Evaluation of Impairment in Cognitive Function by Temporal Exposure of Arsenic and Post Exposure Recovery.

Master of Science (MS)

By

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Atta Ur Rahman School of Applied Biosciences

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Evaluation of impairment in cognitive function by temporal exposure of arsenic and post exposure recovery.

A thesis submitted in partial fulfilment of the requirement for the degree of Master of Science (MS)

By

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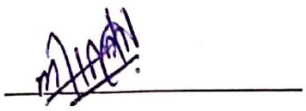

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DEDICATION

I’d like to dedicate this thesis to the two strongest pillars of my life;

My Beloved Parents and My Husband Mudassar.

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All praise to Allah and His blessings for the thesis completion. I thank Allah for all the chances, and courage that I have received to conclude my thesis. My most sincere appreciation is to Muhammad (Peace be with him) who has always guided me in his manner of life.

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I dedicate this thesis to all those fathers who work effortlessly to educate their daughters and support them to achieve their dreams and those working mothers incredibly wonderful who manage to raise their kids to the best alongside their job and household.

List of Acronyms

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

List of Figures

List of Tables

List of Tables

Abstract

Arsenic is a metalloid reported to have carcinogenic and neurotoxic effect. The high prevalence of arsenic in water is leading to multiple health issues including neurotoxicity. This study is aimed at evaluating the effect of arsenic exposure on brain tissues specifically on hippocampus and cortex; the effect of metal consumption on higher cognitive functions i.e. learning and memory. The test subjects are exposed to arsenic in drinking water to develop model of total exposure of 932mg/kg over the time period of 20, 35, 50-days with the exposure of 188mg/kg/day, 106.4mg/kg/day and 76mg/kg/day respectively, the behavioral analysis of subjects was carried out after 20-days, 35-days, 50-days of exposure using the Y-Maze, Hole board analysis, Morris water test and social interaction test. After As exposure was over animals were given normal water for the recovery time of 20-days and again evaluated for the behavioral changes. The conclusions are impaired learning and memory of the arsenic exposed subjects with the most deficit in learning and memory of 50-day exposure and 20-day exposure group with very slight recovery of the damage in recovery period and metal leach out in both the groups, while comparatively less decline in cognitive functions of the 35-days group and better overall recovery in comparison to the 50-day exposure and 20-day exposure group.

Keywords: Arsenic, Metal exposure, Behavior analysis, Cognitive impairment, Memory defect, Learning and memory.

CHAPTER 1

INTRODUCTION

Introduction

1.1 Metals Classification

In chemistry, metal is the material which has the tendency to donate its electron from the valance shell. They can be divided into different categories dependent upon the nature and valance shell according to the periodic table. One of the important class studied vastly includes the heavy metals which belongs to group d and f in the periodic table. When compared to water, heavy metals are defined as metallic components having a somewhat high thickness (Boros et al., 2020). Substantial metals also include metalloids, such as arsenic, which can cause harmfulness at low levels of openness, on the assumption that greatness and poisonousness are associated. Natural tainting by these metals has recently become a growing biological and global public health problem (Deng et al., 2019). Furthermore, due of a considerable increase in their use in a variety of contemporary, farming, domestic, and mechanical applications, human openness has increased dramatically. Geogenic, mechanical, horticultural, drug, homegrown effluents, and air sources are all detailed wellsprings of considerable metals in the environment. In point source areas such as mining, foundries, and smelters, as well as other metal-based contemporary operations, environmental pollution is particularly obvious(Natasha et al., 2021).

1.1.1 Physiological and Toxicological Effects of Heavy Metals

In plants and animals, the fundamental heavy metals have biochemical and physiological impacts. They are important components of a few essential compounds and play crucial roles in oxidation-decrease reactions (B. Sharma et al., 2014). Catalase, superoxide dismutase, peroxidase, cytochrome c oxidases, ferroxidases, monoamine oxidase, and dopamine-monooxygenase are just a few of the oxidative pressure-related compounds that copper plays a role in. Since a result, it's

far from a basic supplement, as it's linked to metalloenzymes involved in haemoglobin formation, carb digestion, catecholamine production, and collagen, elastin, and hair keratin cross-linking. Cuproenzymes involved in redox reactions take use of copper's ability to cycle between an oxidised state, Cu(II), and a reduced form, Cu(I). In any event, it is this quality of copper that makes it potentially toxic, since advancements between Cu(II) and Cu(I) can usher in a new era of superoxide and hydroxyl revolutionaries (Ordak et al., 2018). Likewise, unreasonable openness to copper has been connected to cell harm prompting Wilson sickness in people. Like copper, a few other fundamental components are needed for biologic working, be that as it may, an abundance measure of such metals produces cell and tissue harm prompting an assortment of unfriendly impacts plus humanoid infections. For some comprising chromium in addition to copper, there is a limited scope of fixations among gainful and poisonous impacts (M & Khan M, 2016). Different metals like aluminum (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) don't have any settled natural capacities and are taken into consideration as insignificant metals (Azeh et al., 2019; Jaishankar et al., 2014; Khan, 2016; B. Sharma et al., 2014). Significant metals were observed to influence cell organelles and components which include the cell layer, mitochondria, lysosome, endoplasmic reticulum, cores, and a few proteins worried in digestion, detoxing, and harm restore in herbal systems. Metal debris had been observed to paintings at the side of cellular segments which includes DNA and atomic proteins, inflicting DNA harm and conformational changes that could lead to cell cycle imbalance, most cancers, or apoptosis (Briffa et al., 2020).

1.2 Heavy Metals and Their Effect on Health

A few investigations from our lab have shown that receptive oxygen species (ROS) creation and oxidative pressure and oxidative pressure play a significant role in the toxicity and cancer-causing characteristics of metals such as arsenic, cadmium, chromium, lead, and mercury (Rama Jyothi, 2020). Because of their high level of toxicity, these five elements are considered essential metals that are vital to one's overall health (Jaishankar et al., 2014). They're generally fundamental poisons that are known to cause an assortment of organ injury, even at low degrees of openness. As per the United States Environmental Protection Agency (US EPA) and the International Agency for Research on Cancer (IARC), these metals are named "known" or "conceivable" human malignant growth causing specialists dependent on epidemiological and clinical examinations that show a connection among transparency and infection event in people and creatures (Azeh Engwa et al., 2019). Metals despite of being dangerous to health have been used by humans for many years in different ways. Exposure to these metals and their health-related issues have been increasing day by day around the world, especially in less-developed countries(Sall et al., 2020). In Pakistan, many examinations and studies have plainly shown the immediate impacts of these poisonous substantial metals on human wellbeing particularly youngsters prompting development and neurological issues. General society in the vast majority of the spaces of Pakistan is at high danger to substantial metals openness through tainted drinking water and air, as per aggregate risk list (HI) examination of poisonous metals (Shakir et al., 2017). 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. 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 aluminum is highest through drinking water in areas of Khyber Pakhtunkhwa. Through the route of air inhalation metal toxicity is highly concentrated in Punjab region (Ashraf et al., 2020; Khalid et al., 2020).

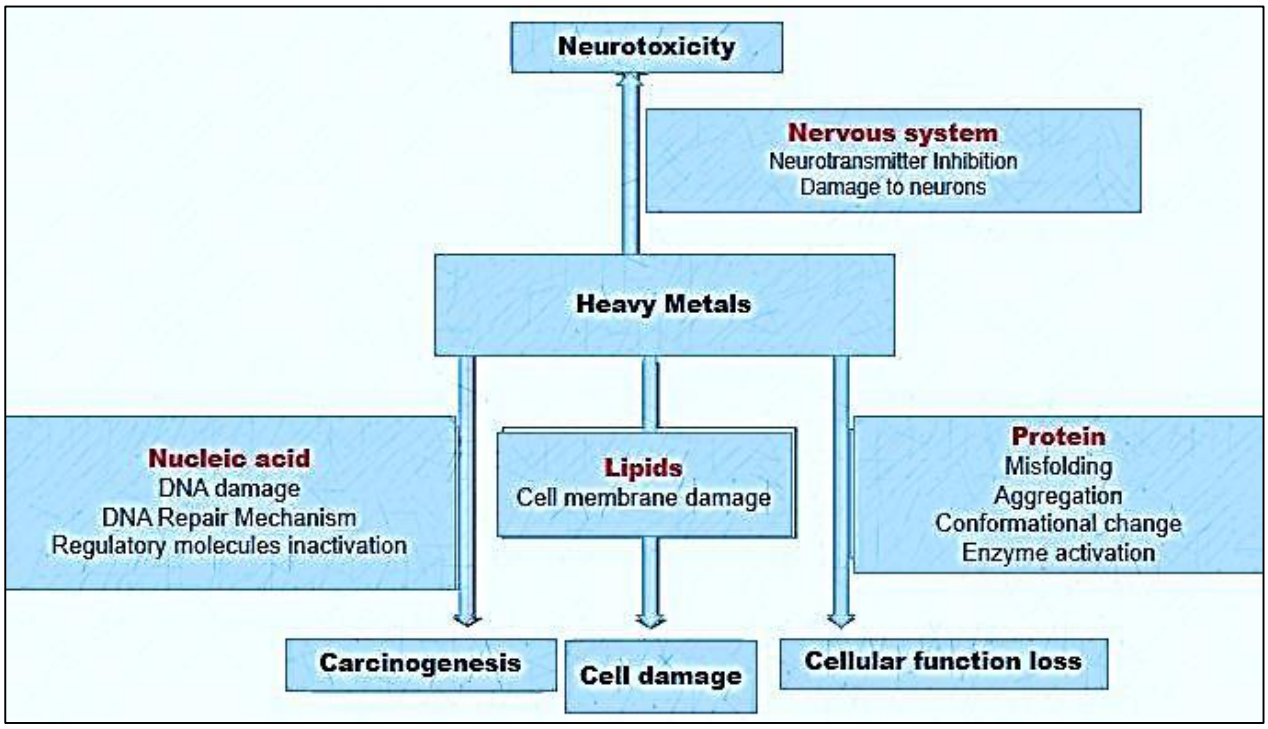


Figure 1: Effect of Heavy metals on the Health of Living Organisms

1.3 Arsenic as Heavy Metal

Arsenic is a chemical element with the atomic number 33 and the symbol As. It can be found in a variety of minerals, usually in association with Sulphur and other elements, but it can also be found as a pure elemental crystal. Arsenic is commonly found in high concentrations in the groundwater in numerous countries.(A. Sharma & Kumar, 2019). Arsenic's inorganic structure makes it particularly dangerous. The most serious threat from arsenic is contaminated water used for

