 ± 1.20) have shown similar learning behavior throughout the next 04 days of training period. However, 50 days exposure group have shown least learning as compared to Control and other exposure groups. Recovery groups have shown significant learning as compared to exposure groups.50 day exposure group (28.20 ± 4.92) have shown minimum learning as compared to control group (10.84 ± 1.03), 20 day exposure group (15.09 ± 1.36) and 35 days exposure group (21.72 ± 2.22). After comparison between exposure groups and recovery groups is done, after the recovery period all the recovery groups perform better. Over all poor spatial learning behavior was observed in 50 day exposure group which did not improved after recovery period

To assess reference memory platform was removed and probe trial was conducted after 5 day training period. Data was analyzed to observe difference in number of entries in target quadrant between all the groups. 20 day exposure group (6.60 ± 0.63) and 50 day exposure group (7.3 ±0.44) showed least number of entries as compared to control (9.2 ± 0.33) and 35 day exposure

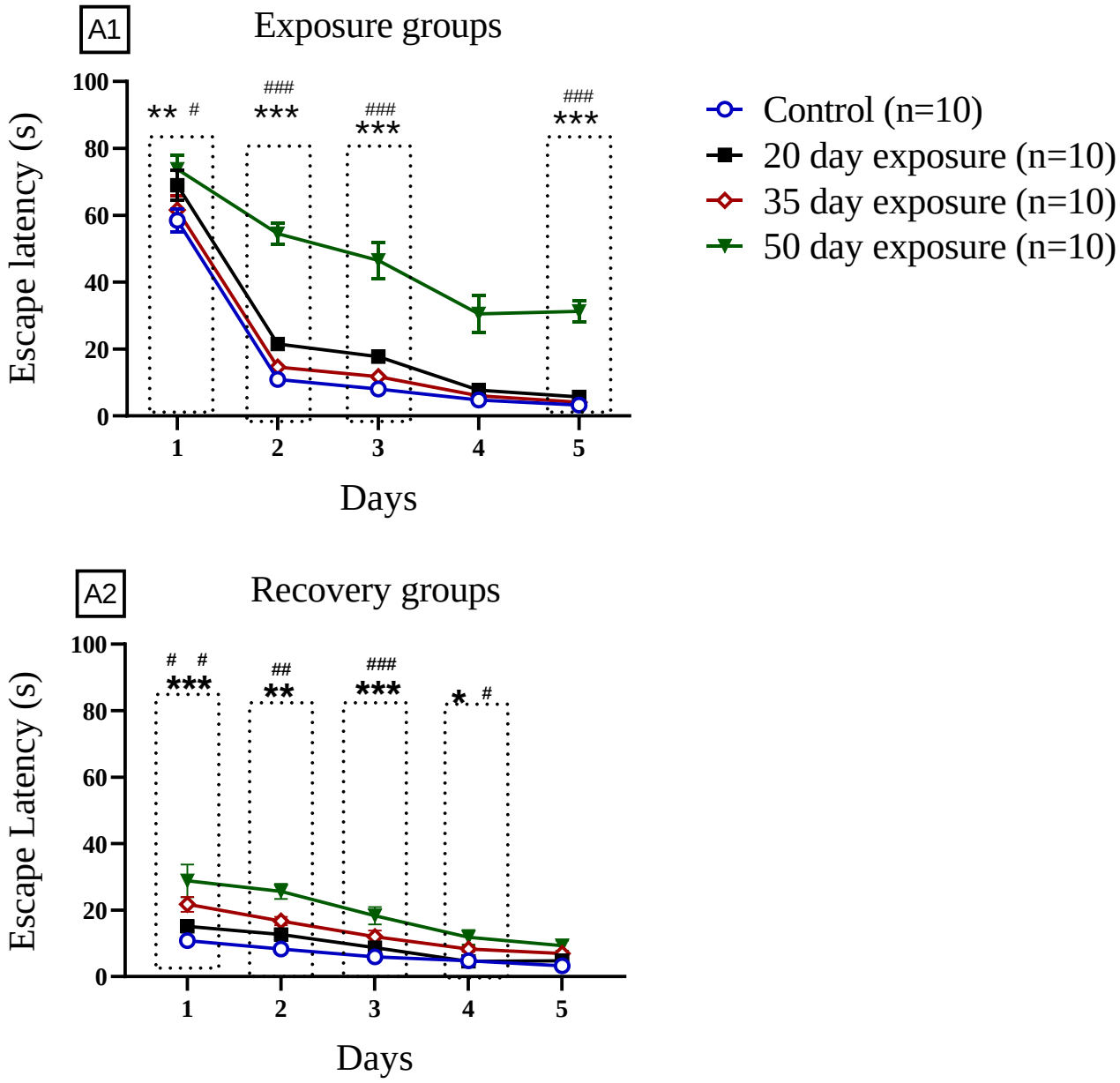


Figure 12. Effect of Cadmium on learning and memory in Morris water maze test; Escape latency. Graph shows escape latency (s) to assess the reference memory and learning among the control, 20 day exposure, 35 day exposure, 50 day exposure and their respective recovery groups. *=p<0.05 is significance between control and Cd treated groups, ## = p< 0.01, ### = p< 0.001 are significance among Cd treated groups. Error bars are represented as mean± SEM.

group (5.80 ± 0.53). After recovery period no significant improvement in memory was observed in any recovery group in comparison with respective exposure groups

The number of platform crossings was recorded and analyzed in probe trial. Within the exposure groups, 20 day exposure group (2.0 ± 0.33) and 50 day exposure group (1.60 ±0.26) showed highest deficit in memory as compared to control group (9.2 ±0.59). After recovery time period improvement in performance was observed in all the groups. 20 day recovery group (5.50 ±0.16) presented highest improvement in memory as compared to its respective 20 day exposure group (2.0 ±0.33)

Time spent in target quadrant (TQ) was analyzed to assess differential deficit between all the groups. 20 day exposure group (34.60 ± 2.8) and 50 day exposure group (34.7 ± 2.58) spent least time in target quadrant as compared to control (64.20 ± 2.64). After recovery period all the three recovery groups spent more time in target quadrant. 20 day recovery group (50.20 ± 1.68) spent highest time in TQ as compared to 50 day recovery group (35.00 ±2.39) and 35 day recovery group (43.60 ± 3.48). 20 day recovery group (50.20 ± 1.68) presented improved referential memory in comparison with its respective 20 day exposure group (34.60 ± 2.8).

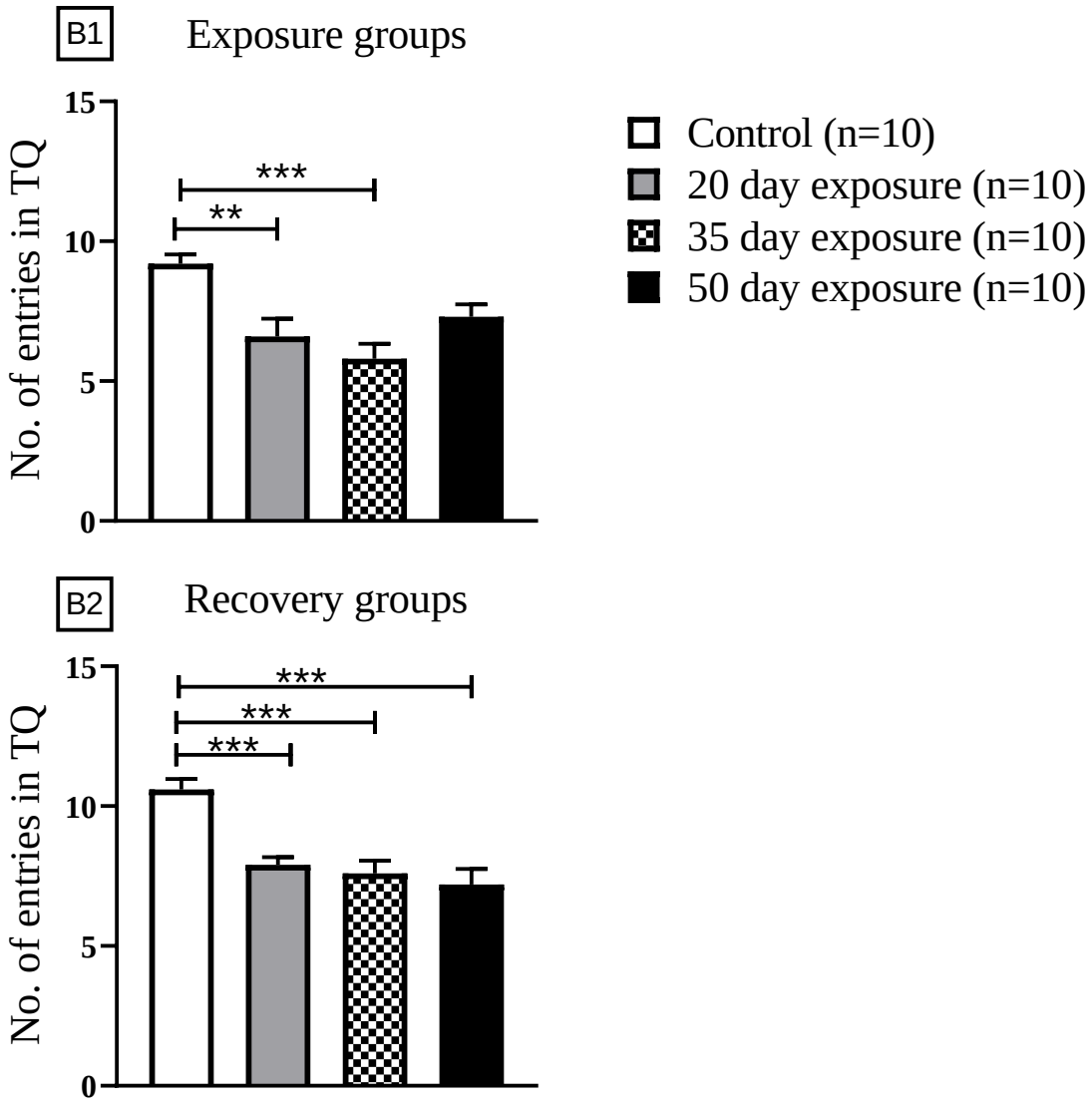


Figure 13. Morris Water Maze; Number of entries in target quadrant. Graph shows the number of platform crossings by all groups. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with *** = p< 0.001 as significance value. s = seconds.

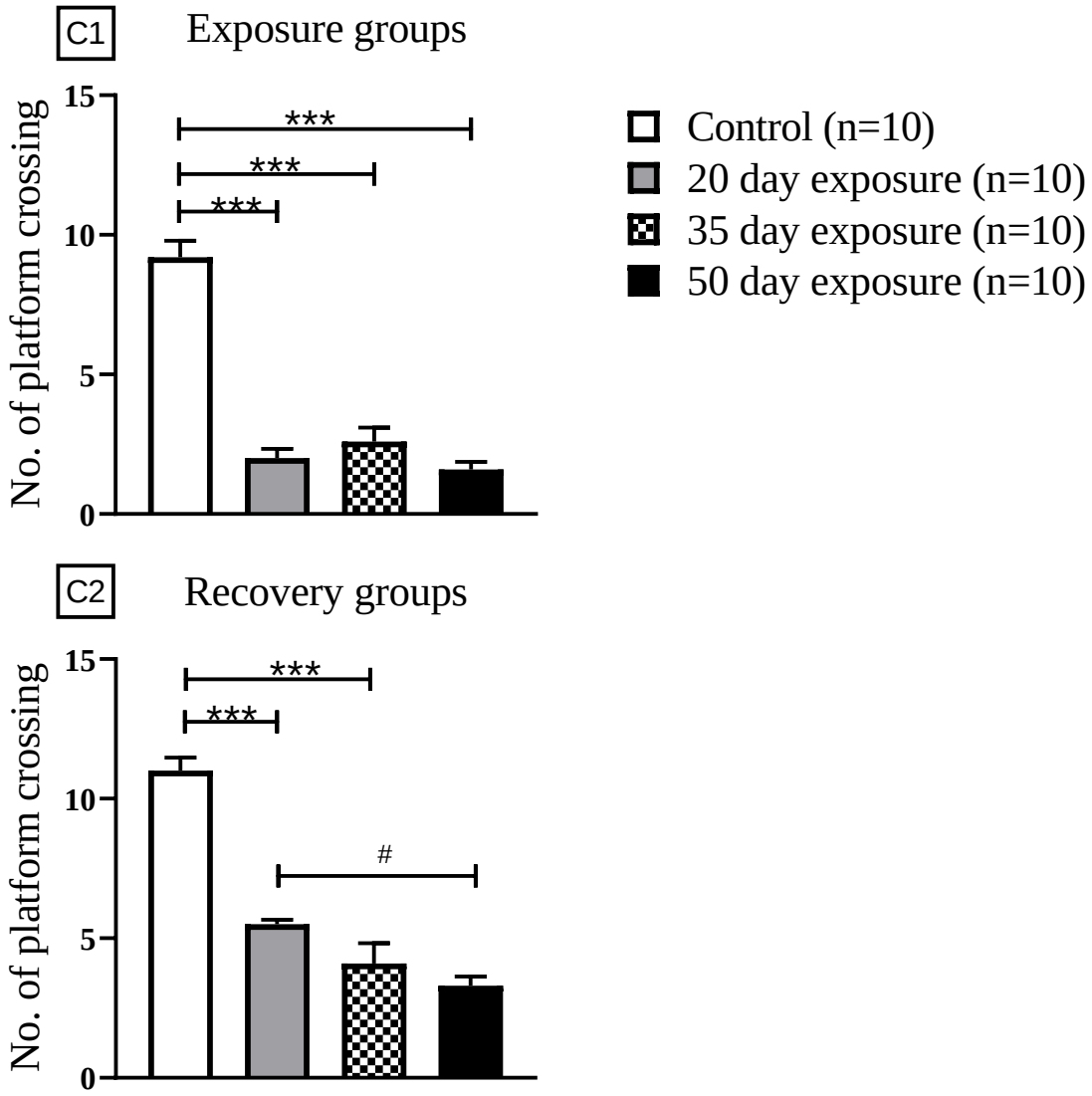


Figure 14. Probe Trial of Morris water maze; platform crossings. It shows the number of platform crossings by all groups. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with #=$p<0.05$ is the significance values. s = seconds.

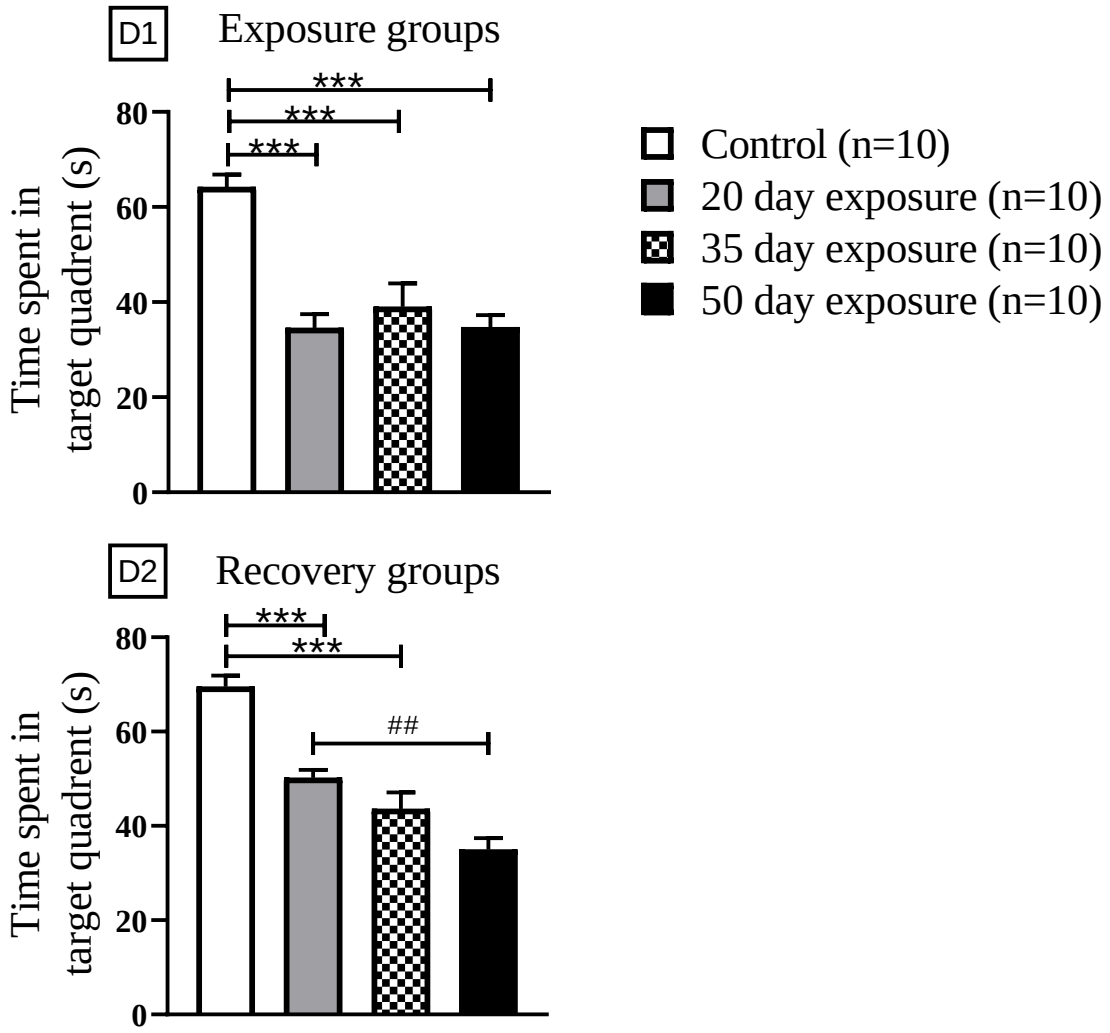


Figure 15. Morris Water Maze; Time spent in target quadrant. Graph shows time spent in target quadrant by all groups. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with *=p<0.05, ** = p< 0.01, are the significance values.. s = seconds.