drinking, food preparation, and irrigation of food crops. Arsenic exposure for longer time duration from drinking water and eating can result in cancerous development and skin damage. It has also been linked to diabetes and cardiovascular infection. In young adults, in utero and youth openness has a negative impact on intellectual development and broadened passage. The key goal in impacted networks is to prevent more arsenic exposure by establishing a protected water supply. (Majumdar & Guha Mazumder, 2012). Arsenic is a normally happening component on the planet's external layer, and it is generally circulated all through the environment, including water and land. Inorganic construction makes it very poisonous. Drinking debased water, utilizing messy water in food arranging and water system, mechanical cycling, eating tainted food, and smoking cigarettes are largely ways for individuals to build their inorganic arsenic levels. Arsenic is a universal part found in practically all regular frameworks at low focuses (Saxena, 2020). The trivalent arsenite and the pentavalent arsenate are the two most normal inorganic types of arsenic. The methylated metabolites monomethylarsonic destructive (MMA), dimethylarsinic destructive (DMA), and trimethylarsine oxide are the normal constructions. Arsenic harming of the climate happens because of regular peculiarities like volcanic launches and soil breaking down, just as anthropogenic exercises. A couple of arsenic-containing synthetic substances are accessible today, and they've been utilized to make bug showers, herbicides, fungicides, algicides, sheep plunges, wood added substances, and shading stuffs, in addition to other things. They've additionally been utilized in veterinary medication to treat tapeworm pervasions in sheep and cows. Arsenic compounds have additionally been utilized in the treatment of syphilis, yaws, amoebic looseness of the bowels, and trypanosomiasis in the clinical field for something like a century (Tyler et al., 2014). Arsenic-based medications are as yet used to treat specific tropical diseases, for example, African dozing infection and amoebic the runs, just as parasitic afflictions in creatures, like filariasis in canines and connected pore turkeys and chickens (Kulik-Kupka et al., 2016). The Food

and Medication Organization as of late supported arsenic trioxide as an anticancer expert in the therapy of intense promeylocytic leukemia (Emadi & Gore, 2010; Fang & Zhang, 2020). The enrollment of adjusted cell demise (apoptosis) in leukemia cells has been credited with its therapeutic action.

1.4 Arsenic Poisoning Worldwide

Arsenic tainting of groundwater is inescapable, and there are various places where arsenic pollution of drinking water is serious. Right now, it is assessed that somewhere around 140 million individuals in 50 nations have been drinking water with arsenic levels surpassing the WHO's between time cutoff of 10 g/L (Sun GX et al., 2008). Millions of individuals are at high hazard of raised arsenic openness, essentially through drinking water, just as by mechanical emanations Inorganic arsenic of topographical beginning is found in ground water utilized as savoring water a few pieces of the world High focus of arsenic in groundwater in the north-eastern territories of India has become a significant reason for concern (Tchounwou PB et al., 1999). Presence in ingestion water causes harmful furthermore, cancer-causing impacts on individuals. It is the first metalloid to be distinguished as a human cancer-causing agent and most instances of ongoing arsenicosis are related with constant admission of arsenic contaminated waterArsenic affects people of all ages and genders, although children and teenagers are more vulnerable to it. (Chowdhury et al., 2003). Intake of inorganic arsenic on a regular basis has a negative impact on multiple systems. Significant degrees of arsenic in drinking water or arsenic-sullied water are very hurtful to the liver and pancreas, as well as cardiovascular and renal diseases, skin infections and nerve tissue wounds, ongoing lung disease, cognitive confusion, and malignant growth of the skin, liver, lungs, kidneys, and urinary bladder. Arsenic defiled drinking water is additionally liable for unconstrained fetus removal, stillbirth and newborn child mortality(Kulik-Kupka et al., 2016; Rahman et al., 2009; A. Sharma & Kumar, 2019).

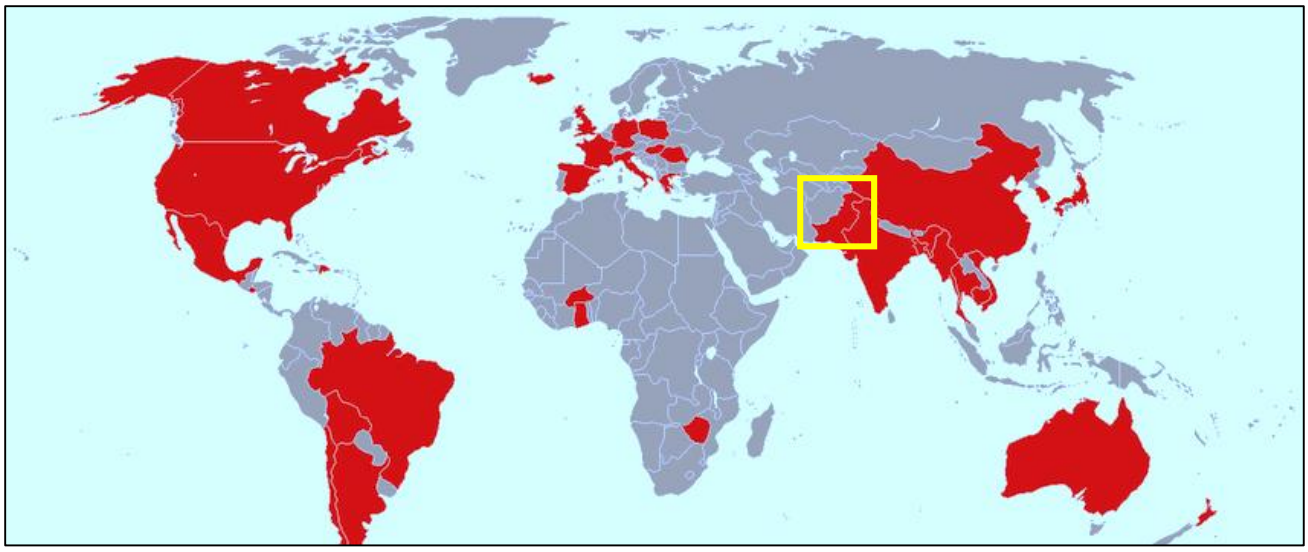


Figure 1.2: Modified image of Arsenic-affected countries (red) around the globe (Smedley et al., 2007).

1.4.1 Permissibility limit of Arsenic

Arsenic affects people of all ages and genders, although children and teenagers are more vulnerable to it. Intake of inorganic arsenic on a regular basis has a negative impact on multiple systems. Undeniable degrees of arsenic in drinking water or arsenic-sullied water are amazingly destructive to the liver and pancreas, as well as cardiovascular and renal diseases, skin infections and nerve tissue wounds, ongoing lung disease, cognitive confusion, and malignant growth of the skin, liver, lungs, kidneys, and urinary bladder (Kuivenhoven & Mason, 2019). Arsenic defiled drinking water is additionally liable for unconstrained fetus removal, stillbirth and newborn child mortality. Most agricultural nations have a standard of 50 g/l, which is several times higher than the MCL and poses a greater risk to the population. The most extreme permissible limit for arsenic in India is 0.05 ppm, whereas the WHO's limit is 0.01 ppm. However, in many arsenic-affected areas of India, arsenic fixation was higher than 0.2 ppm in well and underground water which is utilized as a wellspring of drinking water (Gundert-Remy U et al., 2015). Arsenic levels in savoring water West

Bengal (India) range from 60 to 3700 g/l, influencing around 40 million individuals. The grouping of arsenic in water has been diminished to 50-1354 mg/L, and the predominance of skin injuries has been decreased to 44.80%. It is basic to increase current standards in these nations.

1.5 Arsenic Poisoning in Pakistan

As of late, the assessment on groundwater As pollution country in Pakistan has revealed a significant issue of As defilement, particularly in the spaces associating the Indus Stream. The peril guide of As was made using 9882groundwater models from Pakistan, and it has shown that around 73% of the water tests contained As fixation [10lg/L and around 41% of the water tests have As substance [50lg/L. It was revealed that around 47 million occupants of Pakistan abiding in Sindh and Punjab territories are standing up to real As-spoiling of the springs (Shahid et al., 2018).

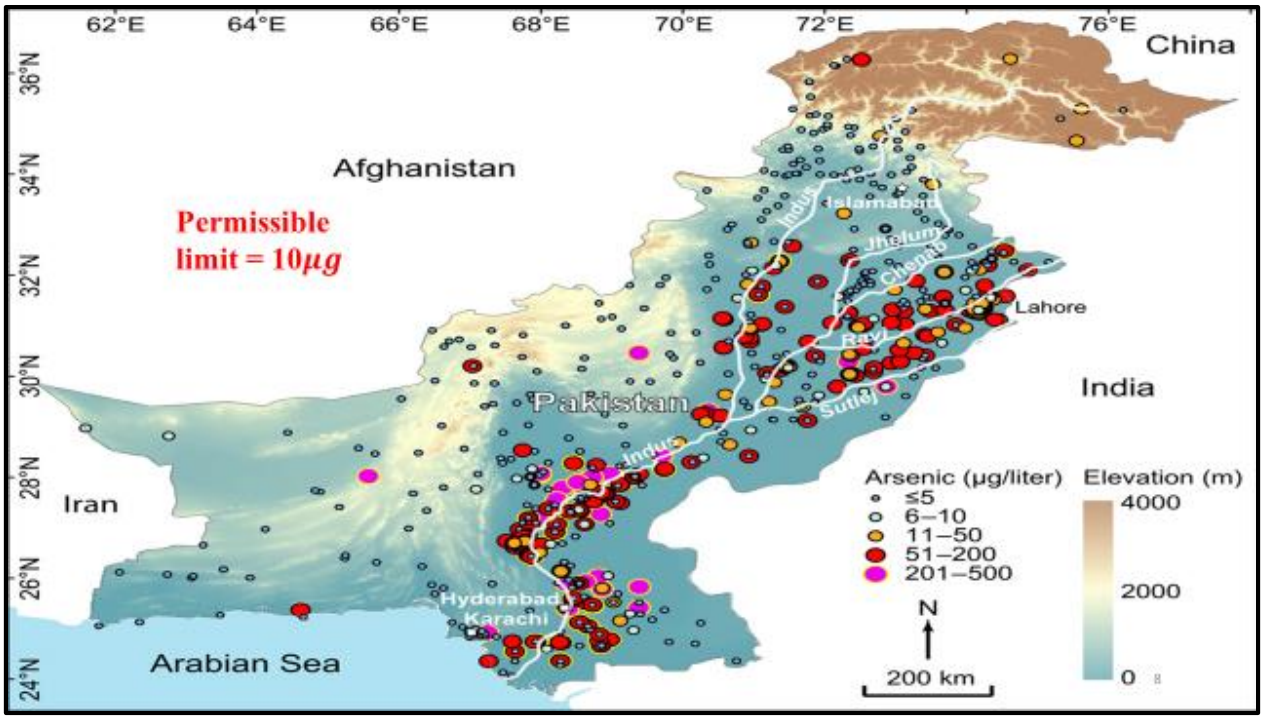


Figure 1.3: Modified image of Arsenic concentrations measured in Pakistan groundwater (Podgorski et al, 2017)

1.6 Cognitive Function

Cognition is the process of acquiring knowledge and comprehending it to perform various everyday life tasks. It forms the basis of our behaviors to achieve various goals through perception, learning, memory and thinking. Higher cognitive functions are executive function performed by brain comprising of thinking, problem solving, attention and decision making. Cognitive functions are modified throughout our life time depending on neuronal plasticity. (Bilotta et al., 2011)

Learning is a process of acquiring new knowledge, information or skill by experience. It can be defined as a process of assembling new information to make sense or alter the behavior accordingly. Learning can happen by paying attention or simply through interaction with new information. Memory is the usage of pre stored information to stride through a situation or achieve certain goal (Brickman., 2009). Memory can outlive the stimulus which triggered it. Long haul, present moment, and working memory are a wide range of recollections that can be delivered. The two kinds of long haul memory are explanatory memory and working (memory of raw numbers) 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). Working memory is limited capacity information storage while performing a task. Information is retained and manipulated to achieve the goal (Forcato et al., 2007).

1.7 Brain regions associated with 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 occurs. 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.

1.7.1 The Hippocampus

The hippocampal formation is in charge of transforming momentary memory to long haul 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 (Ergorul et al., 2004). Many neuroscientists believe that hippocampus is important in forming new memories as it helps in identification of new stimuli event, experience and places. 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. 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 (Bird et al., 2008).

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 (Burgess., 2008).

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 (Squire et al 2001).

1.7.2 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. PFC controls decision making, speech, language, social behavior and complex cognitive behavior. Basic function is the arrangement of thoughts according to person's will (Preston et al 2013).

Working memory, including all executive functions are controlled by PFC. Goldman-rakic determined this creates the representational knowledge which then helps in guiding actions, thoughts and emotions. 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. (Alan et al., 1992)

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 (Euston et al., 2012).

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. Amygdala is located as two almond structures in the brain. Its major role is in generating emotions, processing memory and making decision. 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 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 (Lara et al.,2015).

1.7.3 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 form retrieval of long term fear associated memories. 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 (McGaugh et al., 1992).

Amygdala not only creates fear conditioning but it also creates positive (Appetitive) conditioning through distinct nuclei. Various cores inside amygdala play distinctive part in deciphering appetitive memory. Amygdala also has role in generating reward system. It is influenced by dopamine, primary pheromones and secondarily attractive odorants. Another Important function of amygdala is in memory modulation (Cahill et al 2001). 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 (Bocchio et al., 2017).

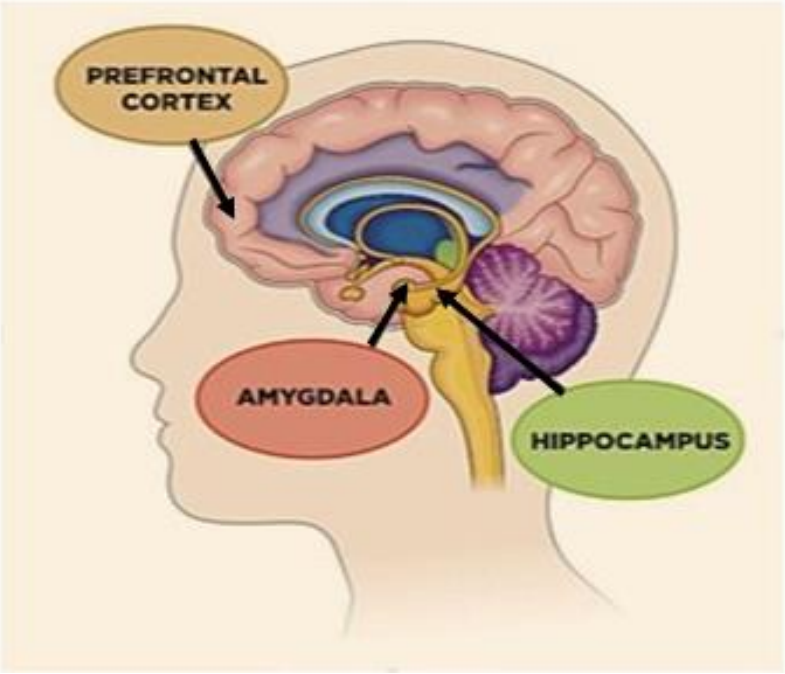


Figure 1.4: Brain regions under consideration in research

1.8 Effect of Arsenic on Cognitive function

One quarter out of top 20 health conditions causing disability are neurological. Around almost 1 million people around the word suffer from these kinds of disabilities. Moreover, the intellectual disability may have a global prevalence of 1% approximately. Developing countries have as twice mental disability rates as developed countries. The mental disability prevalence is influenced by the environmental factors including mother and child healthcare, immunization, and the level of

pollution in environment etc. Thus higher contamination levels of pollutants and poorer health quality contributes to higher prevalence rates (Neal et al., 2012). Arsenic (As) stands on the top among toxicants and based on suspected toxicity, it has potential risks for human health. Currently, arsenic concentration in water is permitted to10µg/L (10ppb). Still many individuals of the world are in danger of openness to harmful centralizations of arsenic in drinking water (in ppm range). Arsenic in all forms i.e. inorganic and methyl containing arsenicals accumulates in different brain portions. These high concentrations can cause delayed growth and deficits in neural tube development (Tyler et al., 2014). Many cases of neuropathies have been reported due to inhalation or ingestion of arsenic. Histological examination has revealed that demyelination of nerves and axonopathy are the main results of arsenic exposure. Significant degrees of As in drinking water have been connected to neurological infections, for example, chemical imbalance, consideration shortage problems, Alzheimer's sickness, dementia, and Parkinson's illness. As gains simple admittance to the CNS under typical physiological conditions and amasses in various mind areas prompting encephalopathy, hindrances of intellectual neurological capacities like learning, memory (both short-term and long-term), language, verbal comprehension, mental confusion, anxiety, irritability, headaches and loss of concentration on arsenic exposure at different concentrations (Rodrıguez et al., 2003).

Different examinations have shown that even at low dosages, arsenic leads to mental issues in youngsters. Arsenic levels in the water or in the urine are linked to poorer execution and results on insight tests, and verbal IQ is by far the most influenced intellectual capacity. These impacts endure into pre-adulthood, to such an extent the total arsenic admission might be a more serious danger factor than intense admission in psychological brokenness (Tolins et al., 2014). Higher centralizations of arsenic openness can adjust development and improvement in youngsters, prompting neurological deficiencies, and females appear to be at more serious danger than guys,

albeit not many investigations have methodically assessed sex contrasts. The comorbidity of various metals with arsenic transparency, the relationship between high arsenic receptiveness and poor monetary standing, and defenseless sustenance in kids presented to arsenic are all possibly muddling angles in these examinations (Tyler & Allan, 2014).

Hippocampus as examined before assumes a significant part in memory advancement that can be transient memory or long haul memory and openness to inorganic arsenic profoundly upsets this capacity of hippocampus displayed through ongoing investigations. Studies recommend that arsenic receptiveness upsets L-cys and L-glu transport in the hippocampus by the up-rule of xCT and EAAC1 and down-rule of GLT1. This altered L-cys and L-glu transport was identified with the negative rule of NR2B subunits and to prevented spatial memory (Ramos-Chávez et al., 2015).

Natural and environmental openness to substantial metals has been connected to neuropathology injury and mental challenges. Arsenic inhibits the growth of neural forebear cells as well as neuronal translocation and cell development. By reacting with sulfhydryl groups, arsenic has a delaying effect on wide protein digestion while also being highly toxic. As a result of this reasoning, arsenic toxicity reduces the level of free thiol in the framework, which is a significant cell reinforcement. Exactly when arsenic-related blends enter in our body, they go through a couple of metabolic pathways in the liver and convert in different kinds of arsenic, for instance, arsenite (As3+), arsenate (As5+), dimethylarsenite (DMA), and monomethylarsonate (MMA). By changing over in these constructions, arsenic streams inside the whole body through blood dispersal (Medda et al., 2020a). According to research, trivalent arsenic compounds (AsIII, MMAIII, and DMA III) cause diabetes. Arsenic digestion in humans occurs through the reduction of trivalent arsenic (As3+) and oxidative methylation to the pentavalent form (As5+). The ageing of trivalent arsenic through digestion or another cycle causes more pronounced poisonousness to

the cells and exhibits more cancer-causing qualities. Along these lines, arsenic exploitation and distinct stages of its digestion may result in diverse arsenic metabolic consequences(Medda et al., 2020b; Zigmond et al., 2014). The abilities of arsenic to tie with decreased thiol in some protein create designated protein poisonousness.

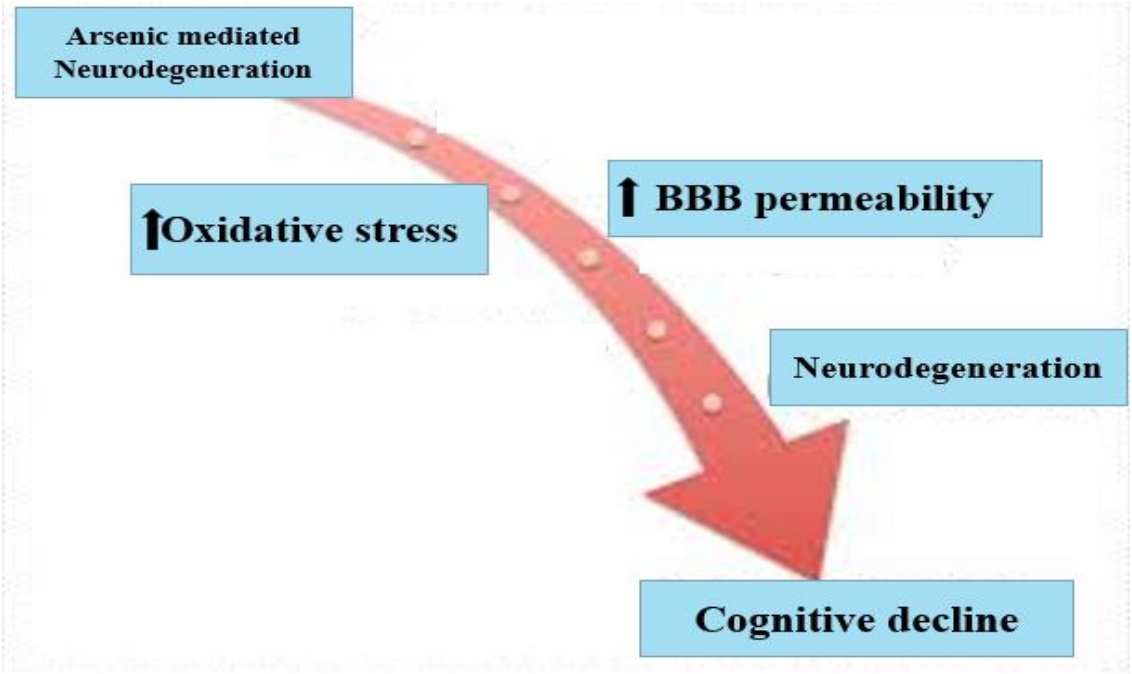


Figure 1.5: Scheme of Arsenic mediated cognition loss

1.9 Arsenic neurotoxicity models

Table- 1 Experimented arsenic neurotoxicity study models

S.No.	Published paper	Year of publication	Reference dose data	Reference
1.	**Arsenic down-regulates the expression of Camk4, an important gene related to cerebellar LTD in mice**	2009	Five groups. G-1= control. G-2 = 1 ppm G-3= 4 ppm As_2O_3, . G-4, G-5 customary both of 4 ppm As_2O_3 for 60 days Total exposure= 240mg	Wang, et al 2009
2.	**Developmental Arsenic Exposure on the Social Behavior and Related Gene Expression in C3H Adult Male Mice**	2019	The subject mice were exposed to sodium arsenite ($NaAsO_2$, 85 mg/L in the ingestion water from gestational day (GD) 8 to 18 Total exposure= 22mg/kg for 10days	Htway, et al 2019
3.	**Perinatal Exposure to Arsenic in Drinking Water Alters Glutamatergic Neurotransmission in the Striatum of C57BL/6 Mice.**	2019	Female virgin C57BL/6 mice were randomly divided into three groups: water treatment (control), 10-mg/L as treatment, 100-mg/L As treatment. Total Exposure= 1050mg/kg for 42 days	Sung, et al 2019
4.	**Effect of chronic arsenic exposure on mouse brain tissue and serum metabolomics.**	2016	12, 3-week-old male C57BL/6J mice arsenite (50 mg/L) via drinking water for 12 weeks. Total exposure=1050mg/kg for 84days	Dai, et al 2016

CHAPTER 2

MATERIALS

AND

METHODS

Material and Methods

The next section will discuss the scheme of study, materials required and methodology which was used to conduct the study.