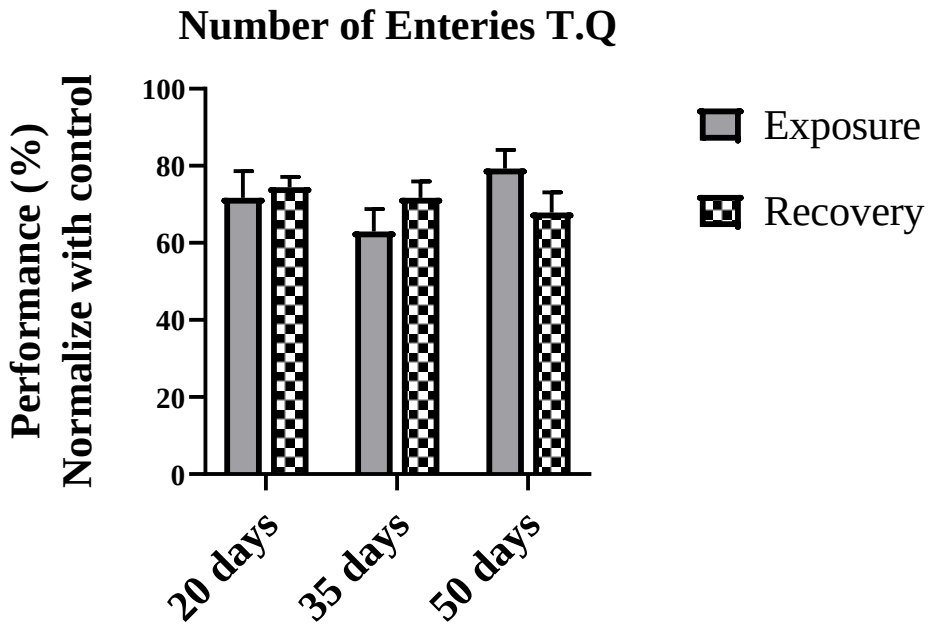


Figure 16 Number of enteries in Target quadrant normalize with control.

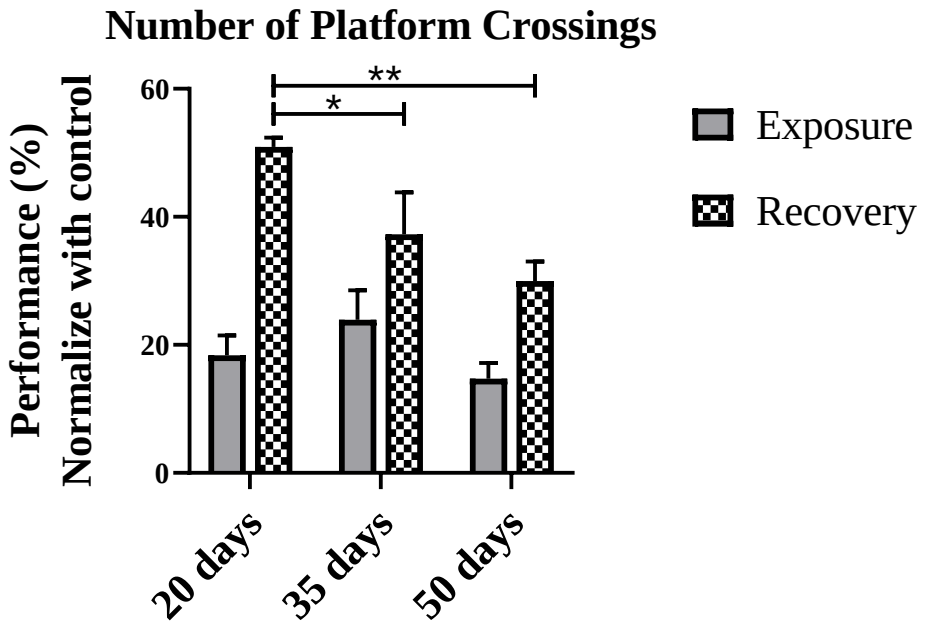


Figure 17 Number of Platform Crossings normalize with control. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with *** = $p < 0.001$ as significance value. s = seconds.

3.2 Y Maze Test (Spontaneous Alternation Test)

Y maze test was employed to assess natural exploratory behavior of mice and to evaluate short term spatial learning memory. Animal hippocampus dependent reference memory was also investigated. All the four groups showed higher preference toward Novel arm through higher number of entries in Novel arm. Least preference was shown by 50 day exposure group (7.60 ± 1.34) as compared to control group (12.00 ± 0.36). After recovery period all groups showed increased number of entries in Novel arm. 50 day recovery group (10.10 ± 0.94) presented highest improvement in spatial memory as compared to its respective 50 day exposure group (7.60 ± 1.34)

Similar trend was observed while assessing time spent in Novel arm. 50 day exposure group (117.80 ± 14.18) presented least preference to novel arm as compared to control group (177.30 ± 4.26), 20 day exposure group (171.70 ± 10.22) and 35 day exposure group (134.20 ± 18.72). After recovery period no significant improvement in spatial memory was observed in recovery groups as compared to exposure groups. Only control showed enhanced spatial learning due to experiment repetition.

Spontaneous alternations performance and Alternate Arm Repeats (%) were calculated to assess impairment in spatial memory. Spontaneous alternation performance showed memory deficit in Cadmium treated groups. Highest impairment was shown by 20 day exposure group (36.58 ± 2.89) and 35 day exposure group (43.11 ± 7.55) as compared to Control group (67.52 ± 1.41) and 50 day exposure group (54.41 ± 4.81). After recovery time all the groups showed minor improvement in spatial memory except 50 day recovery group (51.50 ± 4.47) which showed decreased spontaneous alternation than 50 day exposure group (54.41 ± 4.81) and Control group (69.30 ±1.45).

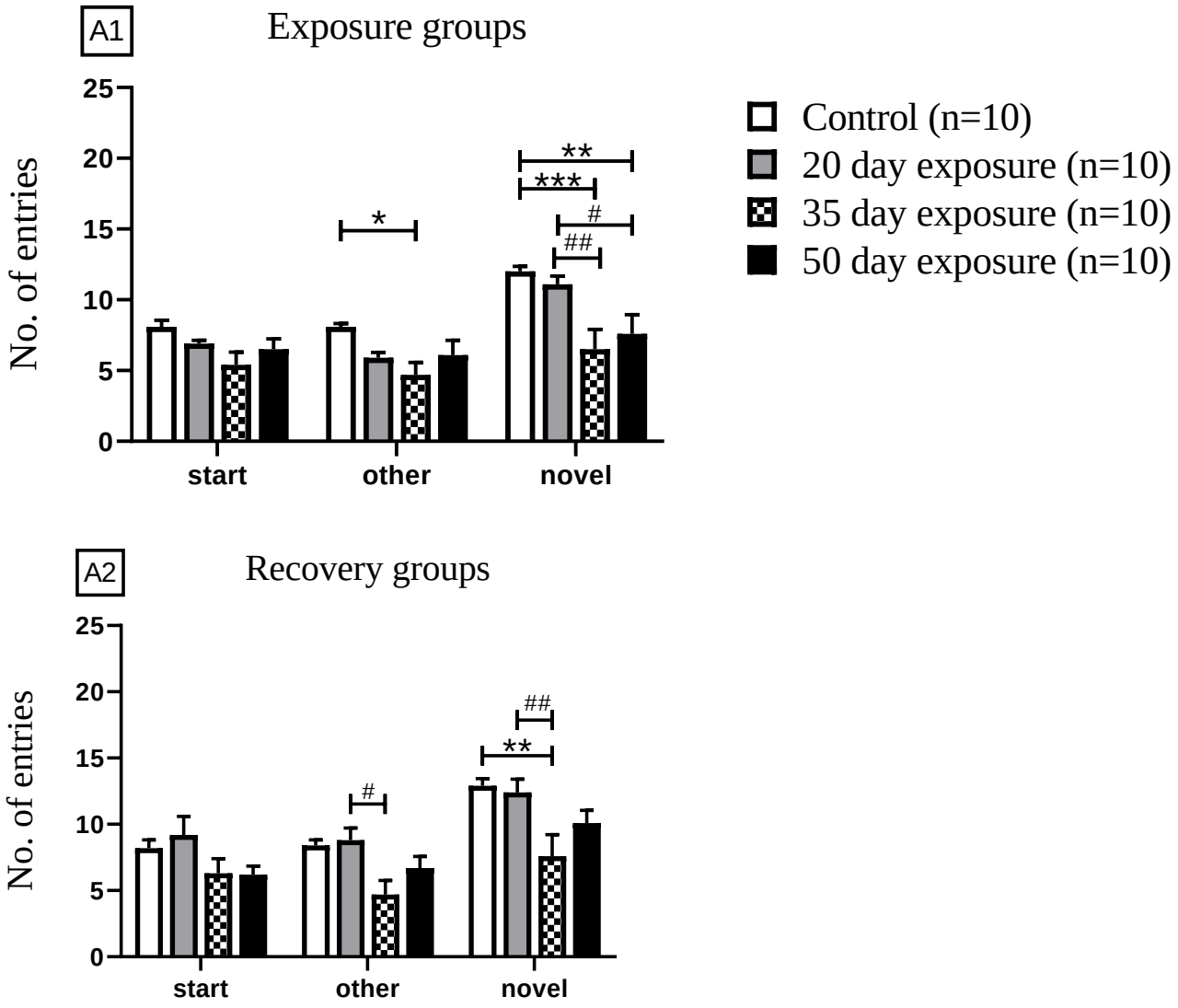


Figure 18 Performance of animals in Y-Maze test. The bar charts depicts the number of entries in each arm, by control, 20 day exposure, 35 day exposure, 50 day exposure, 20 day recovery, 35 day recovery and 50 day recovery groups. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with ** = p< 0.01, *** = p< 0.001, #=p<0.05 ## = p< 0.01, ### = p< 0.001 are the significance values. s = seconds.

Short term memory impairment was observed by calculating Alternate arm repeats (AAR) and same arm repeats (SAR). 20 day exposure group (39.73 ± 3.21), 35 day exposure group (36.51 ± 3.02) and 50 day exposure group (34.59 ± 4.05) showed higher arm repeats thus greater memory impairment as compared to control group (24.27 ± 1.30). Highest deficit was seen in 20 day exposure group (39.73 ± 3.21) as compared to control group (24.27 ± 1.30). After completion of recovery period 50 day recovery group (34.22 ± 1.49) showed no improvement in comparison with 50 day exposure group (34.59 ± 4.05). Highest improvement in spatial memory was seen in 20 day recovery group (32.46 ± 2.79) as compared to 20 day exposure group (39.73 ± 3.21). Control group (0.00 ± 0.00) showed no same arm repeats while 20 day exposure group (1.80 ± 0.32) and 50 day exposure group (1.80 ± 1.06) showed higher spatial memory impairment. After recovery period, highest improvement in spatial memory was observed in 35 day recovery group (0.60 ± 0.16) and 50 day recovery group (0.40 ± 0.16) in comparison with their respective exposure groups.

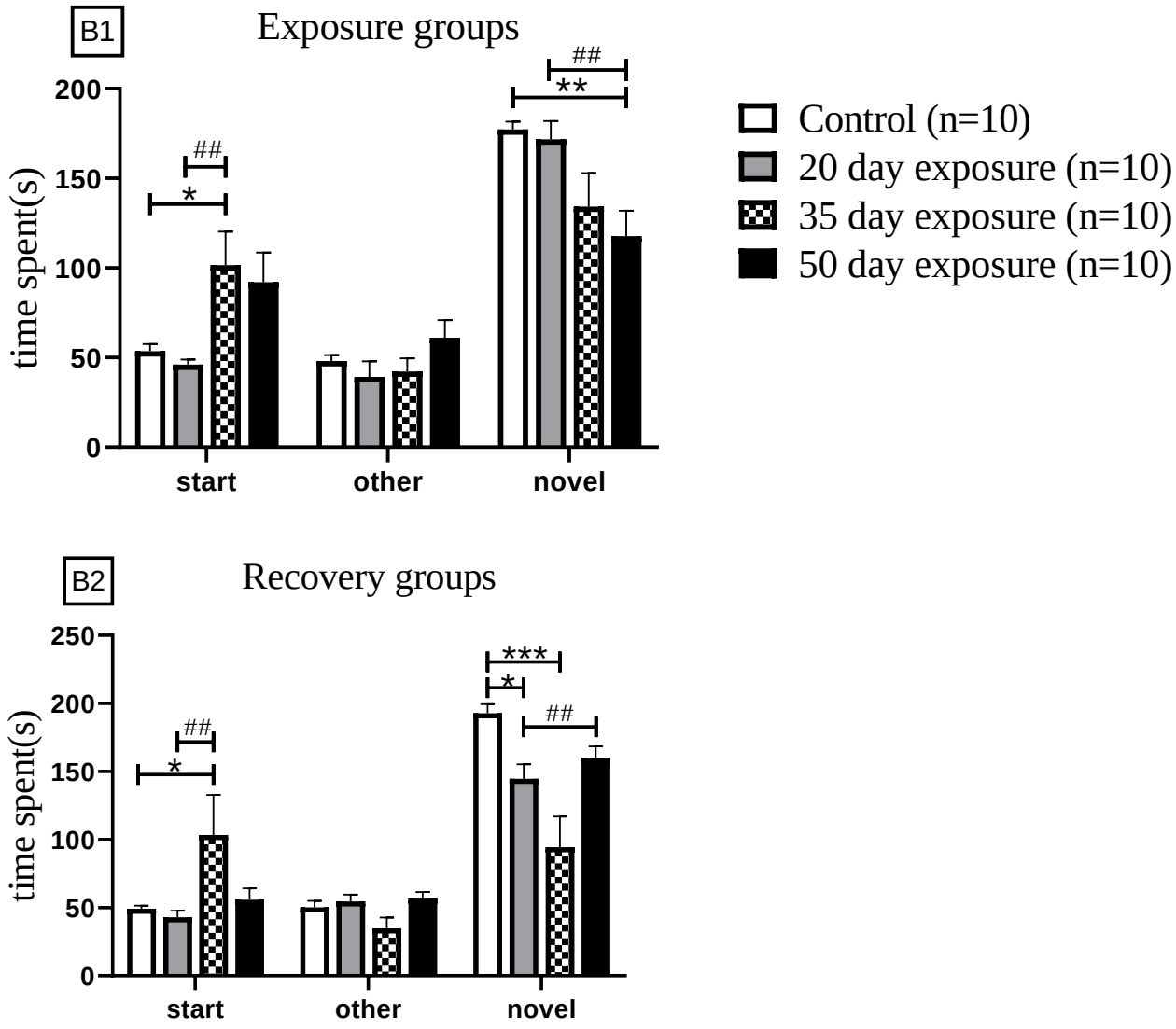


Figure 19 Time spent in each arm by animals in Y-Maze test. The graphs show the time spent in each arm (s) by control, 20 day exposure, 35 day exposure and their respective recovery groups. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with ** = p< 0.01, *** = p< 0.001are the significance values. s = seconds.