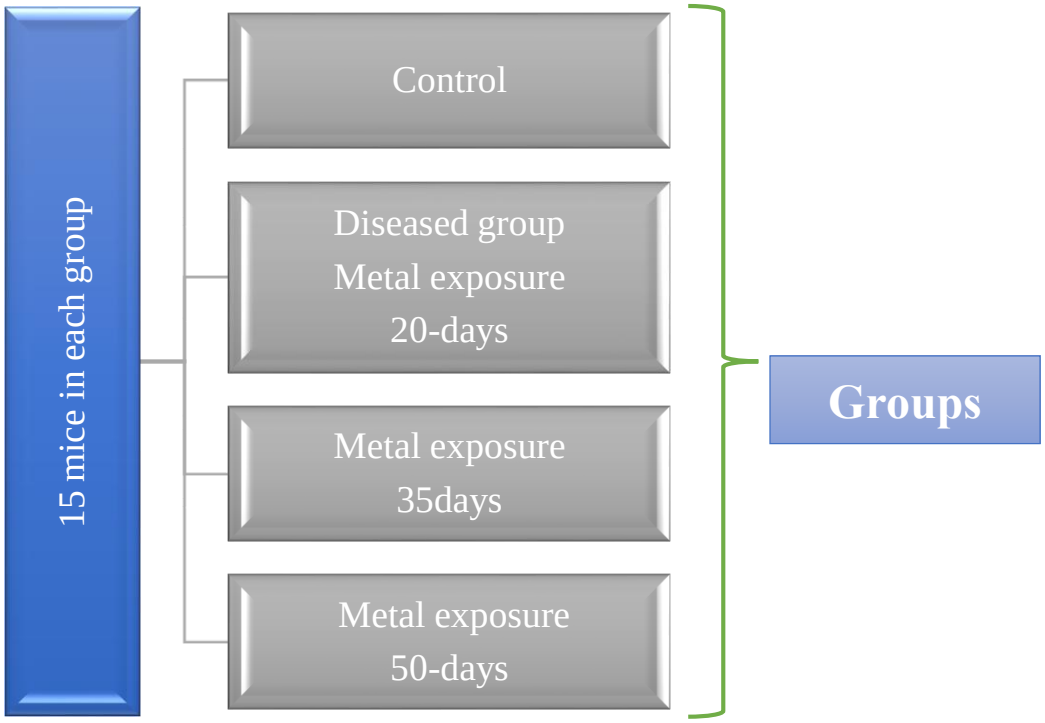


Figure 2(a): General study plan defining the category of groups of animals

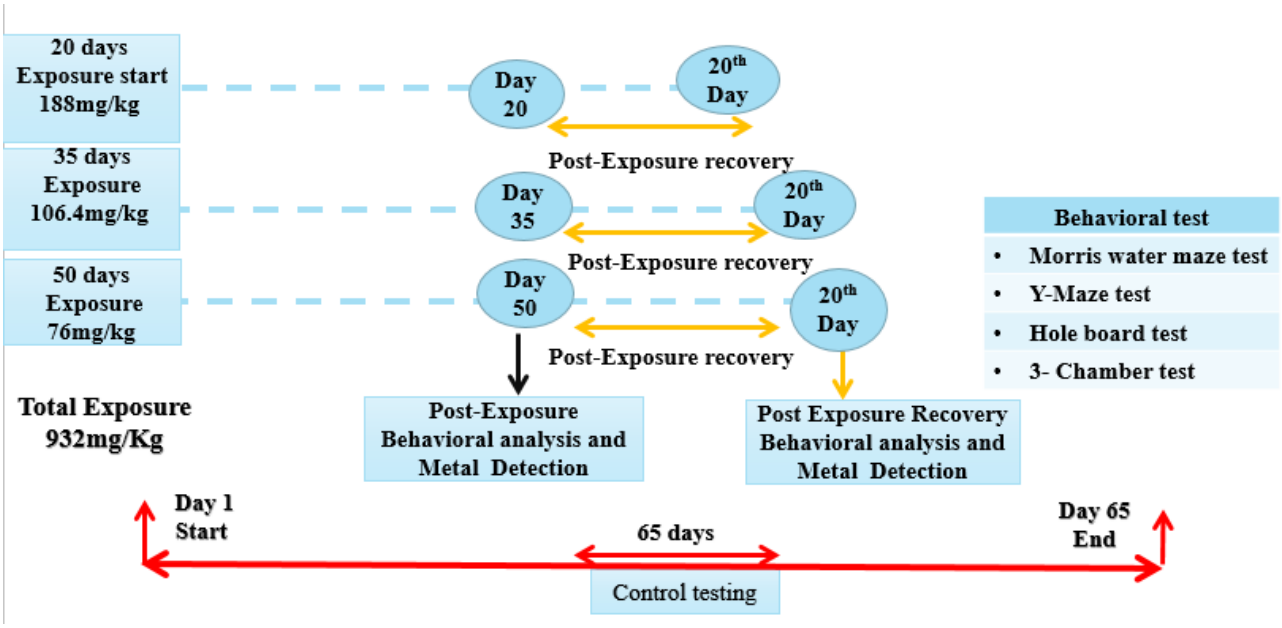


Figure 2(b): Experimental design flow chart explaining the step wise experimentation followed during the course of research tenure.

2. Ethical Approval and policy

All protocols were examined and approved by the ASAB, NUST IRB (Internal Review Board). The mice were maintained under controlled settings in ASAB's Animal House Laboratory. All experiments were conducted in accordance with the findings of the Laboratory Animal Research Institute, Earth and Life Sciences Division, National Institute of Health, USA. (Guide for the Care and Use of Laboratory Animals: Eighth Edition, 2011).

2.1 Animals

All tests and procedures were carried out in accord with the requirements of the National Institute of Health's Institute of Laboratory Animal Research, Division of Earth and Life Sciences. The animal was kept in a regular habitat at the Atta Ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST) laboratory animal house. The Institution Review Board of NUST's Atta Ur Rahman School of Applied Biosciences (IRB # 135) authorized all of the study's testing methodologies and procedures. Male Balb/c mice were utilized in the review, with 40 given by Laboratory Animal House, ASAB, NUST, and 50 acquired from the National Institute of Health (NIH), Islamabad. The animals were housed in 14 plastic cages (40cm25cm15cm) under regular conditions. In each cage, 5 animals stood retained with soft wood shavings as bedding. Housing conditions be situated at 22°C and a 12-hour light/dark cycle, with conventional feed and water supplies.

2.2 Chemicals

Arsenic Salt Aldrich. Solutions were made using standard distilled water.

2.3 Arsenic exposure and study design

We studied temporal effect of Arsenic on learning and memory in various time dependent group with same total exposure to Arsenic. Control group was sustained at normal water. 20-day group was provided with Arsenic salt solution in refined water at a portion of 188 (mg/day/liter). 35-day group was given Arsenic salt in distilled water at a measured quantity of 106.5 (mg/day/liter). And 50-day group was sustained at Arsenic salt in refined water at a portion of 76 (mg/day/liter). Thus, total dose for all the exposure groups were 932 (mg/kg). All the groups were given standard feed. All the exposure groups were given Arsenic 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 groups animals were given normal water and feed.

Table 2: 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

2.3.1. Morris water maze test (MWM Test)

Morris water labyrinth test strategy was adjusted from Iqbal et al., 2019 and was utilized to analyze hippocampal subordinate spatial learning and memory utilizing spatial signals introduced to the creature in its environmental factors. Morris' 1984 test convention, with minor changes, was used to quantify spatial learning dependent on the time it took the creature to arrive at a lowered covered stage in rehashed preliminaries. Reference memory is investigated by creature propensity toward stage region when the stage is taken out (Vorhees & Williams, 2006). Morris water maze test consist of a circular tank with a platform placed in one of the and is camouflaged due to the opacity of water.

Training period comprised of 5 days in which 5 trials are conducted each day. Animal were dropped in the tank from different directions in each trial Table 2. Each trial is of 90 seconds in which animal is allowed to find the platform by observing cues. 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. After the successful completion of training period probe trial was conducted with platform 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.

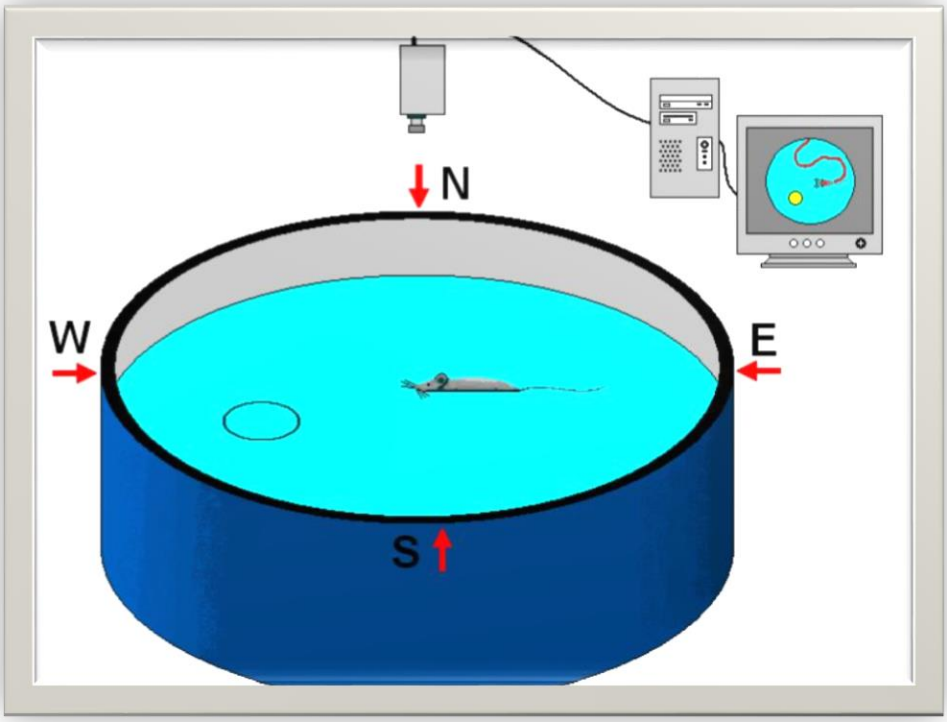


Figure 2.1: Pictorial Diagram of Morris Water test for spatial Memory

Table 3: Training sessions for Morris water maze test.

No. Of Days	Release direction				
	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				

2.3.2 Y-Maze Test

Y muddle test is utilized to examine the presentation of working memory just as acknowledgment memory. The test protocol was adopted from krateur et al., 2019 with minor modification to assess spatial memory. This test depends on rat normal interest to investigate their environment. Spontaneous alternations and exploration of novel arm instead of visiting the already explored arm is the basis of this test. 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 and ends of these three arms are labeled with different white and black pattern to provide spatial cues.

During the habituation stage, the mouse enters the labyrinth from the "Start arm" and faces away from the center. The mouse is then given 15 minutes to investigate the two arms. During the probe trial, the "new arm" is opened, allowing the mouse to freely explore all three arms for 5 minutes. A 30-minute inter-trial period was provided between the habituation and probing trials. A camera mounted above the maze captures the probing trial. Before each experiment, the maze was properly cleaned and wiped down with 70% ethanol.

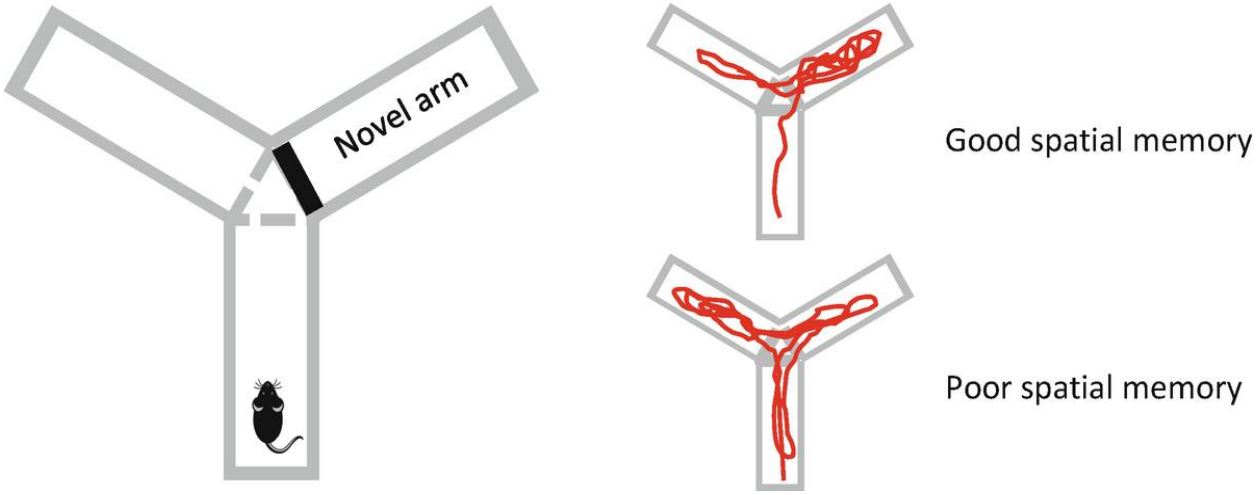


Figure 2.2: Diagram of Y- maze test sensitive to spatial learning evaluation

2.3.3 Social Preference and Novelty Test

This test is employed to assess animal general social interaction and preference for novelty object as pronounced earlier by farhat et al., 2017 with slight amendments. Three chamber analyze is made up of glass rectangular box with three compartments separated by a glass wall with a door like hole in them so that animal can freely move between three chambers. 2 metal wire confines were set in left and right chamber named S1 and S2 in which unfamiliar mice were placed. In habituation period empty cages were placed in chamber. Animal was dropped in the focal chamber and was permitted to investigate unreservedly for 5 minutes. In session 1 wire cage was provided with a stranger mouse (S1) while other wire cage was left empty. Animal was allowed to move and interact freely for ten minutes. Session 2 was 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. Apparatus was cleaned thoroughly with 70% ethanol before start and end of each session

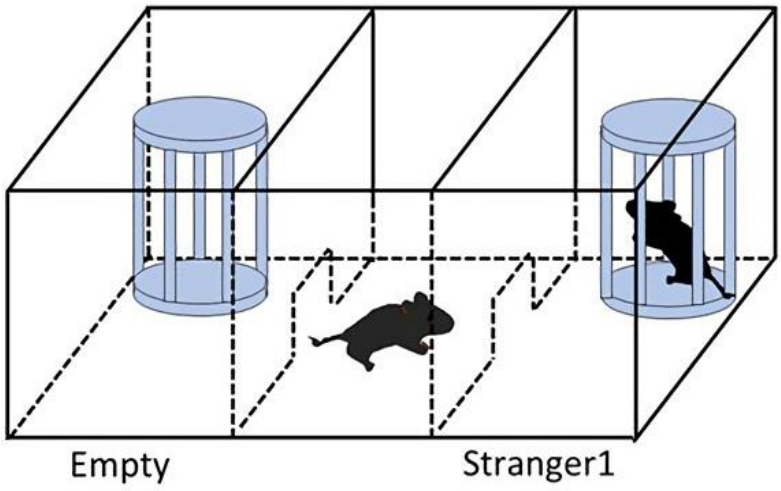


Figure 2.3: Pictorial View of Sociability analysis important for the evaluation of the cognition on basis of social interaction

2.3.4 Hole Board Test

Modified form of hole board test used by Li et al (2009). 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 were considered as reference memory errors (Li et al., 2009). Hole board apparatus is a square box containing 16 holes in it and different spatial cues pasted on the walls. Animals were deprived of feed 24 hours before the start of habituation period in which all hole was 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. Trial sessions were 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 in which same 4 holes were kept baited with 300gm of feed.

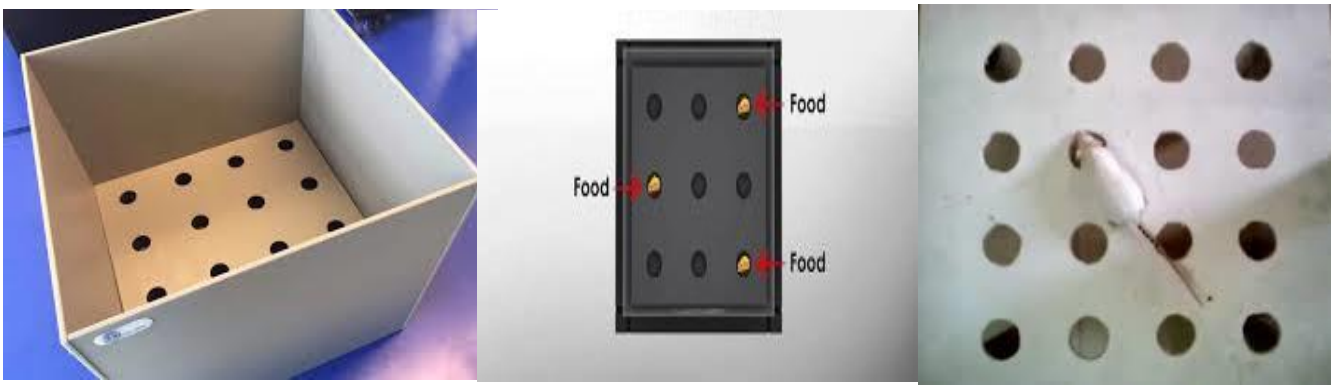


Figure 2.4: Hole board analysis diagram that used to survey spatial learning and memory.

CHAPTER 3
RESULTS

RESULTS

3.1 Morris Water Maze Test

Morris water maze test was used to assess the impact of temporal exposure of Arsenic (932mg/kg) on the long term memory and learning. Memory deficit caused by Ar in various temporal groups was analyzed through escape latency parameter. On first day all the three exposure groups have shown significant deficit in spatial learning as compared to control (58.94±3.43). Highest deficit was seen in 50-day exposure group (77.30 ± 4.495) and 20-day exposure group (76.35 ± 4.89). Control group (58.49 ± 3.43), 20-day exposure (76.35 ± 4.89) and 35-day exposure group (67.70±4.40) have shown almost similar learning behavior through all the next 4 days of training period. However, 50-day exposure group 20-day exposure group has shown decreased learning as compared to control group and other two exposure groups. Recovery groups have shown significant learning as compared to exposure groups. 50-day recovery group (40.90± 7.18) has shown least learning as compared to control (10.84 ±3.2), 20-day recovery (25.20 ±10.25) and 35-day recovery group (21.60 ±7.82). Comparison between exposure groups and recovery groups depict enhanced learning and memory after recovery period in all the recovery groups. Over All poor spatial learning behavior was observed in 50-day exposure group and 20-day exposure group which did not improved much after recovery period (Figure 3.1: A).