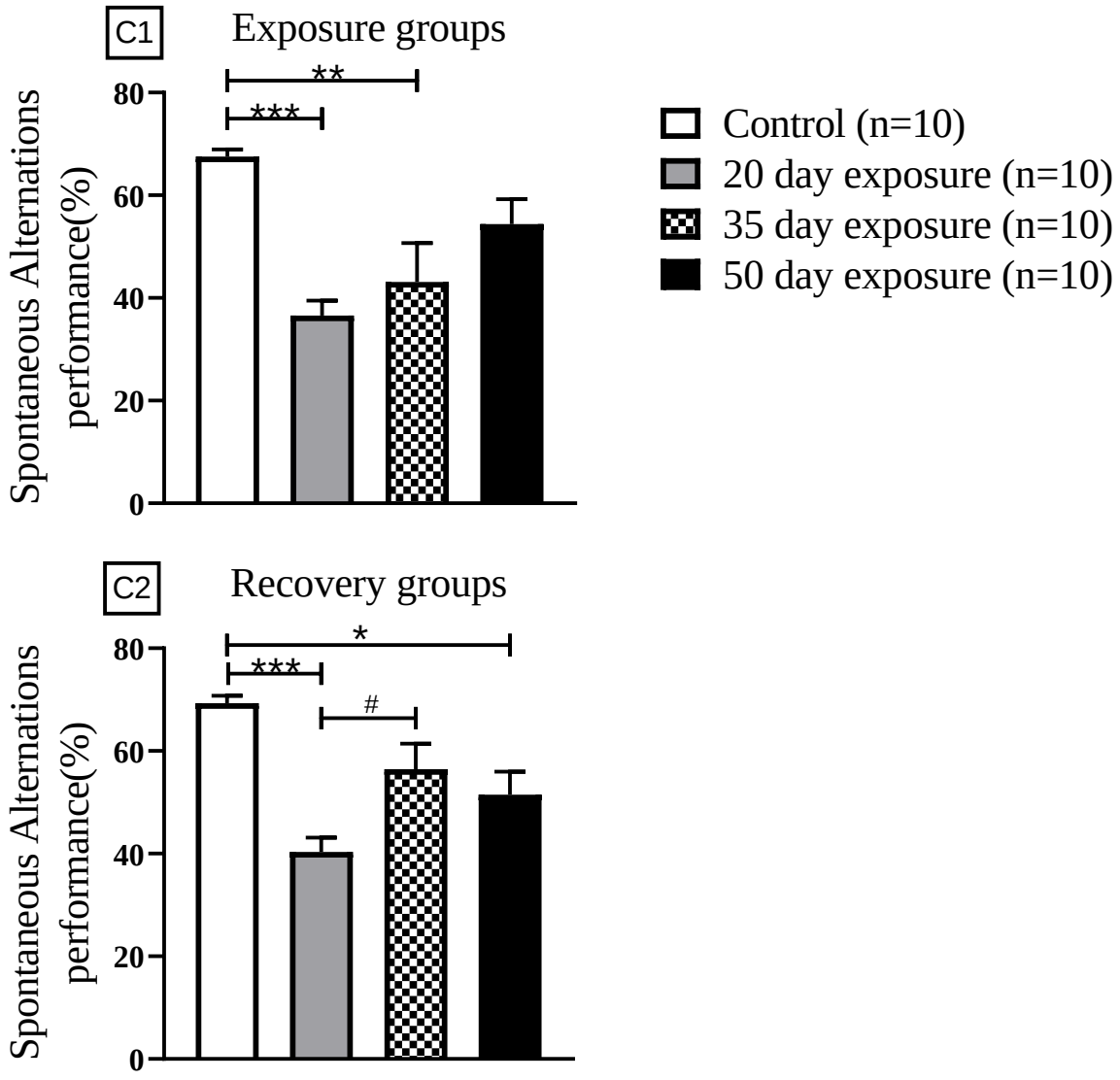


Figure 20 Effect of Cadmium on reference and working memory. Graph shows Spontaneous Alternation (%) in all groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with *=p<0.05, ** = p< 0.01 for exposure group and #=p<0.05 ## = p< 0.01, ### = p< 0.001 are the significance values for recovery group. s = seconds.

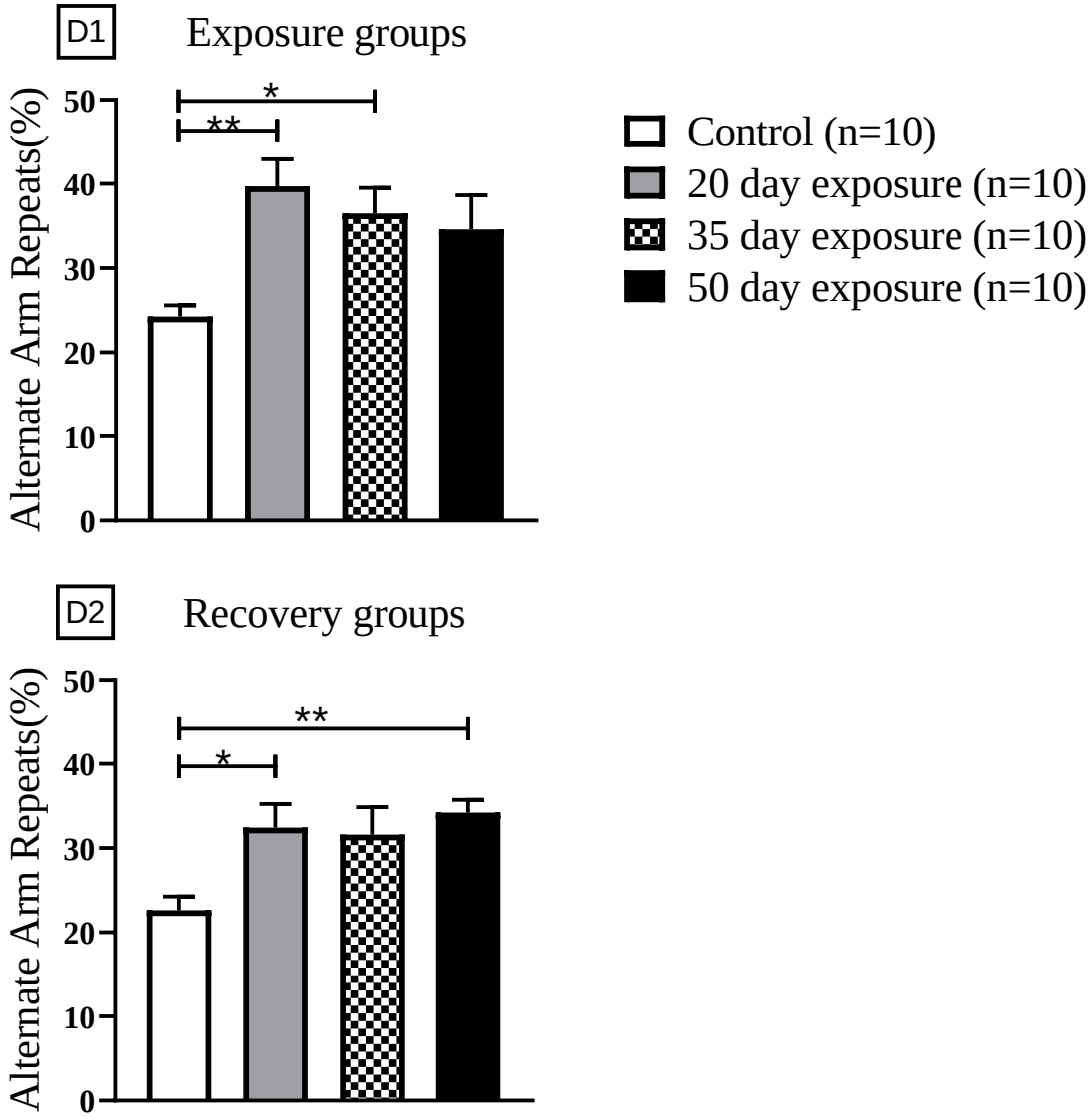


Figure 21 Effect of Cadmium on working memory. Graph shows the Alternate arm repeats (%) by all groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with *=p<0.05, ** = p< 0.01, *** = p< 0.001 for exposure group and #=p<0.05 ## = p< 0.01, ### = p< 0.001 are the significance values for recovery group. s = seconds.

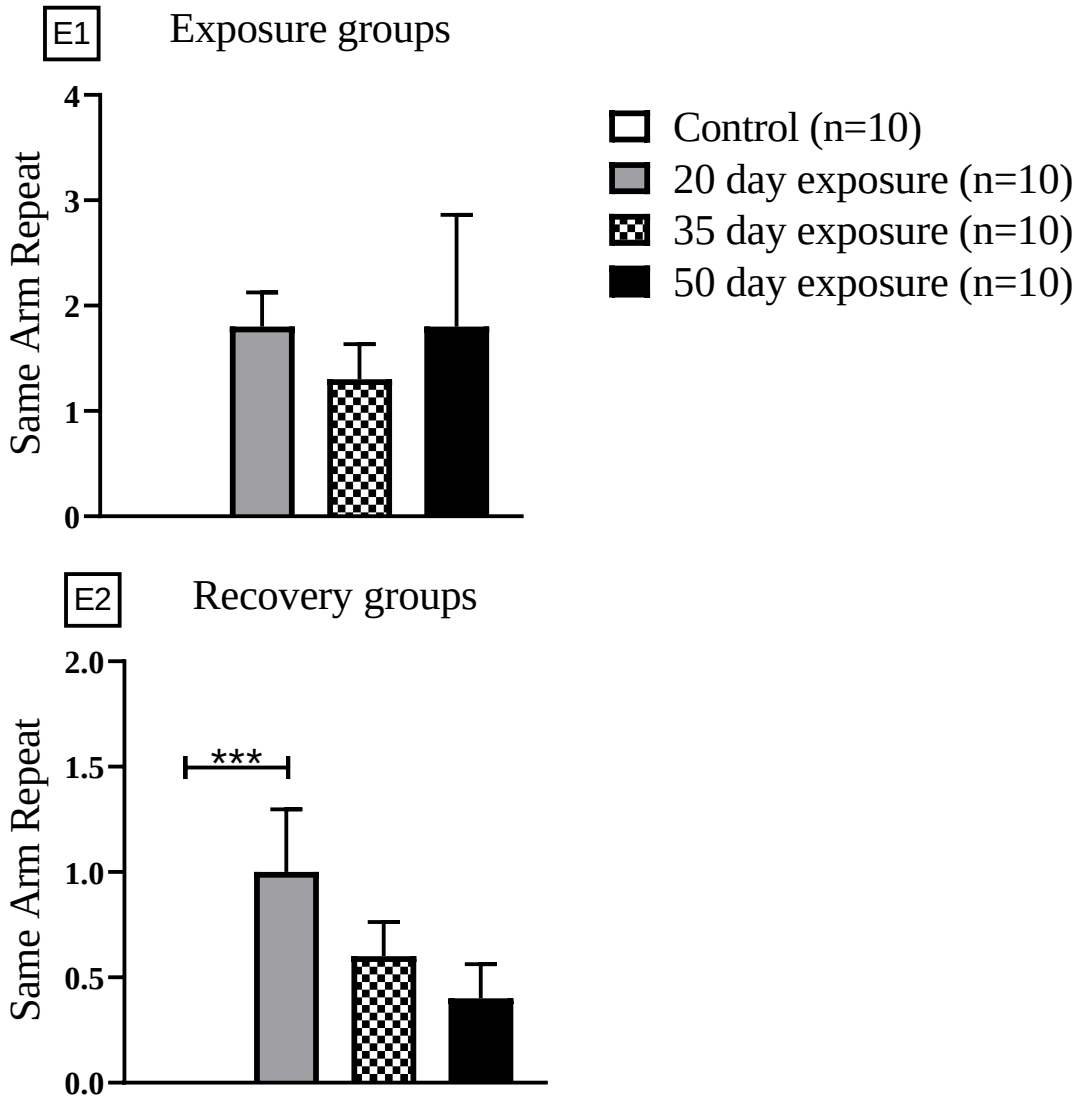


Figure 22 Same arm repeats; Y maze test. Graph shows the same arm repeats, by exposure and recovery groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with *=p<0.05, ** = p< 0.01, *** = p< 0.001 for exposure group and #=p<0.05 ## = p< 0.01, ### = p< 0.001 are the significance values for recovery group. s = seconds.

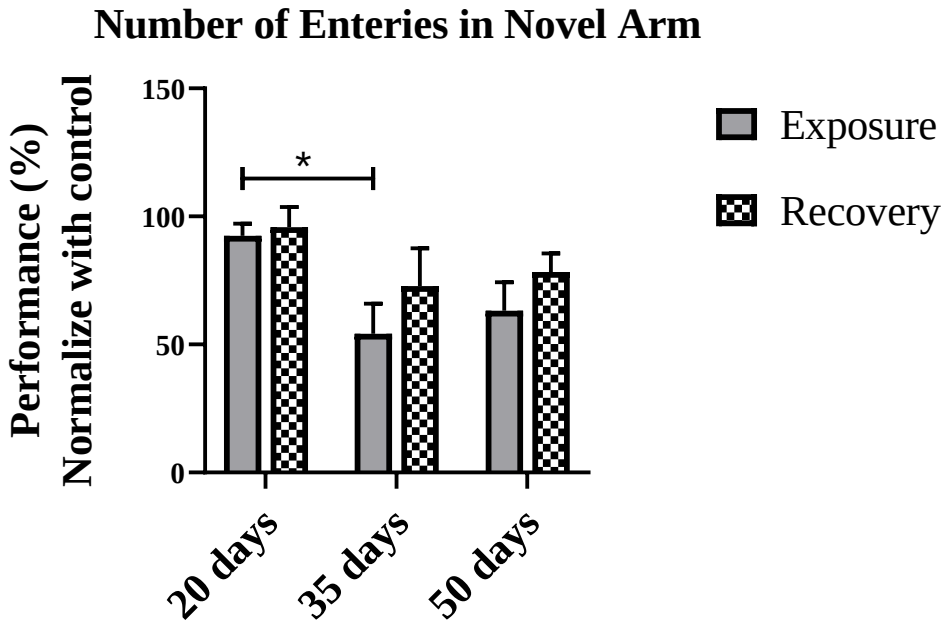


Figure 23 Figure Number of enteries in Novel arm normalize with Control.

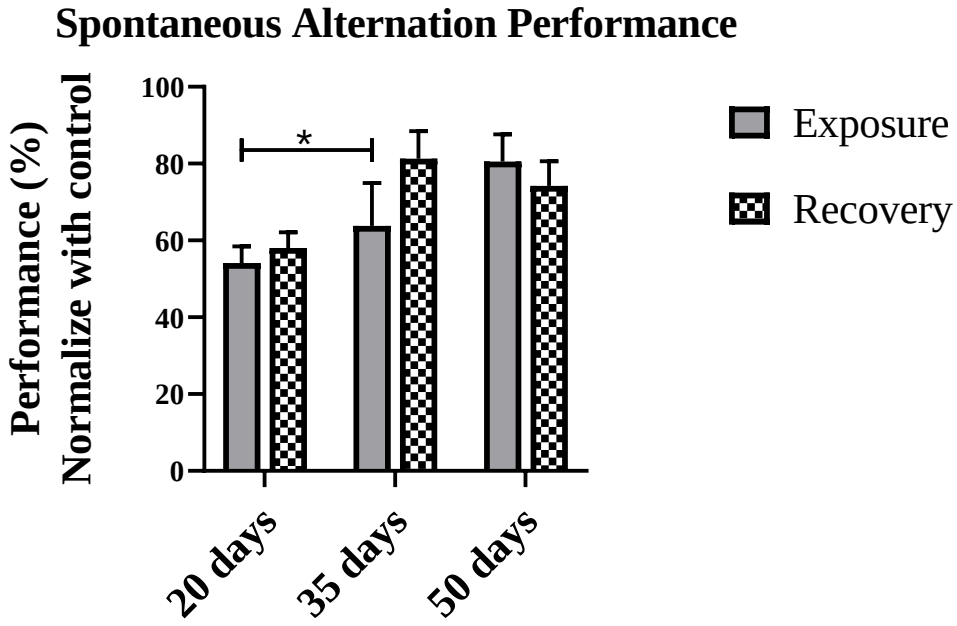


Figure 24 Spontaneous Alternation Performance normalize with control. * is used for significant difference between control and Cd treated groups. # is used for significance among Cd treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with *** = p< 0.001 as significance value. s = seconds.

3.4 Hole Board Test:

Hole board test was used in order to evaluate the effect of Cadmium exposure on long term memory and short term memory in different temporal groups. Locomotion activity of mice was also assessed by calculating Activity/ min in hole board from day 1 to day 4. On day 1, 20 day exposure group (5.13 ± 0.27), 35 day exposure group (5.42 ± 0.34) and 50 day exposure group (4.09 ± 0.46) showed less locomotion than control group (5.94 ± 0.14). On day 4 similar trend was observed but overall locomotion activity was decreased in in control group (3.18 ± 0.17), 20 day exposure group (2.55 ± 0.12), 35 day exposure group (2.65 ± 0.33) and 50 day exposure group (2.28 ± 0.294). Least locomotion activity was observed in 50 day exposure group from day 1 to day 4. However, after recovery period 50 day recovery group (5.63 ± 0.74) showed increased locomotion than 50 day exposure group (4.09 ± 0.46) from day 1 to day 4.