(A)

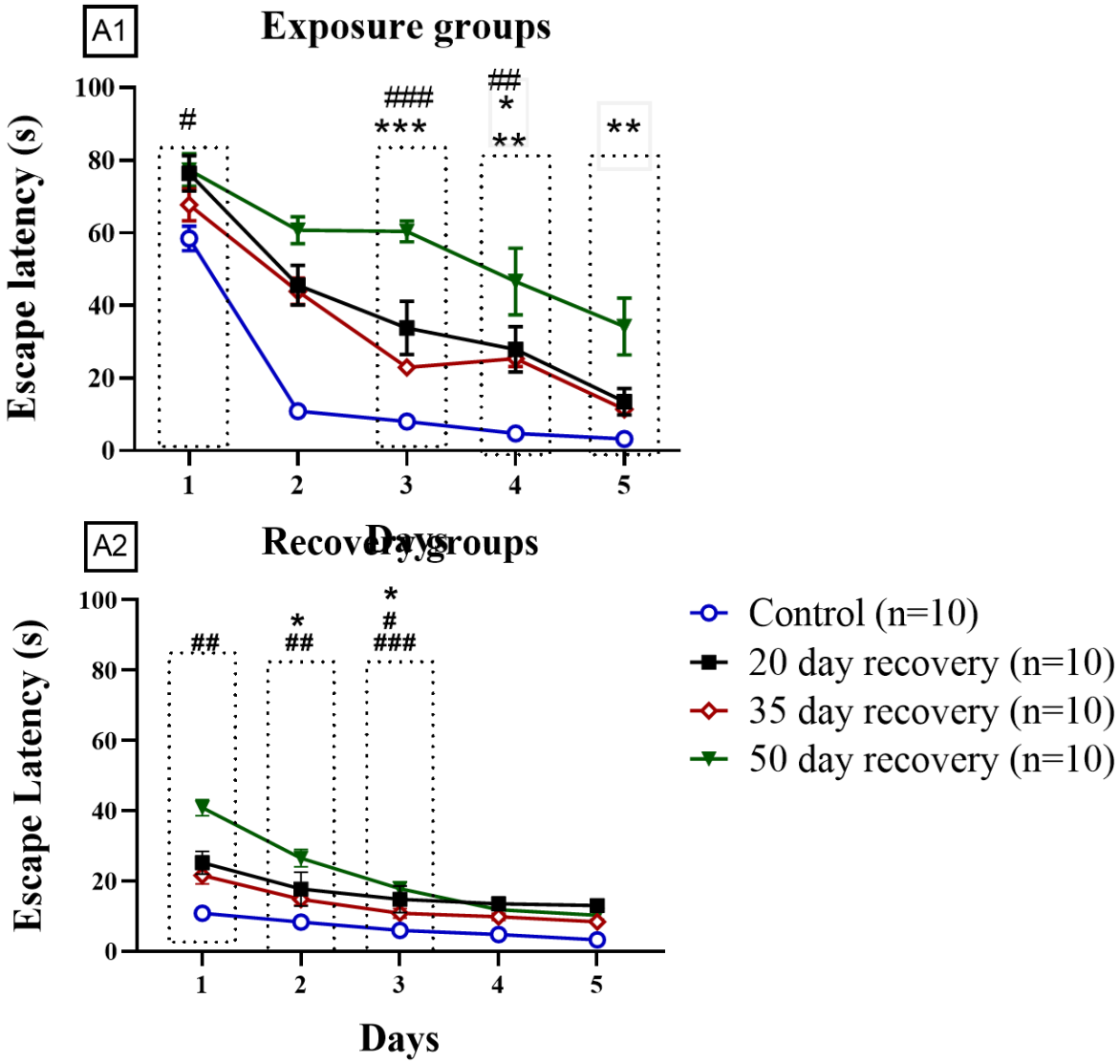


Figure 3.1 A: Effect of Arsenic on learning and memory in Morris water maze test; Escape latency. Graph shows escape latency (s) to assess the reference memory and learning among the control, 20-day exposure, 35-day exposure, 50-day exposure and their respective recovery groups. # =p<0.05 is significance among control and As treated groups, * = p< 0.01, are significance among As treated groups. Error bars are represented as mean± SEM.

After a 5-day training period, the reference memory stage was taken out and an examining preliminary was done. The data was evaluated to see if there was a distinction in the quantity of entries in the target quadrant among sets.20-day exposure group (7.20 ± 0.84) and 50-day exposure group (7.1±0.86) showed least number of entries as compared to control (9.2 ± 0.33) and 35-day exposure group (7.8 ±0.74). After recovery period no significant improvement in memory was observed in any recovery group in comparison with respective exposure groups (Figure 3.1 B)**.** The number of platform crossings was recorded and analyzed in probe trial. Within the exposure groups, 20-day exposure group (1.5 ± 0.28) and 50-day exposure group (1.7 ±0.30) showed highest deficit in memory as compared to control group (9.2 ±0.59). After recovery time period improvement in performance in the 20-day exposure group (4.2±0.38), this was seen and 35-day recovery group from exposure (1.9±0.34) to recovery testing (5.5 ±0.31) presented highest improvement in memory as compared to its respective 50-day exposure group showing more deficit in performance (1.4 ±0.34) (Figure 3.1 C). Time spent in target quadrant (TQ) was scrutinized to calculate differential deficit between All the groups. 20-day exposure group (32.60 ± 1.92) and 50-day exposure group (38.9±3.49) spent least time spent in the target quadrant as a percentage of period disbursed by the control (64.20 ± 2.64). After recovery period all the three recovery groups spent more time in target quadrant. 35-day recovery group (47.7 ± 3.33) spent highest time in TQ as compared to 15-day recovery group (39.9±3.9) and50-day recovery group (43.10 ± 4.23). 35-day recovery group (47.7 ± 3.33) presented enhanced referential memory in contrast with its respective 35-day exposure group (44.3 ±2.42) (Figure 3.1)

(B)

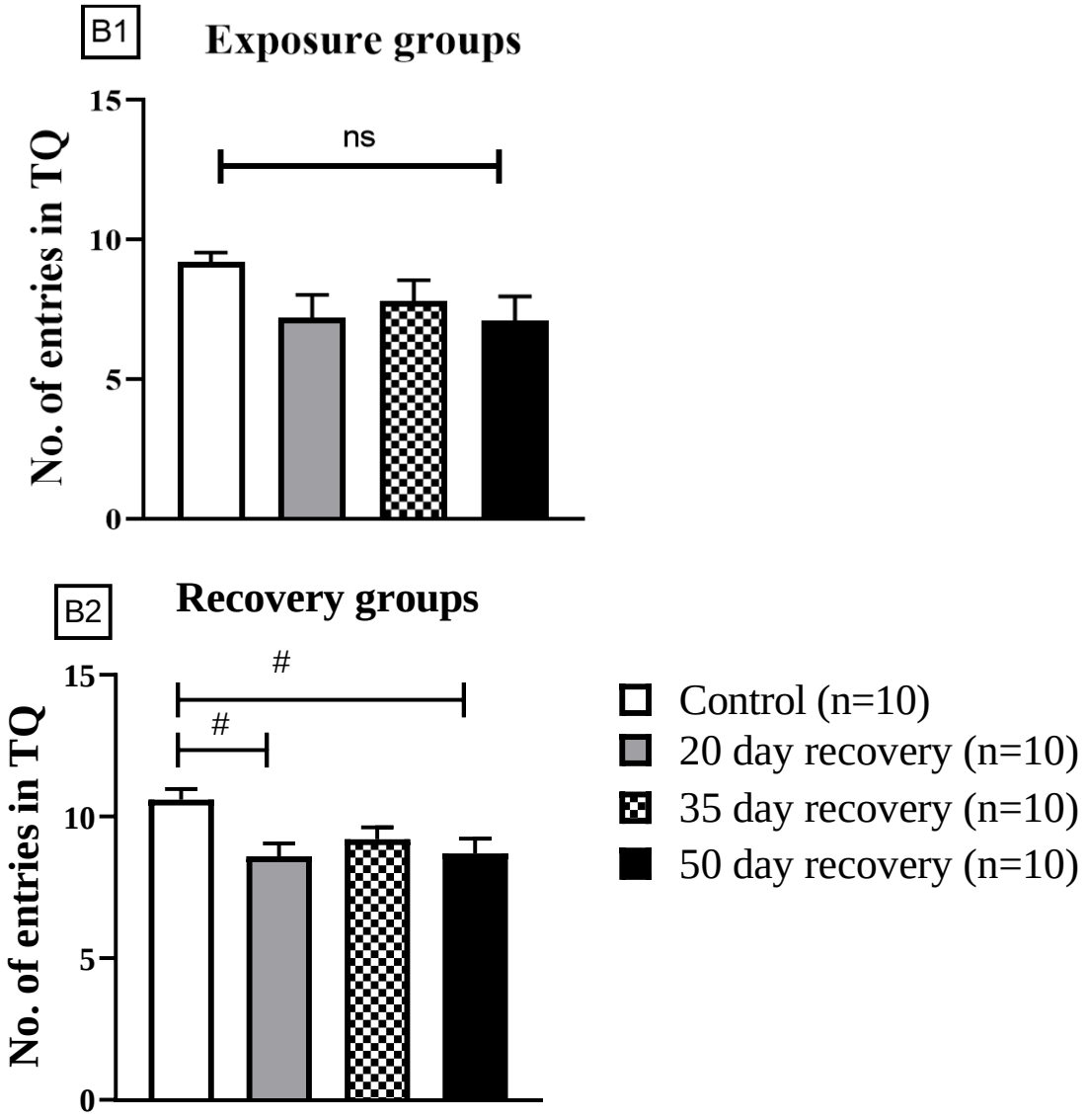


Figure 3.1 B: Morris Water Maze; Number of entries in target quadrant. Graph shows the number of platform crossings by all groups. # is used for noteworthy variance between control and As treated groups. * is used for significance among As treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with **** = p< 0.0001 as significance value. s = seconds

(C)

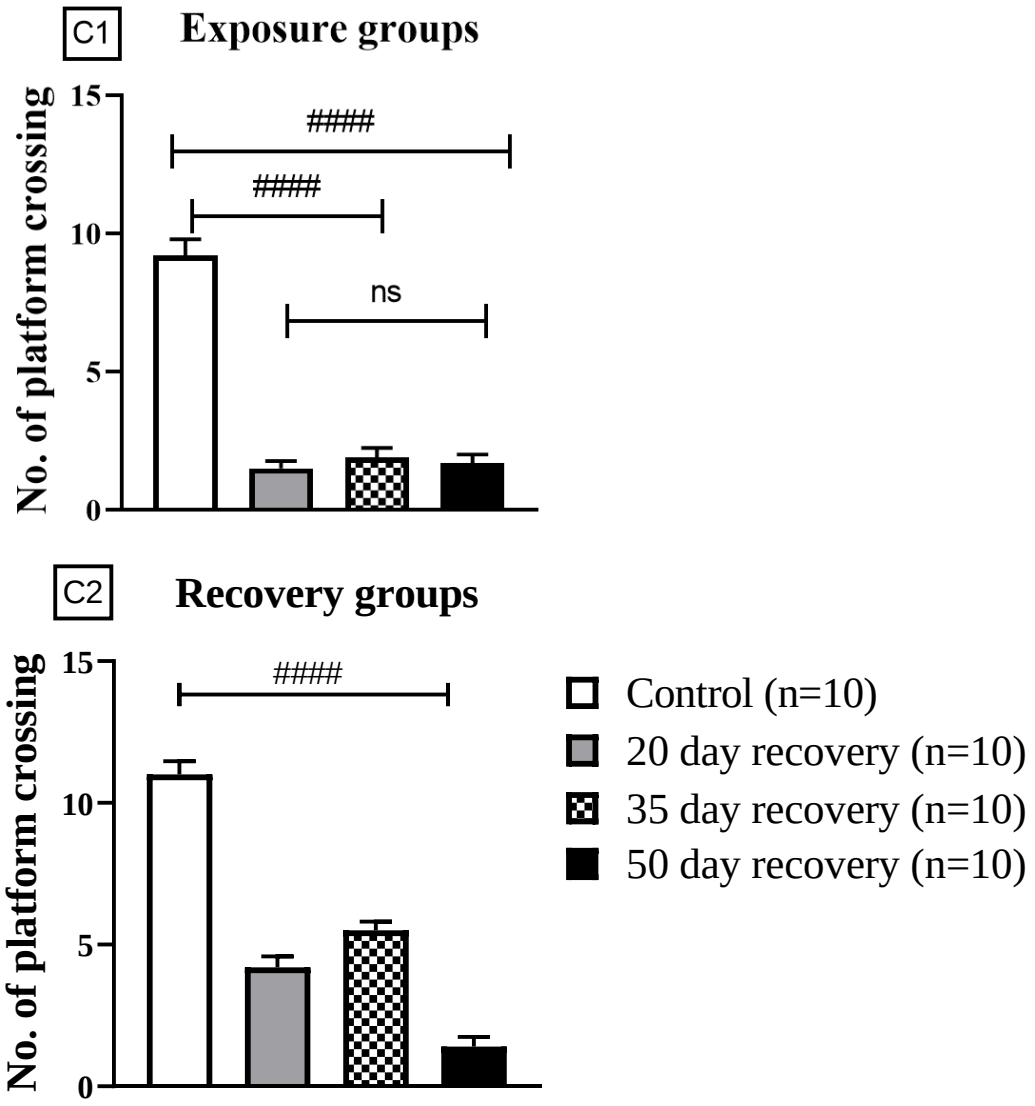


Figure 3.1 C: Probe Trial of Morris water maze; platform crossings. It shows the number of platform crossings by all groups. # is used for significant difference between control and As treated groups. * is used for significance among As treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with ####=p<0.0001 is the significance values. s = seconds.

(D)

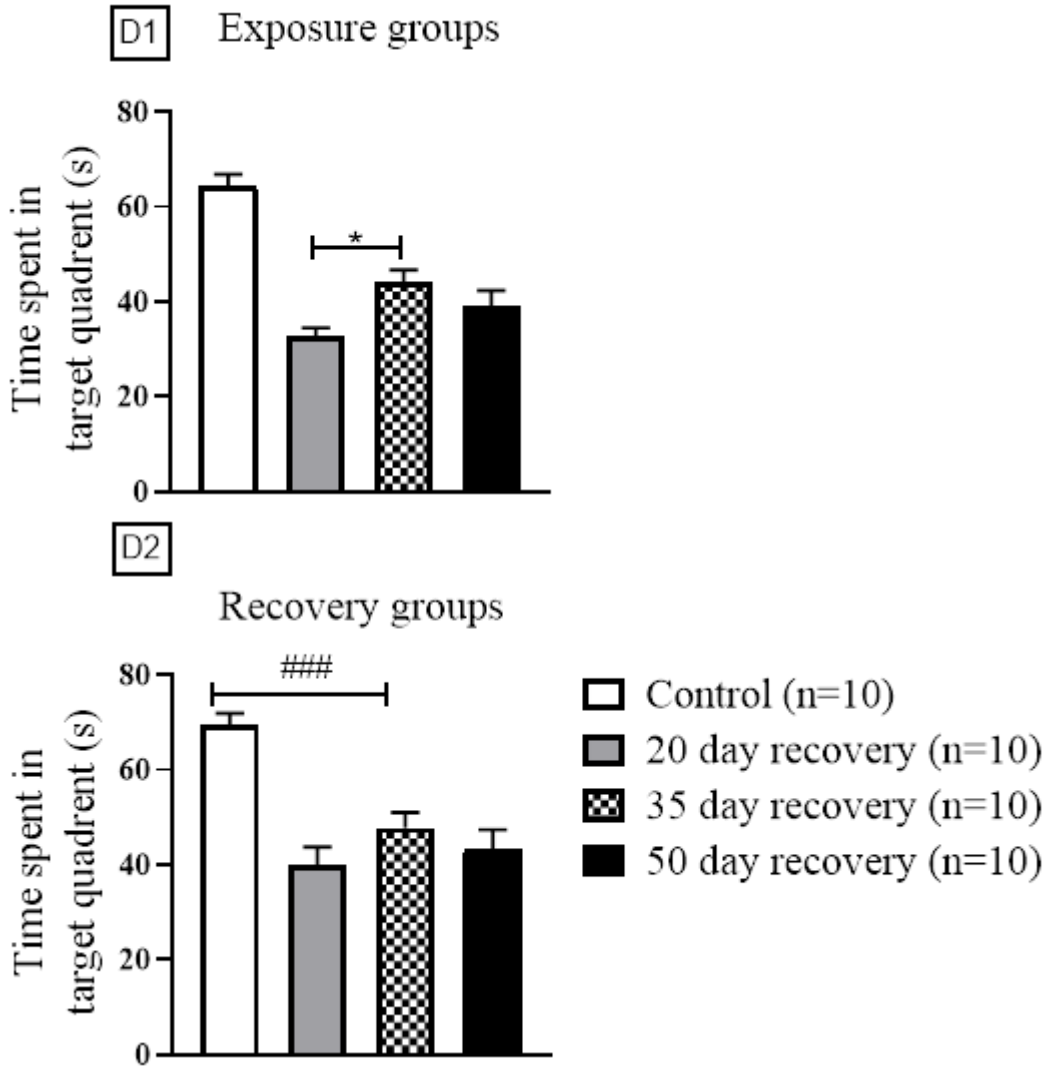


Figure 3.1 D: Morris Water Maze; Time spent in target quadrant. Graph shows time spent in target quadrant by all groups. # is used for significant difference between control and As treated groups. *isused for significance among As treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with ### =p<0.005, are the significance values. s = seconds.

(E)

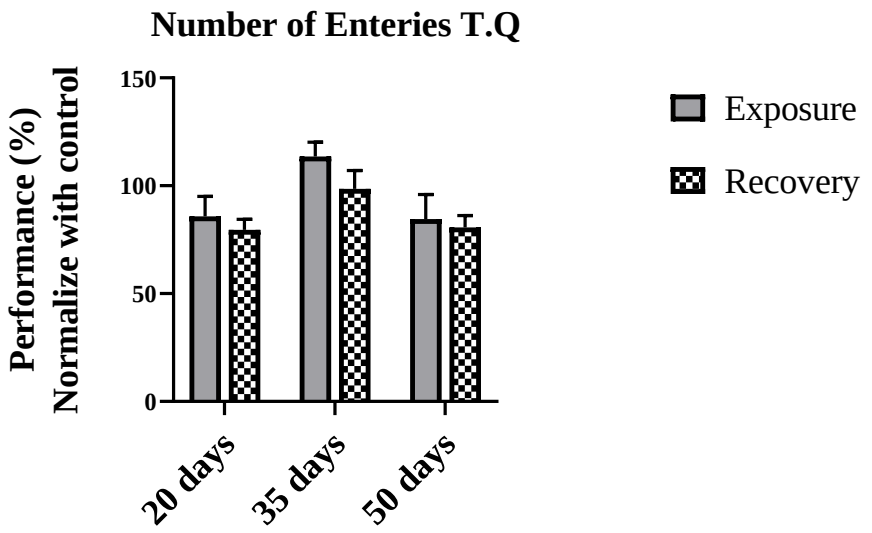


Figure 3.1 (E) Number of entries in Target quadrant normalize with control.

(F)

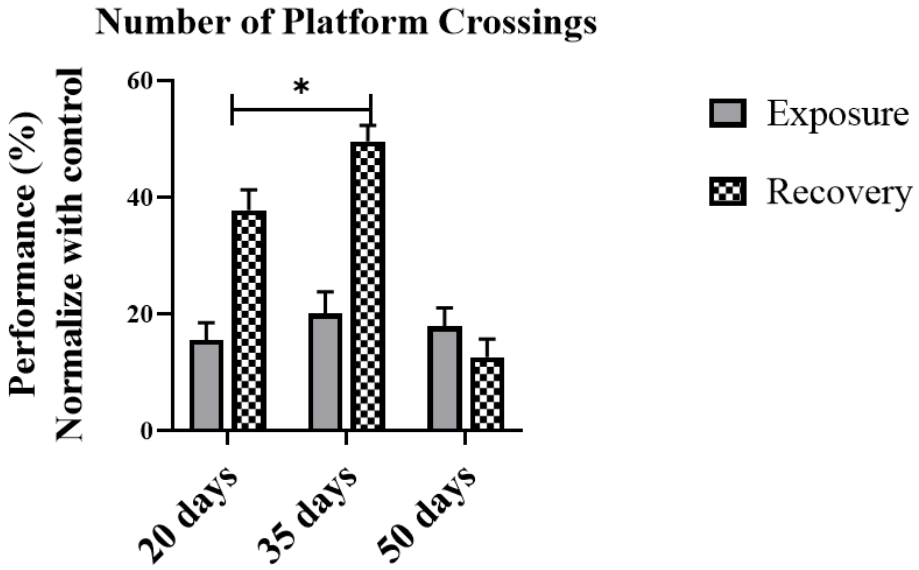
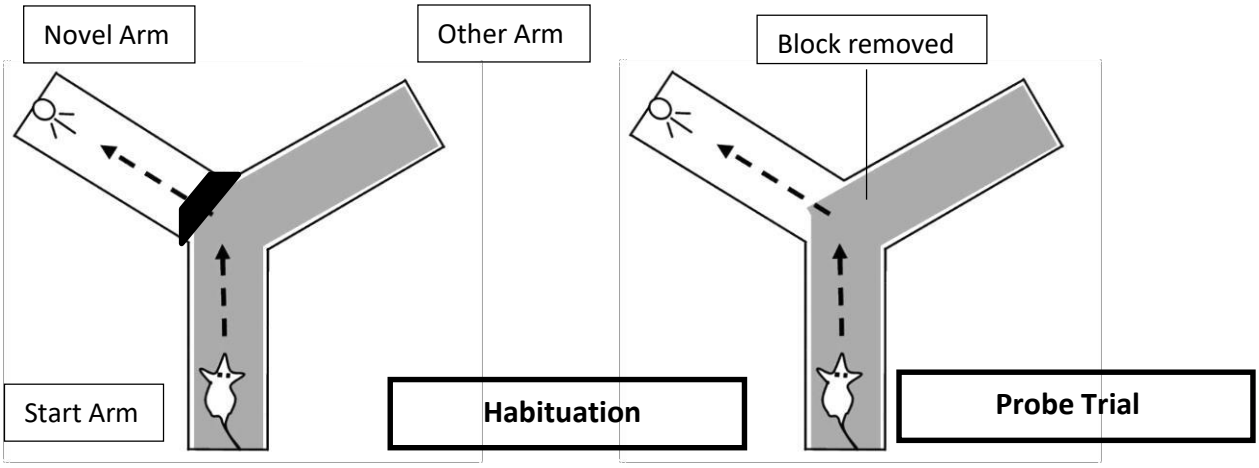


Figure 3.1 (F) Number of Platform Crossings normalize with control. # is used for significant difference between control and As treated groups. * is used for significance among As treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with *** = p< 0.001 as significance value. s = seconds.