Latency to visit the first hole (baited or nonbaited) was calculated to evaluate anxiety level in mice. On day 1 control group (9.8 ± 1.05) showed least latency thus less anxiety as compared to 20 day exposure group (14.98 ± 0.69), 35 day exposure group (13.35 ± 1.44) and 50 day exposure group (12.95 ± 1.23). Highest level of anxiety was observed in 20 day exposure group (14.98 ± 0.69). After recovery period performance of 20 day recovery group (11.14 ± 0.82), 35 day recovery group (9.95 ± 0.81) and 50 day recovery group (10.81 ± 1.10) was improved. Similar trends were seen on day 4 with control group (4.52 ± 0.31) showing least anxiety level as compared to 20 day exposure group (7.91 ± 0.21), 35 day exposure group (6.63 ± 0.51) and 50 day exposure group (5.71 ± 0.35). After recovery period performance on day 4 was improved in all the groups with anxiety level lower in 50 day recovery group (5.71 ± 0.35).

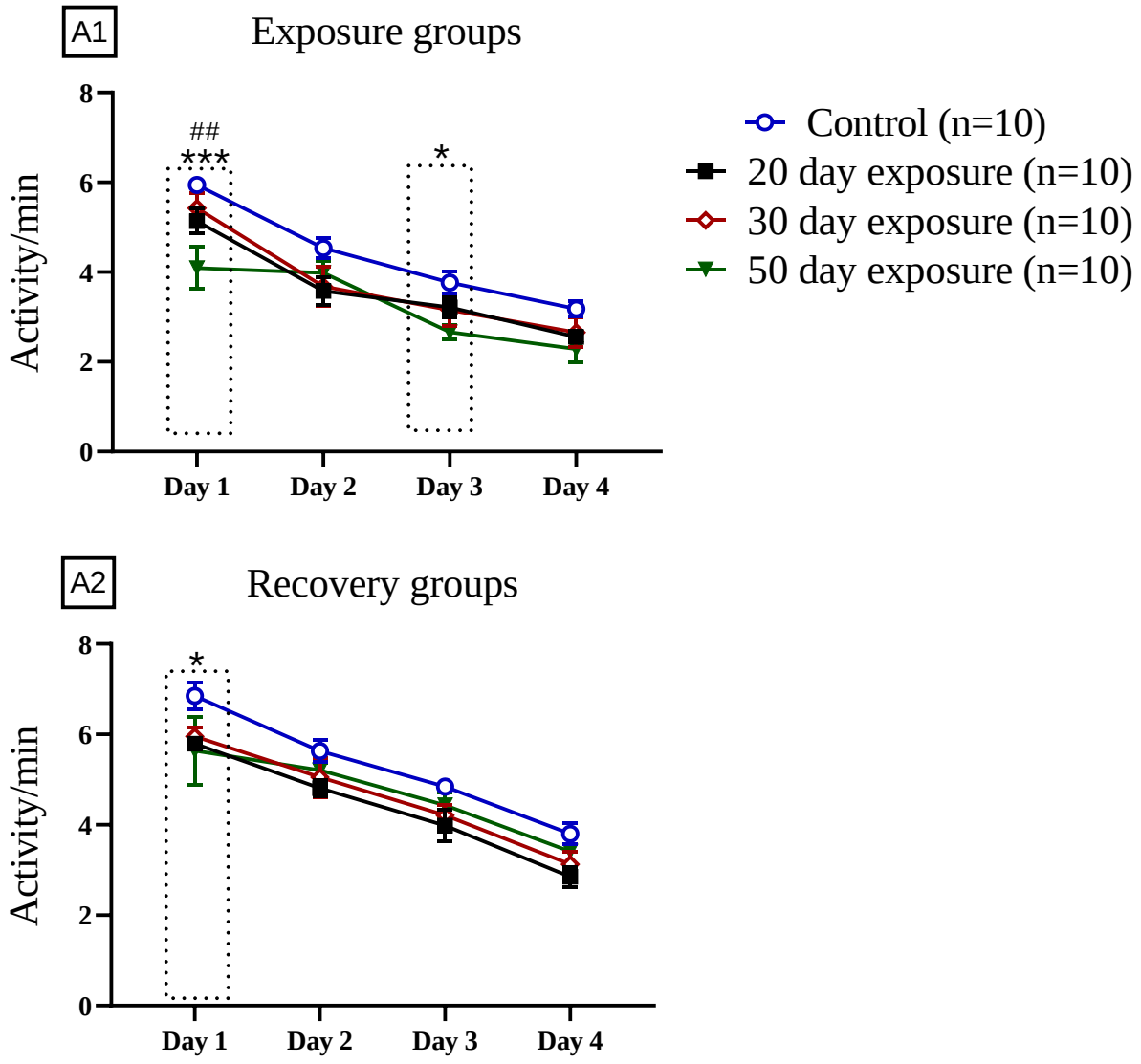


Figure 25 Effect of Cadmium exposure on locomotion activity. Graph shows activity per minute Control, 20 day exposure, 35 day exposure and 50 day exposure and their respective recovery groups. Error bars are represented as mean± SEM by 2 way ANOVA test. s= seconds.

To evaluate reference memory throughout 4 days, Reference memory error (RME) was calculated. On day 1, 20 day exposure group (20.33 ± 1.13), 35 day exposure group (18.14 ±1.36) and 50 day exposure group (22.18 ± 0.90) showed higher impairment in referential memory as compared to control group (7.07 ± 0.43). Highest reference memory errors were observed in 50 day exposure group (22.18 ± 0.90). Reference memory errors were gradually decreased in all the groups from day 1 to day 4. After recovery period it was observed that control group (5.8 ± 0.85), 20 day recovery group (11.48 ± 0.65) and 35 day recovery group (12.55 ± 1.84) have improved reference memory as compared to 50 day recovery group (19.68 ± 1.22). Similar trend was observed at day 4, 50 day recovery group (12.82 ± 1.22) showed higher number of reference memory error as compared to control (2.07 ± 0.32), 20 day recovery group (8.11 ± 0.25) and 35 day recovery group (6.51 ± 0.48).

For all 4 days working memory error (WME) were also calculated to asses short term memory and learning through the test. On day 1 control group (2.37 ± 0.19) showed least working memory error as compared to 20 day exposure group (7.20 ± 0.37), 35 day exposure group (8.45 ± 0.54) and 50 day exposure group (10.45 ± 0.49). Highest impairment of short term memory was observed in 50 day exposure group (10.45 ± 0.49). WME were reduced in all the groups from day 1 to day 4. Highest number of working memory errors were seen in 50 day exposure group (4.82 ± 0.30) at day 4. After recovery period working memory (short term memory) on day 1 was improved in 20 day recovery group (4.87 ± 0.35) and 35 day recovery group (5.87 ± 0.29). 50 day recovery group (10.35 ± 0.51) did not show any improvement as compared to 50 day exposure group (10.45 ± 0.41=9).

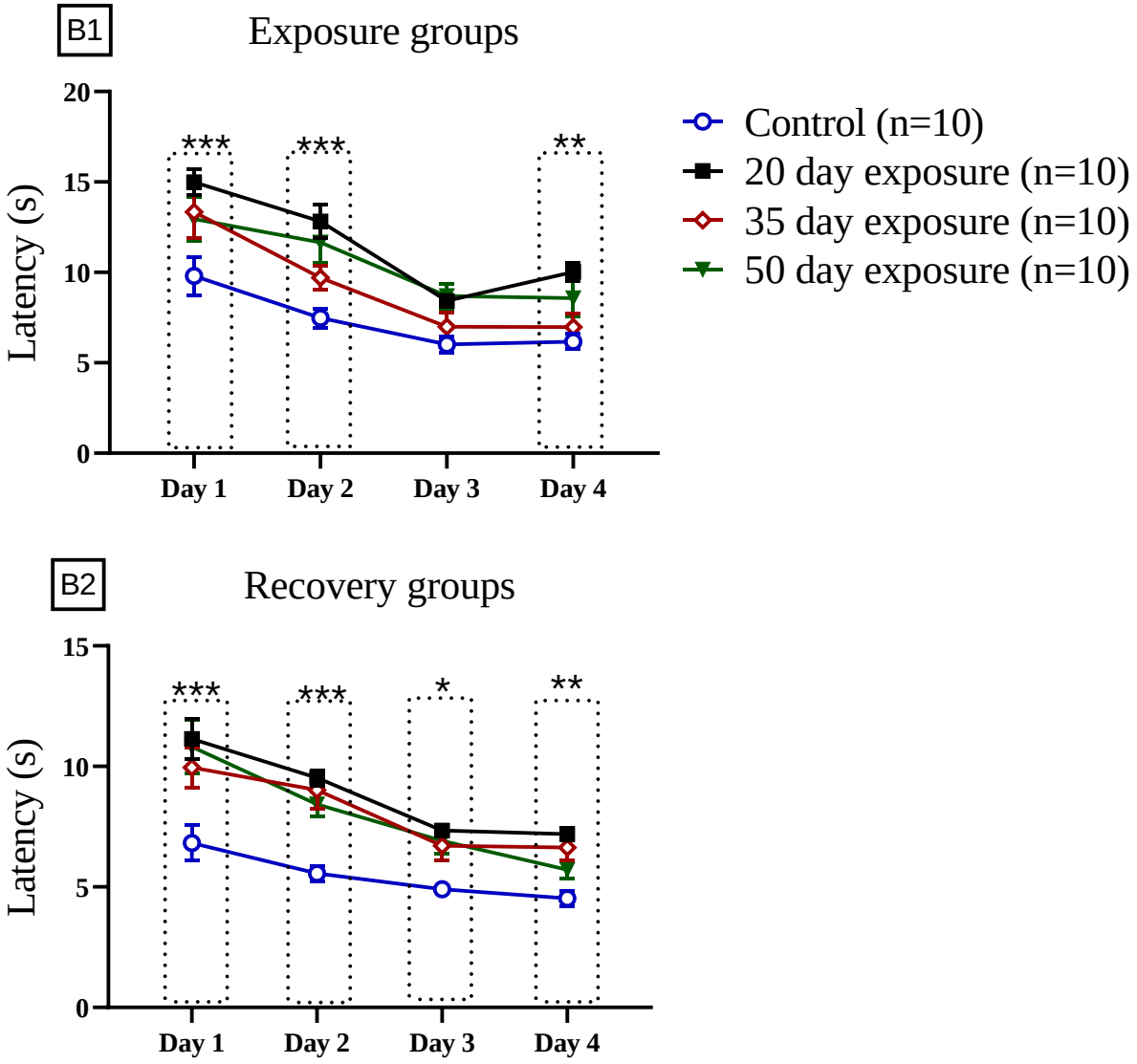


Figure 26 Effect of Cadmium exposure on anxiety levels. Graph shows the latency (s) for visit to first hole,by the groups Control, 20 day exposure, 35 day exposure and 50 day exposure and their respective recovery groups.*= $p < 0.05$, is the significant value between Control and Aluminum treated groups. Error bars are represented as mean± SEM by 2 way ANOVA test. s= seconds.

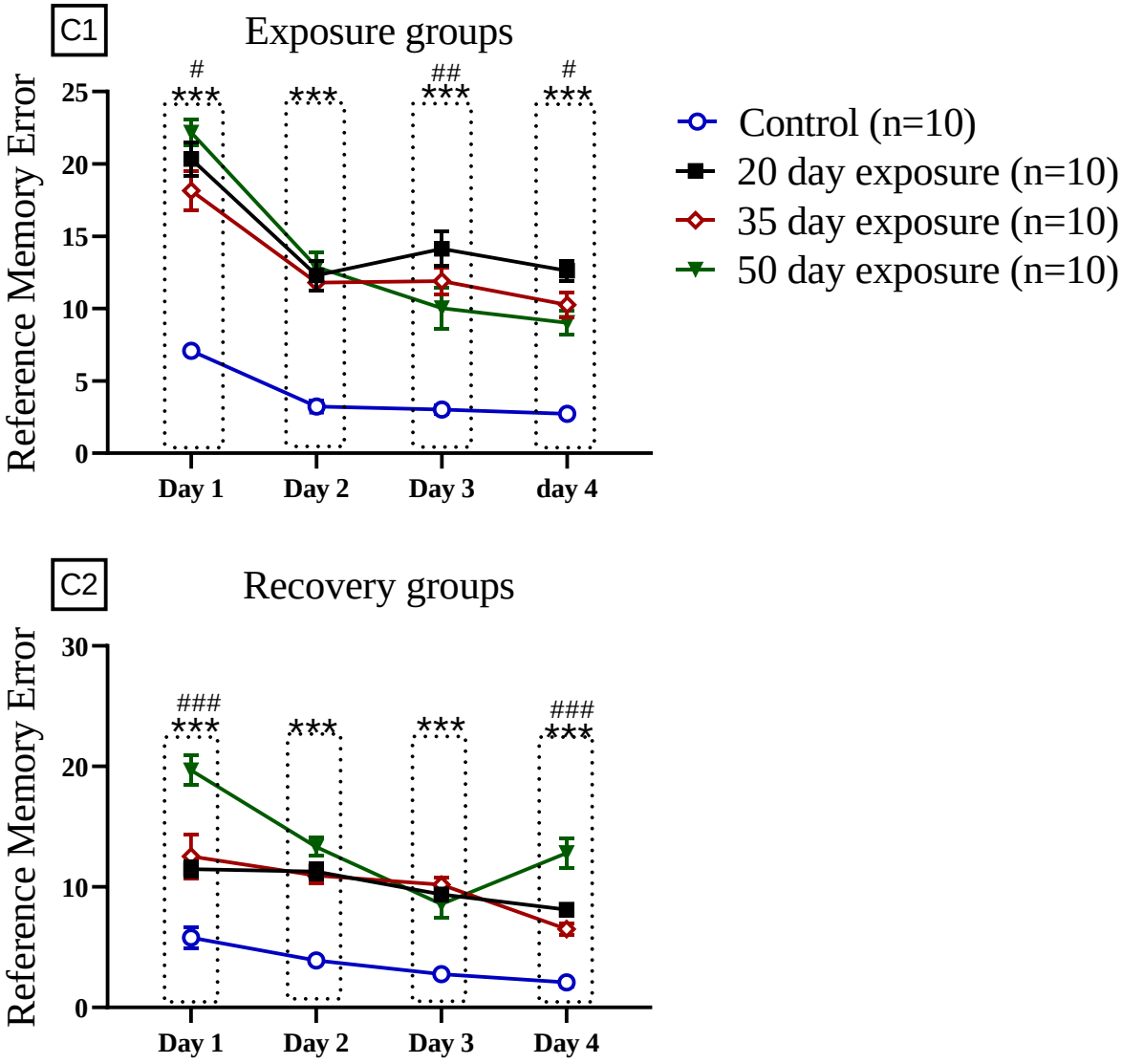


Figure 27 Effect of Cadmium temporal exposure on reference memory. Graph shows reference memory errors in Control, 20 day exposure, 35 day exposure and 50 day exposure and their respective recovery groups.*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ is the significant value between Control and Aluminum treated groups and #= $p < 0.05$, ##= $p < 0.01$ among Aluminum treated groups. Error bars are represented as mean± SEM by 2 way ANOVA test.

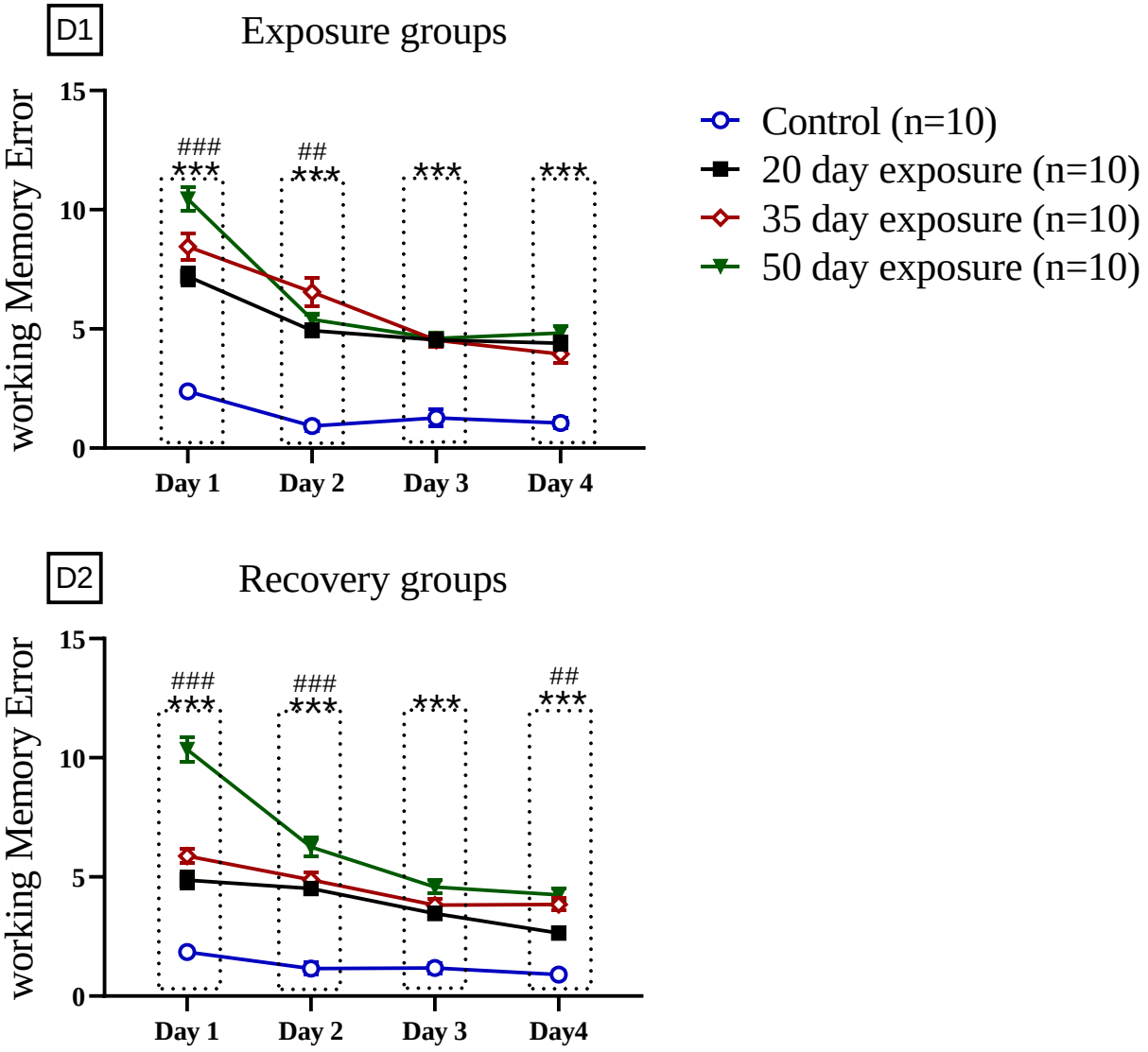


Figure 28 Effect of Cadmium temporal exposure on working memory. Graph shows working memory errors in Control, 20 day exposure, 35 day exposure and 50 day exposure and their respective recovery groups.*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ is the significant value between Control and Aluminum treated groups and #= $p < 0.05$, ##= $p < 0.01$ among Aluminum treated groups. Error bars are represented as mean± SEM by 2 way ANOVA test.

3.3 Social Preference and Novelty Test:

Sociability and social preference was evaluated in session I of test and session II determined social novelty preference. interaction time of animal with empty cage, mouse 1 and mouse 2 was analyzed as well as total time spent in three chambers i.e. mouse 1, mouse 2 and center.

In session I, all the groups showed higher preference for mouse 1 as compared to Empty cage. However, Cadmium exposed 20 day exposure group (36.40 ± 7.22), 35 day exposure group (65.80 ± 8.27) and 50 day exposure group (54.20 ± 5.97) showed low social preference for mouse 1 as compared to control group (146.40 ± 9.65). 20 day exposure group (36.40 ± 7.22) presented least interaction time with mouse 1 as compared to other exposure groups. After recovery period, 20 day recovery group (45.60 ± 6.33) showed improved sociability in comparison with 20 day exposure group (36.40 ± 7.22). However, 35 day recovery group(51.60 ± 7.32) and 50 day recovery group (31.00 ± 5.17) did not show any improvement in comparison to 35 day exposure group (65.80 ± 8.27) and 50 day exposure group (54.20 ± 5.97) respectively.

In session II all the groups showed higher social novelty preference i.e. more interaction time with mouse 2 as compared to mouse 1. But in comparison with control group (138.30 ±11.99) social novelty preference was low in 50 day exposure group (51.50 ± 5.69), 35 day exposure group (93.70 ± 18.11) and 20 day exposure group (53.10 ± 5.01) with least in 50 day exposure group (51.50 ±5.69). After recovery time, social novelty preference was enhanced showing higher interaction with Mouse 2 in control group (153.00 ±13.58), 20 day group (113.60 ± 8.13) and 35 day group (96.80 ± 3,48) as compared to their respective exposure groups. 50 day

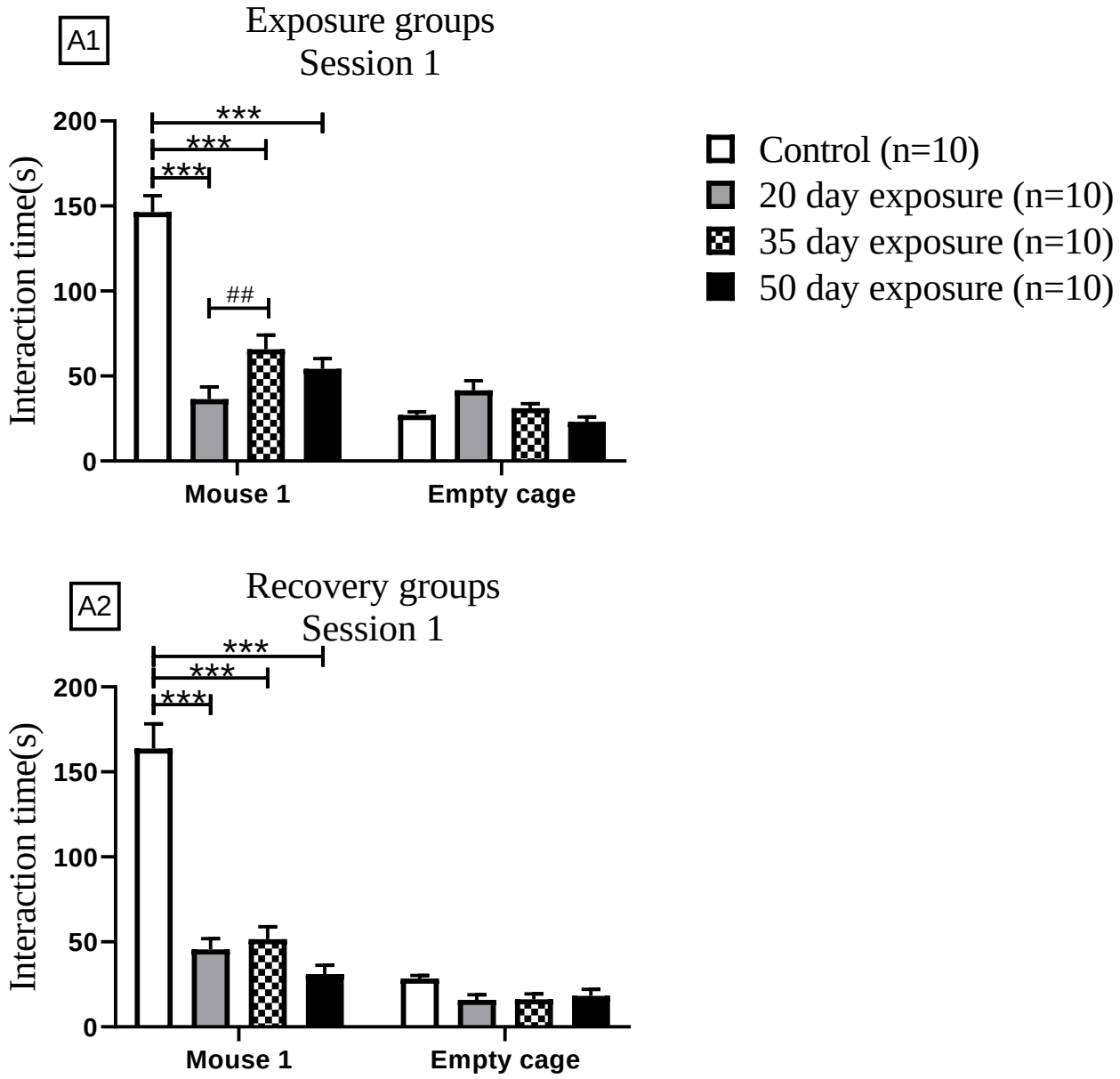


Figure 29 Effect of Temporal exposure of Cadmium on Sociability behavior (Session- I): Graph shows interaction time during session I (s) by the Control, 20 day exposure, 35 day exposure, 50 day exposure and their respective recovery groups.****=p<0.0001, show significance between control and Cd treated groups and #### = p< 0.0001 among Cd treated groups.

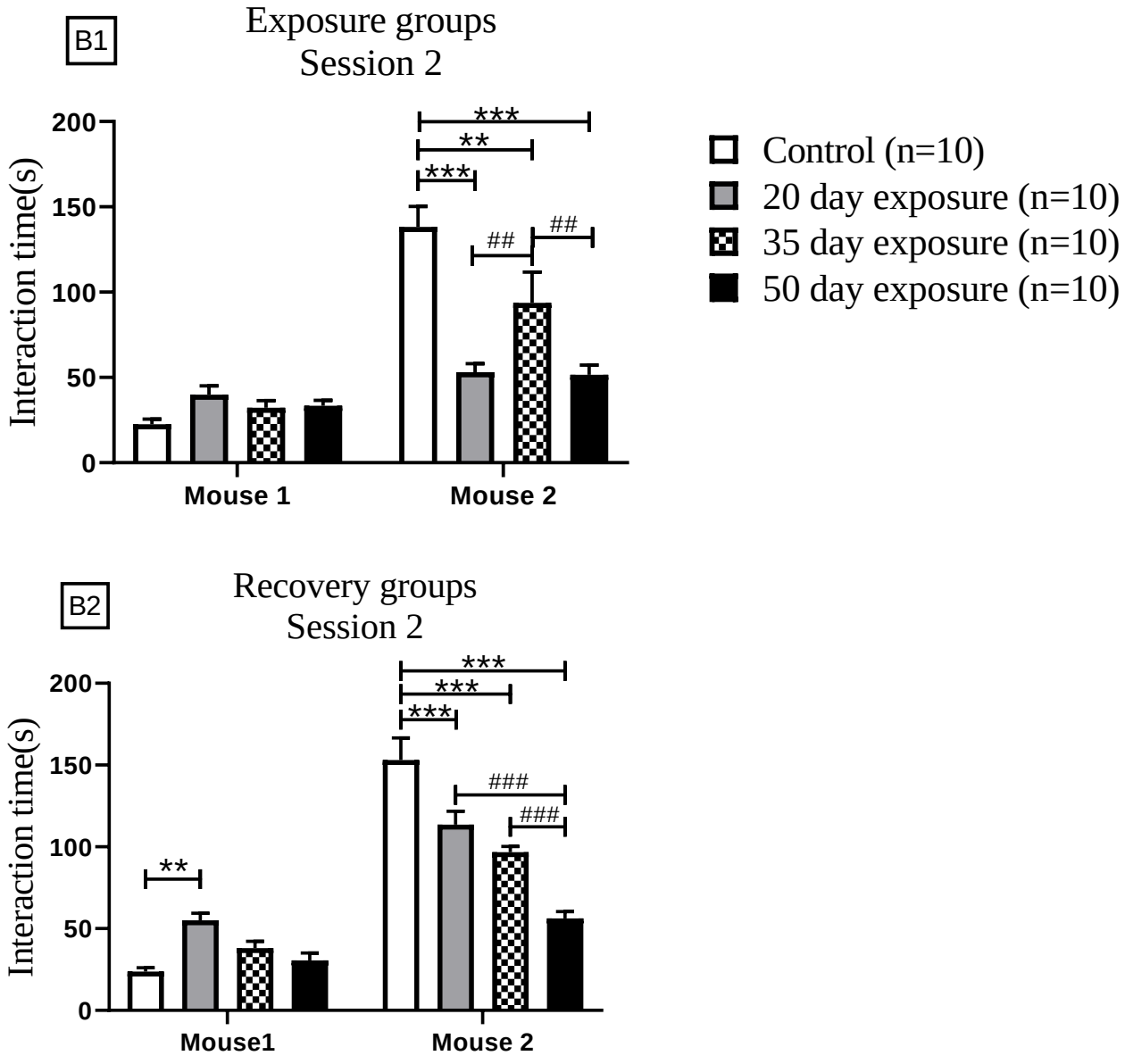


Figure 30 Effect of Temporal exposure of Cadmium on Sociability behavior (Session II): Graph shows interaction time during session II (s) by the Control, 20 day exposure, 35 day exposure, 50 day exposure and their respective recovery groups.*=p<0.05,***=p<0.001 show significance between control and Cd treated groups,## = p< 0.01, ### = p< 0.001.

Recovery group (56.30 ± 4.06) showed less improvement in social novelty preference as compared to 50 day exposure group (51.50 ± 5.69).

Similar trends were observed in sociability assessment by calculating time spent in mouse 1, mouse 2 and center chamber. In Session I, it was observed that all the groups, control (343.4 ± 11.28), 20 day exposure group (254.20 ± 20.33), 35 day exposure group (301.90 ± 21.44) and 50 day exposure group (282.50 ± 13.19) spent higher time in Mouse 1 chamber as compared to center and empty cage chamber. 20 day exposure group (254.20 ± 20.33) spent least time with mouse 1 as compared to Control group (343.4 ± 11.28). After recovery period, 35 day recovery group (337.20 ± 40.37) showed improved sociability as compared to 35 day exposure group (301.90 ± 21.44). 20 day recovery group (192.90 ± 14.92) and 50 day recovery group (208.80 ± 11.98) did not show any improvement in sociability after recovery.

In session II control group (337.60 ± 15.27), 20 day exposure group (250.50 ± 17.51) and 35 day exposure group (287.00 ± 31.78) showed higher social novelty preference. In comparison with control group, all Cadmium exposed group showed less social novelty preference. After recovery period, it was observed that 20 day recovery group (299.80 ± 26.49) and 35 day recovery group (329.80 ± 20.13) spent more time with mouse 2 as compared to their respective exposure groups. Though, 50 day recovery group (268.75 ± 55.23) showed less improvement in social novelty preference as compared to 50 day exposure group (243.40 ± 33.51) by spending less time in mouse 2 chamber.

Percentage discrimination index clearly shows that all Cadmium exposure groups, 20 day exposure group (58.91 ± 2.67), 35 day exposure group (68.46 ± 6.72) and 50 day exposure group (62.31 ± 3.95) interacted less with novel mouse (mouse 2) as compared to control group (85.22 ± 2.30). 20 day exposure group showed least preference for novelty. After recovery, moderate improvement in performance was observed in 50 day recovery group (70.47 ± 6.10) and 35 day recovery group (77.03 ± 3.75). Very little progress was observed in 20 day recovery group (59.17 ± 6.26).

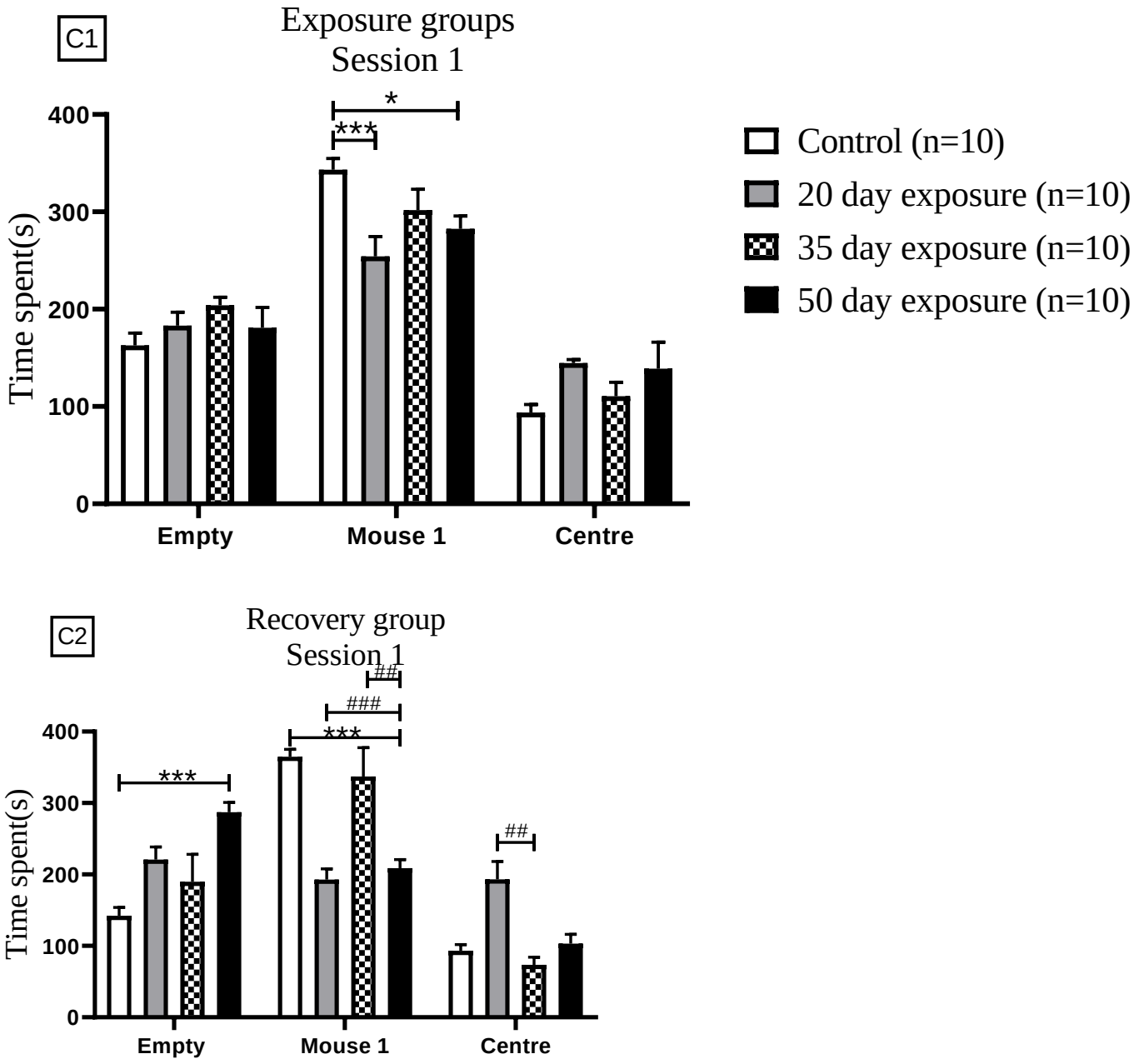


Figure 31 Social Novelty preference (Session-I): Graph shows time spent (s) in each chamber during session I all groups. *=p<0.05, **=p<0.01 show significance between control and Cd treated groups and # = p< 0.05 are the significance values among Cd treated groups.

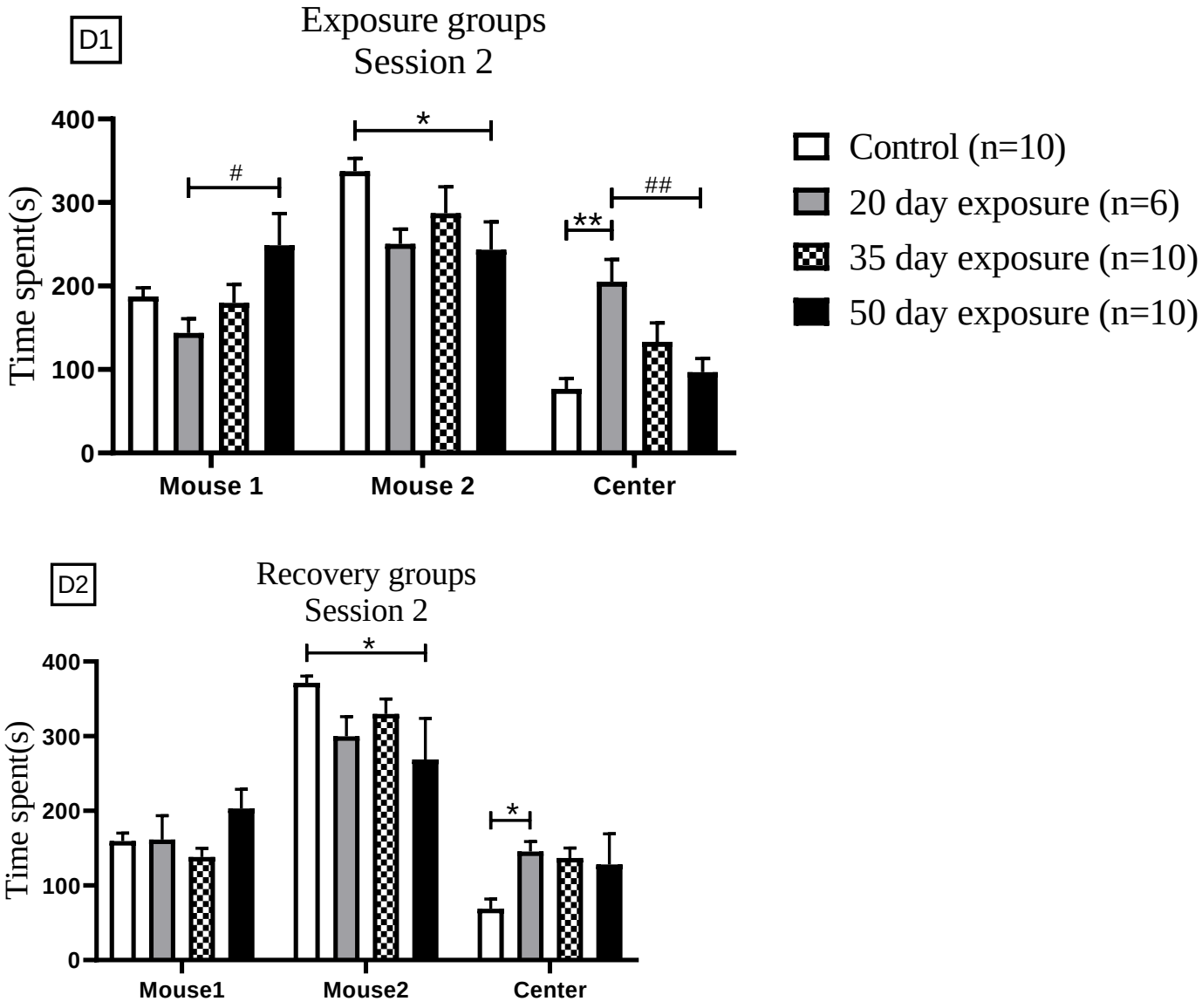


Figure 32 Social Novelty preference (Session-II): Graph shows time spent (s) in each chamber during session II by the Control, 20 day exposure, 35 day exposure, 50 day exposure and recovery groups. *=p<0.05 show significance between control and Cd treated groups,## = p< 0.01, ### = p< 0.001 are the significance values among Cd treated groups.

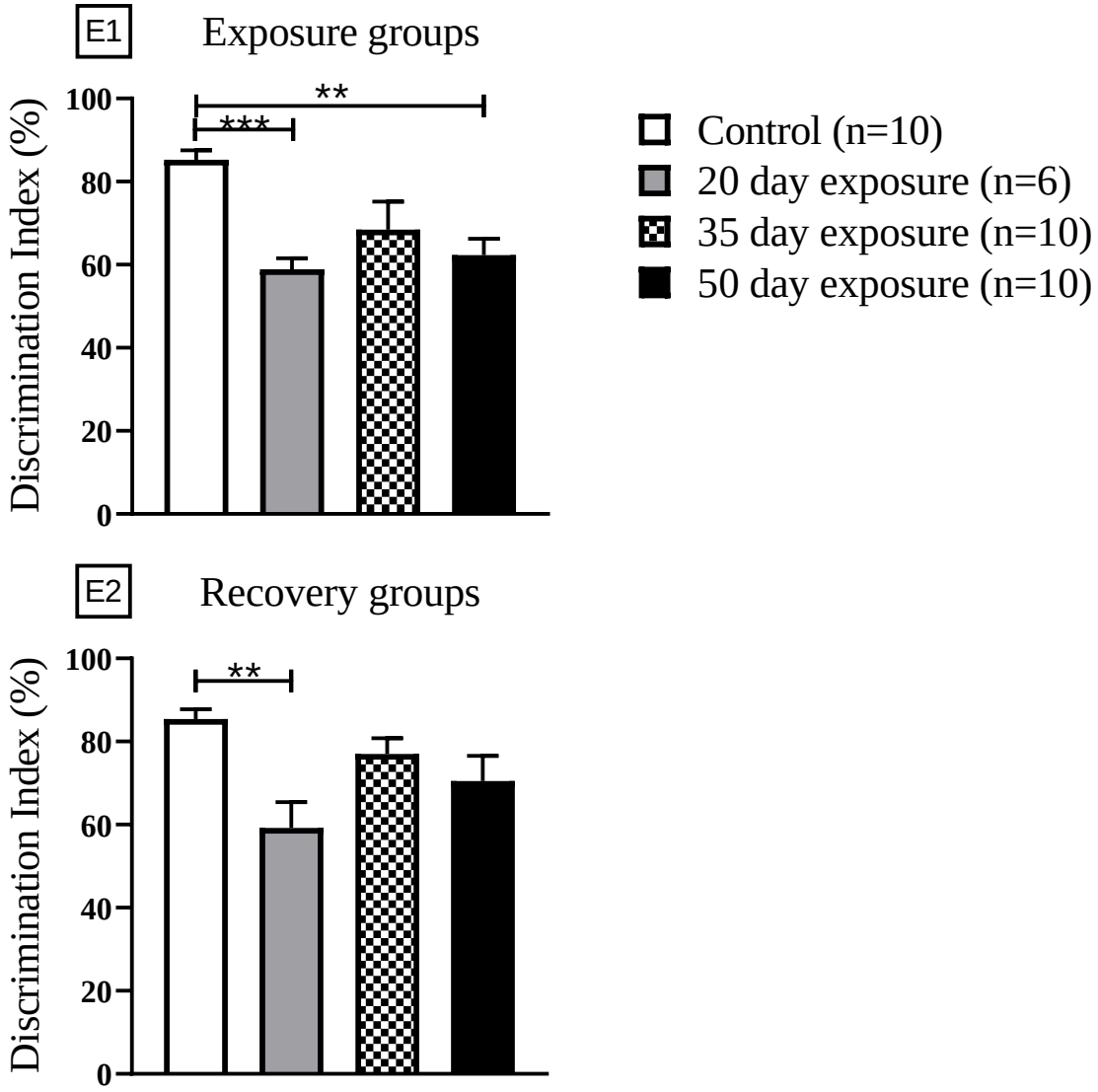


Figure 33 Outcome of Cadmium on social novelty test: Graph present percentage Discrimination Index during session II by the Control, 20 day exposure, 35 day exposure, 50 day exposure and recovery groups. *=p<0.05,** = p< 0.01 show significance between control and Al treated groups through One way ANOVA and post hoc Bonferroni's test.

CHAPTER 4

DISCUSSION

Chapter 4
DISCUSSION

Modification of behavior by the process of acquiring new information called as learning and the remembrance of past experiences called as memory, are exceptional traits of higher organisms (Cassilhas et al., 2016). Short term memory and long term memory, spatial and referential learning and memory are associated deeply with the Hippocampal, prefrontal cortex and amygdala region of the brain (Noble & Kanoski, 2016). Any decline in learning and memory can result into various neurodegenerative diseases like Alzheimer's, Parkinson disease and dementia (Wang et al., 2017). Neuronal inflammation, oxidative stress, neurofibrillary tangles formation and tau protein accumulation are the major roots of decline in cognitive functions (learning and memory).

Cadmium is foremost present in cigarettes, food and drinking water. It is a noxious metal that causes oxidative stress in most of the tissues such as liver, kidney, and hippocampus (Oguzturk et al., 2012). It retains in the tissues for a long time and causes serious pathological ramification (Bagchi et al., 1997). The mechanism behind the damage caused by cadmium is to create reactive oxygen species and amend the normal functioning of the cell by disrupting genetic information and mitochondrial activity (Patrick, 2002)

Cadmium is toxic even at very low doses. Cadmium toxicity results in damaging effects on human health (Wang and Du 2013). It has been suggested that the nutrients and nutritional status of an individual may modify susceptibility to metal toxicity (Fernstrom and Fernstrom, 2007). The brain is one of the major organs affected by cadmium toxicity (Manca et al., 1991; Nemmiche et al., 2007). Cadmium can cross the blood-brain barrier, accumulate in the brain and cause toxicity to the neurons. Several studies have unveiled the neurotoxic effect of cadmium and possible mechanisms of its toxicity (Flora et al., 2008; Méndez-Armenta and Ríos, 2007).

Current study is focused on how different temporal groups provided with same total exposure of Cadmium exhibit different level of learning and memory and impairment and whether the exposure caused permanent impairment or not in each group. Animals were divided into 4 groups i.e. Control, 20 day exposure (45 mg /kg/day), 35 day exposure (25.71 mg/kg) and 50 day exposure (18 mg/kg/day) groups given. After successful completion of exposure times

behavioral tests were performed on these animals. Further all these groups were given recovery period of 20 days in which normal water and feed was given. Behavioral tests were again performed after recovery period to assess recovery in learning and memory impairment in different time groups.

Morris Water Maze test (MWM) was performed in order to assess the effect of Cadmium on long term memory and spatial memory. MWM is the most widely accepted test to evaluate hippocampal functioning in spatial learning and long term memory in mice. Animal tries to find platform in round tub with equipped with spatial cues in training sessions when dropped from different directions (Vorhees & Williams, 2006). Previous study by (Batool et al., 2019) had shown that rats treated with 500 mg/kg/week of Cadmium in drinking water for 28 days have shown reduced spatial reference memory as compared to Control group animals in MWM test. Our result also matched with this study as 50 day group (18 mg /kg/day) showed impaired spatial and reference memory in MWM escape latency task as well as platform crossings (probe trial). In training session task, 50 day exposure group (18 mg/kg/day) showed highest deficit in spatial learning and memory as compared to Control group.

20 day exposure group (45 mg /kg/day) showed slightly less impairment than 50 day exposure group (18 mg/kg/day). While 35 day exposure group (25.71 mg/kg/day) has shown least impairment. Slight recovery was observed in performance of all recovery groups in comparison with their respective groups. This may indicate that long term exposure or high dose exposure to Cadmium cause higher impairment in learning and memory as compared to slow and low dose exposure. In probe trial highest impairment of long term memory and spatial learning was shown by 50 day exposure group then 20 day exposure group and least by 35 day exposure group in comparison with Control group. Highest recovery in spatial memory and learning was observed in 35 day group and least or no prominent recovery was seen in 50 day exposure group. The results may indicate that long term exposure or high dose exposure to Cadmium can cause higher impairment in reference and spatial learning and long term memory as compared to slow and low dose exposure.

Animal natural exploratory behavior is recognized by Y-maze test. It is employed to evaluate both spatial working memory and reference memory. Intact working memory is associated with prefrontal cortex and spatial reference memory with hippocampal functioning (Kraeuter et al.,

2019). Rodents are naturally inclined to visit unexplored novel arm more than start and other arm. To define reference memory impairment time spent in Novel arm and No. of entries in Novel arm was assessed. Result were consistent with MWM test result as 50 day exposure group and 20 day exposure group showed highest impairment than Control and 35 day exposure. Very low or no significant improvement in reference memory was observed in 20 day recovery and 50 day recovery group however improvement was observed in 35 day recovery group. Spontaneous alternations, alternate arm repeats and same arm repeats were calculated to assess short-term memory. Rodents employ their working memory and generally visit arm that is visited least recently i.e. alternative visits between three arms. Rodents generally tend to explore the arm visited least recently, and so, normally they are expected to alternatively visit the three arms (Wietrzych et al., 2005). Least number of spontaneous alternations was observed in 20 day exposure group implicating impaired working memory. Other than that no significant impairment or recovery was observed. Alternate arm repeats data showed highest impairment in 20 day group and 50 day group as compared to 35 day and Control group. After recovery 20 day recovery group did not show any improvement in performance while 35 day recovery group presented improved working memory. Same arm repeat data result was consistent with alternate arm repeats result with 20 day exposure group showing highest impairment in working memory. Slight recovery was observed in 35 day recovery group and 50 day recovery group but no significant improvement was observed in 20 day recovery group.

Three chamber sociability test was performed to assess sociability in three phases. First is habituation, second is Session I to assess sociability in animal and third is Session II to evaluate social novelty preference (Moy et al., 2004). In Session I 20 day exposure group spent least time in Mouse 1 chamber and also interacted more with empty cage as compared to Mouse 1 while all other groups interacted more with Mouse 1 than empty cage. Impairment in sociability was not recovered in 20 day recovery group after recovery period. But 35 day recovery presented better performance in both time in Mouse 1 chamber and interaction with Mouse 1. In Session II highest impairment of social novelty preference was seen in 20 day exposure group as it preferred to interact more with Mouse 1 than Mouse 2 as compared to Control group. 50 day exposure group and 35 day exposure group showed less social novelty preference than Control but still interacted and spent more time with Mouse 2 than Mouse 1. After recovery period performance of 35 day recovery group regarding interaction with Mouse 2 was significantly

improved. But no significant improvement was observed in 50 day recovery group. These results indicate that long term exposure and high exposure of Cadmium can cause deficit in sociability of mice and novel object recognition. Percentage discrimination results were also consistent with the other parameters. 50 day exposure group showed least discrimination between novel mouse (Mouse 2) and Mouse 1 as compared to control with least recovery after recovery period.

Hole board test was employed to assess anxiety level and locomotion activity in rodents as well as to evaluate working memory and spatial reference memory. Anxiety was assessed by calculating the time taken by animal to poke the first hole whether baited or unbaited and locomotion performance was assessed by calculating activity/min. Throughout the 4 days least locomotion activity was presented by 20 day exposure group, then 50 day exposure and then 35 day exposure group. No significant improvement was seen in 20 day recovery group. However, slight improvement in performance was observed in 50 day recovery and 35 day recovery group. Our results of working memory error were consistent with the factor that anxiety interferes with the working memory by interfering with the tasks involving complex attention and coordination (Salthouse, 1996). It builds tension and nervousness which result in poor perception resulting in poor performance of working memory (Wetherell et al., 2002). Reference memory errors and working memory errors were calculated to assess reference memory (long term memory) and working memory (short term memory) respectively. RME were highest in 20 day exposure group as compared to control, 50 day exposure and 35 day exposure group. On day 4 highest RME were observed in 20 day exposure group then 50 day exposure group and least in 35 day exposure group. After recovery period slight improvement was seen in all groups except 50 day recovery group in which RME did not decrease. Working memory errors result was consistent with reference memory error result with highest WME in 50 day exposure group and 20 day exposure group on day 1 and day 2 respectively. Very low or non-significant improvement in WME was noted after completion of recovery time period. Hence, we can assume that long term exposure of Cadmium low dose (18 mg/kg/day) and short term exposure of Cadmium high dose (45 mg/kg/day) both with same total exposure (900 mg/kg) results in impairment of hippocampal dependent learning and memory which cannot be recovered significantly on its own especially in long term exposure case.

Table 6. Interpretation of results.

Test	Brain region involved	Behavior	Damage	Recovery
Y maze test	Hippocampus prefrontal cortex	spatial learning and memory	20 day exposure group>50 day exposure group > 35 day exposure group	35 day recovery group >20 day recovery group > 50 day recovery group
Morris water maze	Hippocampus	spatial learning and memory	50 day exposure group >20 day exposure group > 35 day exposure group	35 day recovery group >20 day recovery group > 50 day recovery group
Hole board test	Hippocampus Amygdala	exploratory behavior/anxiety	50 day exposure group >35 day exposure group > 20 day exposure group	35 day recovery group >50 day recovery group > 20 day recovery group
Three chamber assay	prefrontal cortex	sociability	50 day exposure group >20 day exposure group > 35 day exposure group	35 day recovery group >20 day recovery group > 50 day recovery group

CONCLUSION

Conclusion

Increased exposure to Cadmium contaminated drinking water has led to impairment in learning and memory process. This study presents how different time dependent exposures of same total dose of Cadmium can cause differential impairment in hippocampal dependent learning and memory, which can be reversible on its own. Results of the study clearly presents that long term exposure of Cadmium with low dose and short term exposure with high dose both can cause learning and memory impairment that cannot be reversed on its own without any treatment. However, moderate dose exposure of Cadmium for moderate period of time cause low level of learning and memory impairment that can be reversed slightly on its own. Still further research is needed to establish the exact mechanism of temporal exposure of Cadmium on higher cognitive functions.

REFERENCES

REFERENCES

Adekunle, I. M., Adetunji, M. T., Gbadebo, A. M., & Banjoko, O. B. (2007). Assessment of groundwater quality in a typical rural settlement in Southwest Nigeria. *International journal of environmental research and public health*, *4*(4), 307-318.

Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N. J., Habel, U., Schneider, F., &Zilles, K. (2005, Dec). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. *AnatEmbryol (Berl), 210*(5-6), 343-352.

Asubiojo, O. I., Nkono, N. A., Ogunsua, A. O., Oluwole, A. F., Ward, N. I., Akanle, O. A., & Spyrou, N. M. (1997). Trace elements in drinking and groundwater samples in Southern Nigeria. *Science of the total environment*, *208*(1-2), 1-8.

Baars, B. J., & Gage, N. M. (2010). *Cognition, brain, and consciousness: Introduction to cognitive neuroscience*. Academic Press.Baddeley, A., Logie, R., Bressi, S., Sala, S. D., &Spinnler, H. (1986, 1986/11/01). Dementia and Working Memory. *The Quarterly Journal of Experimental Psychology Section A, 38*(4), 603-618.

Baddeley, A., Logie, R., Bressi, S., Sala, S. D., &Spinnler, H. (1986, 1986/11/01). Dementia and Working Memory. *The Quarterly Journal of Experimental Psychology Section A, 38*(4), 603-618.

Bagchi, D., Bagchi, M., Stohs, S. J., Das, D. K., Ray, S. D., Kuszynski, C. A., ... & Pruess, H. G. (2000). Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology*, *148*(2-3), 187-197.

Baig, J. A., Kazi, T. G., Shah, A. Q., Kandhro, G. A., Afridi, H. I., Khan, S., ... & Wadhwa, S. K. (2012). Arsenic speciation and other parameters of surface and ground water samples of Jamshoro, Pakistan. *International Journal of Environmental Analytical Chemistry*, *92*(1), 28-42.

Batool, Z., Agha, F., Ahmad, S., Liaquat, L., Tabassum, S., Khaliq, S., ... & Haider, S. (2017). Attenuation of cadmium-induced decline in spatial, habituation and recognition memory by long-term administration of almond and walnut supplementation: Role of cholinergic function. *Pak J Pharm Sci*, *30*(1 Suppl), 273-279.

Batool, Z., Agha, F., Tabassum, S., Batool, T. S., Siddiqui, R. A., & Haider, S. (2019). Prevention of cadmium-induced neurotoxicity in rats by essential nutrients present in nuts. *ActaNeurobiolExp*, *79*(2), 169-183.

Bernard, A. (2008). Cadmium & its adverse effects on human health. *Indian Journal of Medical Research*, *128*(4), 557.

Branca, J. J. V., Morucci, G., & Pacini, A. (2018). Cadmium-induced neurotoxicity: still much ado. *Neural regeneration research*, *13*(11), 1879.

Cassilhas, R. C., Tufik, S., & de Mello, M. T. (2016, Mar). Physical exercise, neuroplasticity, spatial learning and memory. *Cell Mol Life Sci*, *73*(5), 975-983.

Chen, L., Xu, B., Liu, L., Luo, Y., Zhou, H., Chen, W., ... & Huang, S. (2011). Cadmium induction of reactive oxygen species activates the mTOR pathway, leading to neuronal cell death. *Free Radical Biology and Medicine*, *50*(5), 624-632.

Cohen, N. J., & Eichenbaum, H. (1993). *Memory, amnesia, and the hippocampal system*. The MIT Press.

Davison, A. G., Taylor, A. N., Darbyshire, J., Chettle, D. R., Guthrie, C. J. G., O'Malley, D., ... & Gompertz, D. (1988). Cadmium fume inhalation and emphysema. *The Lancet*, *331*(8587), 663-667.

de Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, David H., Kopeikina, Kathy J., Pitstick, R., Sahara, N., Ashe, Karen H., Carlson, George A., Spires-Jones, Tara L., & Hyman, Bradley T. (2012, 2012/02/23/). Propagation of Tau Pathology in a Model of Early Alzheimer's Disease. *Neuron*, *73*(4), 685-697.

DeYoung, C. G., Hirsh, J. B., Shane, M. S., Papademetris, X., Rajeevan, N., & Gray, J. R. (2010, Jun). Testing predictions from personality neuroscience. Brain structure and the big five. *PsycholSci*, *21*(6), 820-828.

Di Gennaro, G., Grammaldo, L. G., Quarato, P. P., Esposito, V., Mascia, A., Sparano, A., Meldolesi, G. N., &Picardi, A. (2006, Jun). Severe amnesia following bilateral medial temporal lobe damage occurring on two distinct occasions. *NeurolSci*, *27*(2), 129-133.

Fern, R. O. B. E. R. T., Black, J. A., Ransom, B. R., & Waxman, S. G. (1996). Cd (2+)-induced injury in CNS white matter. *Journal of neurophysiology*, *76*(5), 3264-3273.

Fernstrom, J. D., & Fernstrom, M. H. (2007). Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *The Journal of nutrition*, *137*(6), 1539S-1547S.

Fernstrom, J. D., &Fernstrom, M. H. (2007). Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *The Journal of nutrition*, *137*(6), 1539S-1547S.

Flora, S. J. S., Mittal, M., & Mehta, A. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian Journal of Medical Research*, *128*(4), 501.

Flora, S. J., & Agrawal, S. (2017). Arsenic, cadmium, and lead. In *Reproductive and developmental toxicology* (pp. 537-566). Academic Press.

Friberg, L., Nordberg, G. F., & Vouk, V. B. (1979). *Handbook on the Toxicology of Metals*. Elsevier/North-Holland Biomedical Press, 335 Jan van Galenstraat, 1061 AZ Amsterdam, The Netherlands.

Gabrieli, J. D., Poldrack, R. A., & Desmond, J. E. (1998, Feb 3). The role of left prefrontal cortex in language and memory. *ProcNatl Acad Sci U S A, 95*(3), 906-913.

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., & Groneberg, D. A. (2006). The toxicity of cadmium and resulting hazards for human health. *Journal of occupational medicine and toxicology, 1*(1), 1-6.

Gross, R. (2015). *Psychology: The science of mind and behaviour 7th edition*. Hodder Education.

Guerrero, A., Piasecki, M., Alicata, D., & Jacobs, N. (2008). *Problem-based behavioral science and psychiatry*: Springer.

Hoffman, P. (2019, Jan 31). Reductions in prefrontal activation predict off-topic utterances during speech production. *Nat Commun, 10*(1), 515.

Iqbal, G., & Ahmed, T. (2019). Co-exposure of metals and high fat diet causes aging like neuropathological changes in non-aged mice brain. *Brain research bulletin, 147*, 148-158.

Jaishankar, M., Mathew, B. B., Shah, M. S., & Gowda, K. R. S. (2014). Biosorption of few heavy metal ions using agricultural wastes. *Journal of Environment Pollution and Human Health, 2*(1), 1-6.

Jeneson, A., & Squire, L. R. (2012). Working memory, long-term memory, and medial temporal lobe function. *Learning & memory, 19*(1), 15-25.

Kaji, M. (2012). Role of experts and public participation in pollution control: the case of Itai-itai disease in Japan1. *Ethics in science and environmental politics*, *12*(2), 99-111.

Kanter, M., Unsal, C., Aktas, C., & Erboga, M. (2016). Neuroprotective effect of quercetin against oxidative damage and neuronal apoptosis caused by cadmium in hippocampus. *Toxicology and industrial health*, *32*(3), 541-550.

Karaca, S., &Eraslan, G. (2013). The effects of flaxseed oil on cadmium-induced oxidative stress in rats. *Biological trace element research*, *155*(3), 423-430.

Knudsen, K. S., Kaufman, D. S., White, S. A., Silva, A. J., Jentsch, D. J., & Bilder, R. M. (2015). Animal creativity: Cross-species studies of cognition. In *Animal creativity and innovation.* (pp. 213-237). Elsevier Academic Press.

Korte, M., & Schmitz, D. (2016, Apr). Cellular and System Biology of Memory: Timing, Molecules, and Beyond. *Physiol Rev, 96*(2), 647-693.

Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Y-maze for assessment of spatial working and reference memory in mice. In *Pre-Clinical Models* (pp. 105-111). Humana Press, New York, NY.

Lalumiere, R. T. (2014). Optogenetic dissection of amygdala functioning. *Front BehavNeurosci*, *8*, 107.

Leung, C. C., Yu, I. T. S., & Chen, W. (2012). Silicosis. *The Lancet*, *379*(9830), 2008-2018.

Lone, M. I., Saleem, S., Mahmood, T., Saifullah, K., & Hussain, G. (2003). Heavy metal contents of vegetables irrigated by sewage/tubewell water. *International journal of Agriculture and Biology*, *5*(4), 533-535.

Manca, D., Ricard, A. C., Trottier, B., & Chevalier, G. (1991). Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. *Toxicology*, *67*(3), 303-323.

Manzoor, S., Shah, M. H., Shaheen, N., Khalique, A., & Jaffar, M. (2006). Multivariate analysis of trace metals in textile effluents in relation to soil and groundwater. *Journal of hazardous materials*, *137*(1), 31-37.

Mascagni, P., Consonni, D., Bregante, G., Chiappino, G., & Toffoletto, F. (2003). Olfactory function in workers exposed to moderate airborne cadmium levels. *Neurotoxicology*, *24*(4-5), 717-724.

Méndez-Armenta, M., & Ríos, C. (2007). Cadmium neurotoxicity. *Environmental Toxicology and Pharmacology*, *23*(3), 350-358.

Méndez-Armenta, M., & Ríos, C. (2007). Cadmium neurotoxicity. *Environmental Toxicology and Pharmacology*, *23*(3), 350-358.

Méndez-Armenta, M., & Ríos, C. (2007). Cadmium neurotoxicity. *Environmental Toxicology and Pharmacology*, *23*(3), 350-358.

Merolla, P. A., Arthur, J. V., Alvarez-Icaza, R., Cassidy, A. S., Sawada, J., Akopyan, F., Jackson, B. L., Imam, N., Guo, C., Nakamura, Y., Brezzo, B., Vo, I., Esser, S. K., Appuswamy, R., Taba, B., Amir, A., Flickner, M. D., Risk, W. P., Manohar, R., & Modha, D. S. (2014). A million spiking-neuron integrated circuit with a scalable communication network and interface. *Science*, *345*(6197), 668.

Meyer, G., & Ciccarelli, S. (2005). *Psychology (Paperback)*: Prentice Hall.

Millan, M. J., Agid, Y., Brüne, M., Bullmore, E. T., Carter, C. S., Clayton, N. S., ... & Young, L. J. (2012). Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nature reviews Drug discovery*, *11*(2), 141-168.

Millan, Mark J., et al. "Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy." *Nature reviews Drug discovery* 11.2 (2012): 141.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annu Rev Neurosci*, *24*, 167-202.

Miller, E. K., Freedman, D. J., & Wallis, J. D. (2002, Aug 29). The prefrontal cortex: categories, concepts and cognition. *Philos Trans R SocLond B BiolSci*, *357*(1424), 1123-1136.

Moy, S. S., Nadler, J. J., Perez, A., Barbaro, R. P., Johns, J. M., Magnuson, T. R., ... & Crawley, J. N. (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes, Brain and Behavior*, *3*(5), 287-302.

Munisamy, R., Ismail, S. N. S., & Praveena, S. M. (2013). Cadmium exposure via food crops: a case study of intensive farming area. *Am J Appl Sci*, *10*(10), 1252-1262.

Mustafa, H. T., Hassan, H., Abo-Melha, A., & Rihan, T. I. (1988). Cadmium and zinc concentrations in the potable water of the Eastern Province of Saudi Arabia. *Bull. Environ. Contam. Toxicol.;(United States)*, *40*(3).

Mustafa, H. T., Hassan, H., Abo-Melha, A., & Rihan, T. I. (1988). Cadmium and zinc concentrations in the potable water of the Eastern Province of Saudi Arabia. *Bull. Environ. Contam. Toxicol.;(United States)*, *40*(3).

Nelson, C. A., de Haan, M., & Thomas, K. M. (2015). *Neuroscience of Cognitive Development: The Role of Experience and the Developing Brain*. Wiley Blackwell.

Nemmiche, S., Chabane-Sari, D., & Guiraud, P. (2007). Role of α-tocopherol in cadmium-induced oxidative stress in Wistar rat's blood, liver and brain. *Chemico-biological interactions*, *170*(3), 221-230.

Nieh, E. H., Kim, S.-Y., Namburi, P., &Tye, K. M. (2013, 2013/05/20/). Optogenetic dissection of neural circuits underlying emotional valence and motivated behaviors. *Brain Research*, *1511*, 73-92.

Noble, E. E., & Kanoski, S. E. (2016). Early life exposure to obesogenic diets and learning and memory dysfunction. *Current opinion in behavioral sciences*, *9*, 7-14.

Oboh, G., Adebayo, A. A., Ademosun, A. O., &Olowokere, O. G. (2020). Rutin restores neurobehavioral deficits via alterations in cadmium bioavailability in the brain of rats exposed to cadmium. *Neurotoxicology*, *77*, 12-19.

Oguzturk, H., Ciftci, O. S. M. A. N., Aydin, M., Timurkaan, N., Beytur, A., & Yilmaz, F. (2012). Ameliorative effects of curcumin against acute cadmium toxicity on male reproductive system in rats. *Andrologia*, *44*(4), 243-249.

O'Neil, E. B., Newsome, R. N., Li, I. H., Thavabalasingam, S., Ito, R., & Lee, A. C. (2015, Nov 11). Examining the Role of the Human Hippocampus in Approach-Avoidance Decision

Making Using a Novel Conflict Paradigm and Multivariate Functional Magnetic Resonance Imaging. *J Neurosci*, *35*(45), 15039-15049.

Park, J. D., Cherrington, N. J., & Klaassen, C. D. (2002). Intestinal absorption of cadmium is associated with divalent metal transporter 1 in rats. *Toxicological Sciences*, *68*(2), 288-294.

Paschal, D. C., Burt, V., Caudill, S. P., Gunter, E. W., Pirkle, J. L., Sampson, E. J., . . . Jackson, R. J. (2000). Exposure of the U.S. population aged 6 years and older to cadmium: 1988-1994. *Archives of environmental contamination and toxicology*, *38*(3), 377-383.

Patrick, L. (2002). Patrick L. Mercury toxicity and antioxidants: Part 1: role of glutathione and alpha-lipoic acid in the treatment... *Alternative Medicine Review*, *7*(6), 456-471.

Renaud, H. J., Cui, J. Y., Khan, M., & Klaassen, C. D. (2011). Tissue distribution and gender-divergent expression of 78 cytochrome P450 mRNAs in mice. *Toxicological sciences*, *124*(2), 261-277.

Satarug, S., Baker, J. R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P. E. B., Williams, D. J., & Moore, M. R. (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicology letters*, *137*(1-2), 65-83.

Schacter, D. L., Gilbert, D. T., & Wegner, D. M. (2009). *Introducing psychology*: Macmillan.

Shakir, S. K., Azizullah, A., Murad, W., Daud, M. K., Nabeela, F., Rahman, H., ... & Häder, D. P. (2016). Toxic metal pollution in Pakistan and its possible risks to public health. *Reviews of Environmental Contamination and Toxicology Volume 242*, 1-60.

Shekhar, T. S., Kiran, B. R., Puttaiah, E. T., Shivaraj, Y., & Mahadevan, K. M. (2008). Phytoplankton as index of water quality with reference to industrial pollution. *Journal of Environmental Biology*, *29*(2), 233.

Sherwood, L. (2015). *Human physiology: from cells to systems*: Cengage learning.

Shi-Lei, S., Guang-Yu, M., Bachelor, L. H., Bachelor, Z. Y., Hong-Mei, D., & Xiao-Hu, X. (2005). Effect of naloxone on aluminum-induced learning and memory impairment in rats. *Neurology India, 53*(1), 79.

Shukla, A., Shukla, G. S., & Srimal, R. C. (1996). Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. *Human & experimental toxicology*, *15*(5), 400-405.

Simpson, J. R. (2014). DSM-5 and neurocognitive disorders. *Journal of the American Academy of Psychiatry and the Law Online*, *42*(2), 159-164.

Singh, A., Sharma, R. K., Agrawal, M., & Marshall, F. M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and chemical toxicology*, *48*(2), 611-619.

Singh, M. R. (2007). Impurities-heavy metals: IR perspective. *International journal of the physical sciences*, *5*(4), 1045-1058.

Solms, M., Turnbull, O., & Sacks, O. (2018). *The brain and the inner world: An introduction to the neuroscience of subjective experience*. Routledge.

Sutherland, R. J., & Rudy, J. W. (1989, 1989/06/01). Configural association theory: The role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology, 17*(2), 129-144.

Taskar, B., Chatalbashev, V., &Koller, D. (2004, July). Learning associative markov networks. In *Proceedings of the twenty-first international conference on Machine learning* (p. 102).

Tomer, R., Slagter, H. A., Christian, B. T., Fox, A. S., King, C. R., Murali, D., Gluck, M. A., & Davidson, R. J. (2014). Love to Win or Hate to Lose? Asymmetry of Dopamine D2 Receptor Binding Predicts Sensitivity to Reward versus Punishment. *Journal of Cognitive Neuroscience*, *26*(5), 1039-1048.

Torpy, J. M., Burke, A. E., & Glass, R. M. (2008). Delirium. *JAMA*, *300*(24), 2936-2936.

Torpy, J. M., Lynm, C., & Glass, R. M. (2010). Dementia. *Jama*, *304*(17), 1972-1972.

Uematsu, A., Tan, B. Z., Ycu, E. A., Cuevas, J. S., Koivumaa, J., Junyent, F., Kremer, E. J., Witten, I. B., Deisseroth, K., & Johansen, J. P. (2017, Nov). Modular organization of the brainstem noradrenaline system coordinates opposing learning states. *Nat Neurosci*, *20*(11), 1602-1611.

Usai, C., Barberis, A., Moccagatta, L., & Marchetti, C. (1999). Pathways of cadmium influx in mammalian neurons. *Journal of neurochemistry*, *72*(5), 2154-2161.

Verma, C., Das, A. J., & Kumar, R. (2017). PGPR-assisted phytoremediation of cadmium: An advancement towards clean environment. *Current Science*, *113*(4), 715.

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*, *1*(2), 848-858.

Wang, B., & Du, Y. (2013). Cadmium and its neurotoxic effects. *Oxidative medicine and cellular longevity*, *2013*.

Wang, D., Zhang, J., Jiang, W., Cao, Z., Zhao, F., Cai, T., Aschner, M., & Luo, W. (2017, May 4). The role of NLRP3-CASP1 in inflammasome-mediated neuroinflammation and autophagy dysfunction in manganese-induced, hippocampal-dependent impairment of learning and memory ability. *Autophagy*, *13*(5), 914-927.

Wetherell, J. L., Reynolds, C. A., Gatz, M., & Pedersen, N. L. (2002). Anxiety, cognitive performance, and cognitive decline in normal aging. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *57*(3), P246-P255.

Wietrzych, M., Meziane, H., Sutter, A., Ghyselinck, N., Chapman, P. F., Chambon, P., & Krężel, W. (2005). Working memory deficits in retinoid X receptor γ-deficient mice. *Learning & Memory*, *12*(3), 318-326.

Yang, Y., &Raine, A. (2009, Nov 30). Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: a meta-analysis. *Psychiatry Res*, *174*(2), 81-88.

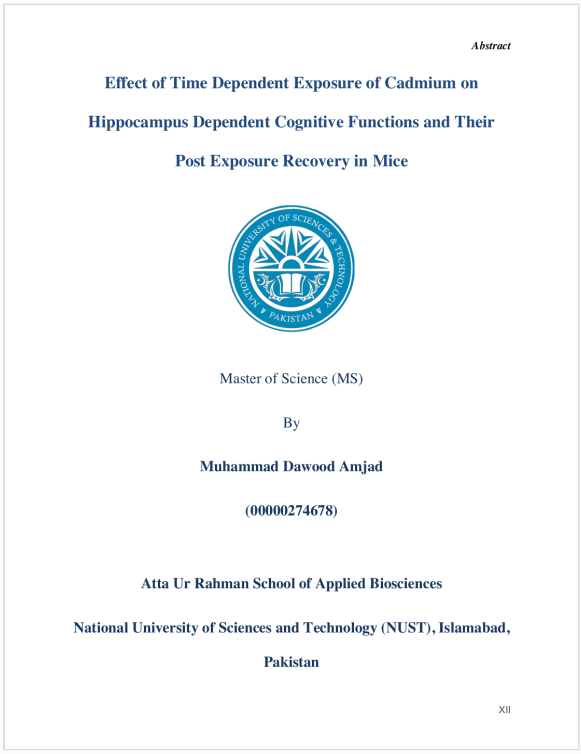
Yochim, B., Yutsis, M., Rosen, A., &Yesavage, J. (2014). Neurocognitive Disorders. *Study Guide to DSM-5®*, 381.

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Touqeer Ahmed
Assignment title:	Dawood Thesis
Submission title:	Dawood MS Thesis
File name:	For_plagirism_Report_Dawood_Final_thesis.docx
File size:	2.95M
Page count:	78
Word count:	12,431
Character count:	65,077
Submission date:	13-Sep-2021 10:21PM (UTC-0700)
Submission ID:	1648072550



Abstract

Effect of Time Dependent Exposure of Cadmium on Hippocampus Dependent Cognitive Functions and Their Post Exposure Recovery in Mice

Master of Science (MS)

By

Muhammad Dawood Amjad

(00000274678)

Atta Ur Rahman School of Applied Biosciences

National University of Sciences and Technology (NUST), Islamabad, Pakistan

XII

Dawood MS Thesis

ORIGINALITY REPORT

12%	10%	9%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Hong Kong Student Paper	1%
2	Submitted to Higher Education Commission Pakistan Student Paper	1%
3	link.springer.com Internet Source	1%
4	www.newscientist.com Internet Source	1%
5	www.ncbi.nlm.nih.gov Internet Source	1%
6	Ganiyu Oboh, Adeniyi A. Adebayo, Ayokunle O. Ademosun, Olanike G. Olowokere. "Rutin alleviates cadmium-induced neurotoxicity in Wistar rats: involvement of modulation of nucleotide-degrading enzymes and monoamine oxidase", Metabolic Brain Disease, 2019 Publication	<1%

7	Zhi-Jian Wang, Xiao-Hong Liu, Li Jin, De-Yong Pu, Jing Huang, Yao-Guang Zhang. "Transcriptome profiling analysis of rare minnow (Gobiocypris rarus) gills after waterborne cadmium exposure", Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 2016 Publication	<1 %
8	academic.oup.com Internet Source	<1 %
9	multiplesclerosisnewsblog.blogspot.com Internet Source	<1 %
10	mafiadoc.com Internet Source	<1 %
11	vtechworks.lib.vt.edu Internet Source	<1 %
12	www.ijbmb.org Internet Source	<1 %
13	pvj.com.pk Internet Source	<1 %
14	"Animal Models of Acute Neurological Injuries II", Springer Science and Business Media LLC, 2012 Publication	<1 %
15	epubs.surrey.ac.uk Internet Source	<1 %

16	Sierksma, Annerieke S.R., Jos Prickaerts, Leonidas Chouliaras, Somayeh Rostamian, Lore Delbroek, Bart P.F. Rutten, Harry W.M. Steinbusch, and Daniel L.A. van den Hove. "Behavioral and neurobiological effects of prenatal stress exposure in male and female APPswe/PS1dE9 mice", Neurobiology of Aging, 2013. Publication	<1 %
17	tessera.spandidos-publications.com Internet Source	<1 %
18	Submitted to Centre for Nutrition Education & Lifestyle Management (CNELM) Student Paper	<1 %
19	www.hindawi.com Internet Source	<1 %
20	www.frontiersin.org Internet Source	<1 %
21	Isyaku Yarube, Joseph Ayo, Rabiu Magaji, Ismail Umar. "Insulin treatment increases brain nitric oxide and oxidative stress, but does not affect memory function in mice", Physiology & Behavior, 2019 Publication	<1 %
22	www.ejh.it Internet Source	<1 %

23	www.mdpi.com Internet Source	<1 %
24	www.tandfonline.com Internet Source	<1 %
25	spandidos-publications.com Internet Source	<1 %
26	escholarship.org Internet Source	<1 %
27	virologyj.biomedcentral.com Internet Source	<1 %
28	Nelson, Randy J.. "An Introduction to Behavioral Endocrinology", Oxford University Press Publication	<1 %
29	www.jneurosci.org Internet Source	<1 %
30	Ashkhen Manukyan, Lilit Hunanyan, Artem Grigoryan, Hayk Harutyunyan, Lilit Sukiasyan, Magdalina Melkonyan. "Adrenergic Alpha-2 Receptor Antagonists Regulate Noise–induced Metabolic and Cognitive Changes in Rats", Research Square, 2020 Publication	<1 %
31	en.wikipedia.org Internet Source	<1 %

32	repository.lib.cuhk.edu.hk Internet Source	<1 %
33	Naderi, N.. "Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice", Pharmacology, Biochemistry and Behavior, 200803 Publication	<1 %
34	Submitted to The University of Manchester Student Paper	<1 %
35	digitalcommons.fiu.edu Internet Source	<1 %
36	scholarbank.nus.edu.sg Internet Source	<1 %
37	www.biorxiv.org Internet Source	<1 %
38	www.e-jar.org Internet Source	<1 %
39	blogs.oswego.edu Internet Source	<1 %
40	eprints.nottingham.ac.uk Internet Source	<1 %
41	peerj.com Internet Source	<1 %
42	roar.uel.ac.uk Internet Source	<1 %

43	www.clinicians-view.com Internet Source	<1 %
44	www.researchsquare.com Internet Source	<1 %
45	Engler-Chiurazzi, E.. "Tonic Premarin dose-dependently enhances memory, affects neurotrophin protein levels and alters gene expression in middle-aged rats", Neurobiology of Aging, 201104 Publication	<1 %
46	In-Kyung Jeong, Seung-Hoon Oh, Byung-Joon Kim, Jae-Hoon Chung, Yong-Ki Min, Myung-Shik Lee, Moon-Kyu Lee, Kwang-Won Kim. "The effects of dexamethasone on insulin release and biosynthesis are dependent on the dose and duration of treatment", Diabetes Research and Clinical Practice, 2001 Publication	<1 %
47	inba.info Internet Source	<1 %
48	kclpure.kcl.ac.uk Internet Source	<1 %
49	publications.idiap.ch Internet Source	<1 %
50	www.qps.com Internet Source	<1 %

51	Hidekazu Miyagawa, Masaya Hasegawa, Taneo Fukuta, Manabu Amano, Kiyofumi Yamada, Toshitaka Nabeshima. "Dissociation of impairment between spatial memory, and motor function and emotional behavior in aged rats", Behavioural Brain Research, 1998 Publication	<1 %
52	Hirohisa Tajima, Masaoki Kawasumi, Tomohiro Chiba, Marina Yamada et al. "A humanin derivative, S14G-HN, prevents amyloid-?-induced memory impairment in mice", Journal of Neuroscience Research, 2005 Publication	<1 %
53	Hua Zheng, Hoai Ton, Lei Yang, Ning Liufu, Yuanlin Dong, Yiying Zhang, Zhongcong Xie. "Acute Fasting Does Not Induce Cognitive Impairment in Mice", Frontiers in Neuroscience, 2019 Publication	<1 %
54	Jie Zhang. "Pentylenetetrazole-induced status epilepticus following training does not impair expression of morphine-induced conditioned place preference", Addiction Biology, 04/2009 Publication	<1 %
55	Submitted to University of Ulster Student Paper	<1 %
56	acervodigital.ufpr.br Internet Source	<1 %

57	eprints.qut.edu.au Internet Source	<1 %
58	oatext.com Internet Source	<1 %
59	tesisenxarxa.net Internet Source	<1 %
60	thriveworks.com Internet Source	<1 %
61	www.nature.com Internet Source	<1 %
62	A. Shipp, G. Lawrence, R. Gentry, T. McDonald, H. Bartow, J. Bounds, N. Macdonald, H. Clewell, B. Allen, C. Van Landingham. "Acrylamide: Review of Toxicity Data and Dose-Response Analyses for Cancer and Noncancer Effects", Critical Reviews in Toxicology, 2008 Publication	<1 %
63	Ashkhen Manukyan, Artem Grigoryan, Lilit Hunanyan, Hayk Harutyunyan, Lilit Sukiasyan, Magdalina Melkonyan. "Adrenergic alpha-2 receptor antagonists regulate noise–induced metabolic and cognitive changes in rats", Research Square, 2020 Publication	<1 %

64	Charisse N. Winston, Anastasia Noël, Aidan Neustadtl, Maia Parsadanian et al. "Dendritic Spine Loss and Chronic White Matter Inflammation in a Mouse Model of Highly Repetitive Head Trauma", The American Journal of Pathology, 2016 Publication	<1 %
65	Kim, D.H.. "Gomisin A improves scopolamine-induced memory impairment in mice", European Journal of Pharmacology, 20060807 Publication	<1 %
66	Muhammad Faisal Ikram, Syeda Mehpara Farhat, Aamra Mahboob, Saeeda Baig, Ahmed Yaqinuddin, Touqeer Ahmed. "Expression of DnMTs and MBDs in AlCl3-Induced Neurotoxicity Mouse Model", Biological Trace Element Research, 2020 Publication	<1 %
67	archive-ouverte.unige.ch Internet Source	<1 %
68	centaur.reading.ac.uk Internet Source	<1 %
69	livrepository.liverpool.ac.uk Internet Source	<1 %
70	www.koreascience.or.kr Internet Source	<1 %

71	www.news-medical.net Internet Source	<1%
72	F.Y. Doré, S. Goulet, A. Gallagher, P.-O. Harvey, J.-F. Cantin, T. D'Aigle, M.-E. Mirault. "Neurobehavioral changes in mice treated with methylmercury at two different stages of fetal development", Neurotoxicology and Teratology, 2001 Publication	<1%
73	Pranay Srivastava, Yogesh K. Dhuriya, Vivek Kumar, Akriti Srivastava et al. "PI3K/Akt/GSK3β induced CREB activation ameliorates arsenic mediated alterations in NMDA receptors and associated signaling in rat hippocampus: Neuroprotective role of curcumin", NeuroToxicology, 2018 Publication	<1%
74	www.exeley.com Internet Source	<1%
75	Lívia Carla Silva de Melo, Ariane Pinheiro Cruz, Saavedra José Rios Valentim, Andresa Rosane Marinho et al. "Δ9-THC administered into the medial prefrontal cortex disrupts the spatial working memory", Psychopharmacology, 2005 Publication	<1%