3.2 Y Maze Test (Spontaneous Alternations Test)

Y maze test was employed to assess natural exploratory behavior of mice and to evaluate short term spatial learning memory. Animal's hippocampus dependent reference memory was also inspected. 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 (8.66 ± 0.42) as compared to control group (12.90 ± 0.36). After recovery period 20-day recovery group (9.3±0.92) showed increased number of entries in Novel arm whilst 35-day recovery group (7.00 ± 0.189) and 50-day recovery group (1.9±0.38) presented decline in spatial memory as compared to its respective 35-day exposure group (8.3 ±1.38) and 20-day exposure group (Figure 3.2 A). The trend observed while assessing time spent in Novel arm exhibited extreme decline in 50-day exposure group (123.80 ±6.76) presented least preference to novel arm as compared to control group (177.30 ± 4.26), 20-day exposure group (165.6 ± 13.9) and 35-day exposure group (135.50 ±12.52). After recovery period enhancement in spatial memory was observed in recovery groups 20-day recovery group (158.6 ± 18.24) and 35-day recovery group (168.1 ±13.49) 50-day recovery group (144.3 ±1 0 . 0 9) as compared to exposure groups. (Figure 3.2 B)



(A)

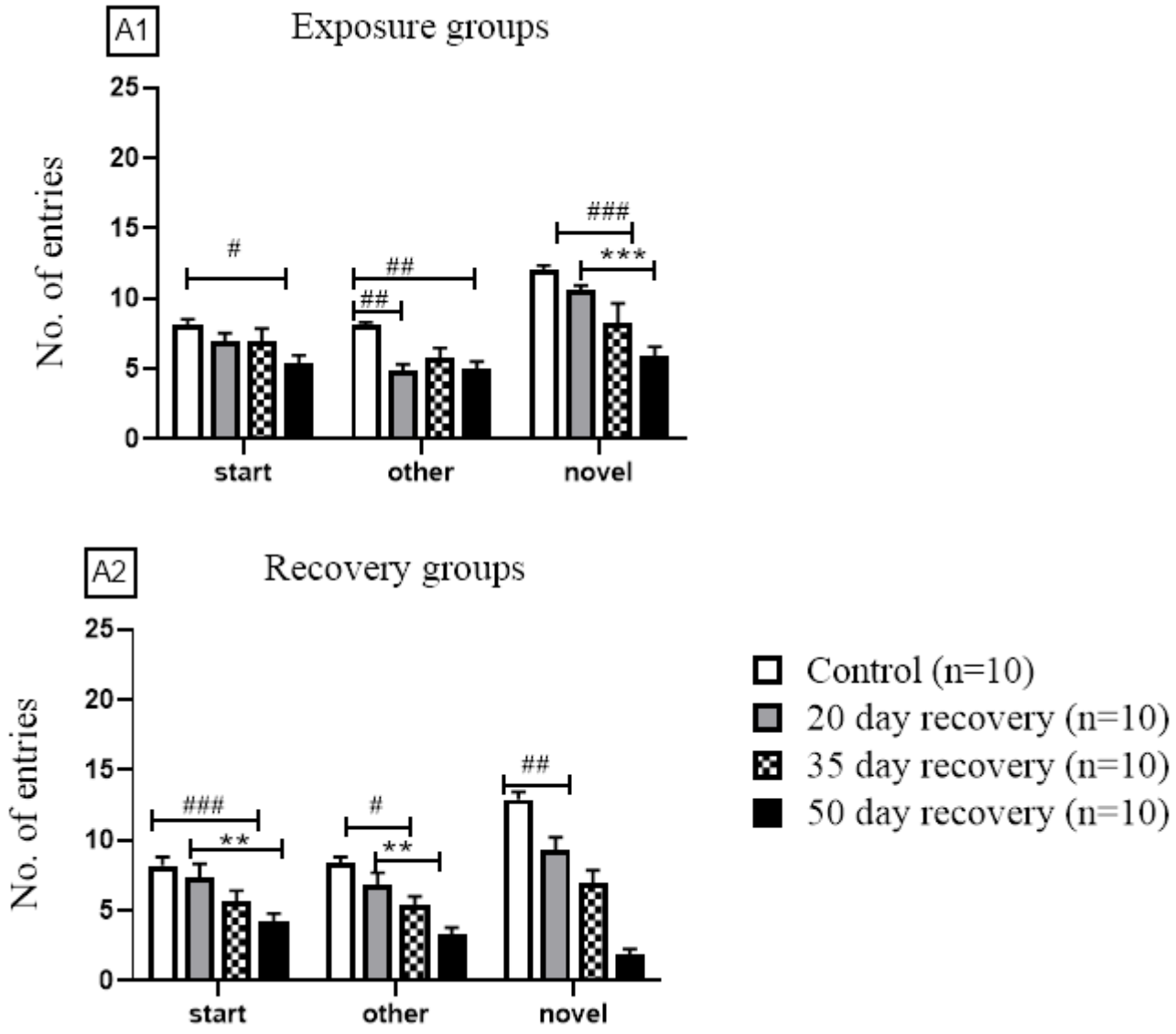


Figure 3.2 A: Performance of animals in Y-Maze test. The bar charts depict the number of entries in each arm, by control, 20-day exposure, 35-day exposure, 50-day exposure, 20-day recovery, 35-day recovery and 50-day recovery groups. # is used for significant difference between control and As treated groups. *is used for significance among As treated groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with ** = $p< 0.01$, # = $p< 0.05$,## = $p< 0.01$, ### = $p< 0.001$ are the significance values. s = seconds.

(B)

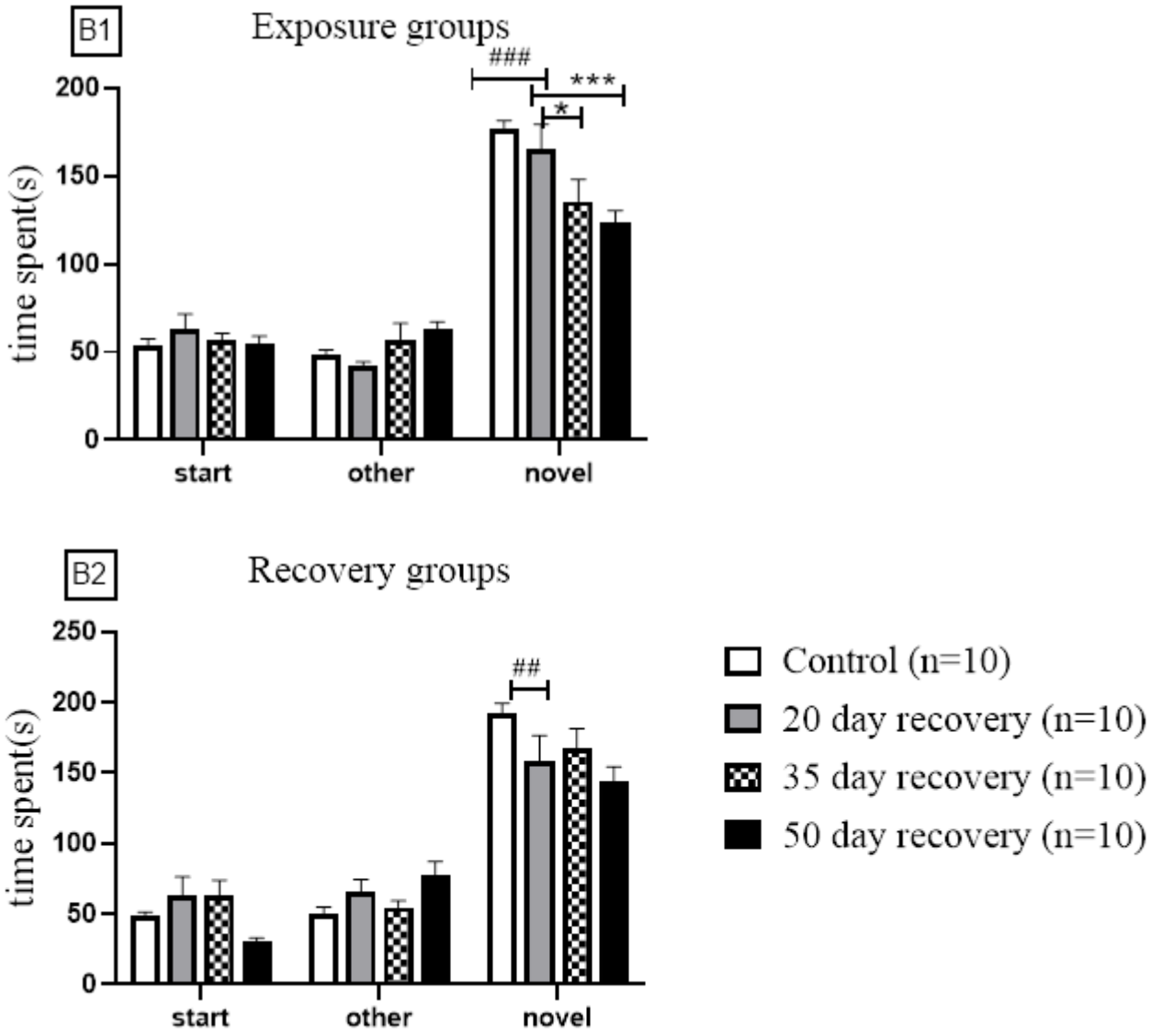


Figure 3.2.B: Time spent in each arm by animals in Y-Maze test. The graphs show the time spent in each arm (s) by control, 20-day exposure, 35-day exposure and their respective recovery groups. #is used for significant difference between control and As treated groups. *is used for significance among As treated groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison tests with ## = $p< 0.01$, are the significance values. s = second

Spontaneous Alternations performance and Alternate Arm Repeats (%) were calculated to assess impairment in spatial memory. Spontaneous Alternations performance showed memory deficit in Arsenic treated groups. Highest impairment was shown by 50-day exposure group (56.27 ± 4.46) and 35-day exposure group (40.62 ±5.05) as compared to Control group (67.52 ± 1.41) and 20-day exposure group (37.89 ± 4.27). After recovery time All the groups showed minor improvement in spatial memory except 50-day recovery group (50.37 ± 3.81) which showed decreased spontaneous Alternations than 35-day recovery group (51.78 ± 3.82), 20-day recovery group (43.07±5.02) and Control group (69.30 ±1.45) (Figure 3.2 C). Short term memory impairment was observed by calculating Alternate arm repeats (AAR) and same arm repeats (SAR). 20-day exposure group (38.06±3.33), 35-day exposure group (41.11 ± 2.45) and 50-day exposure group (38.96 ± 2.63) showed higher arm repeats thus greater memory impairment as compared to control group (24.27 ±1.30). Highest deficit was seen in 35-day exposure group (36.74 ±4.60) as compared to control group (24.27 ± 1.30). After completion of recovery period all groups showed improvement 50-day exposure group (32.28 ±1.79), 35-day recovery group (37.02 ±2.87), 20-day recovery group (32.59 ±2.82), Highest improvement in spatial memory was seen in 20-day recovery group (32.59 ±2.82) as comparedto 20-day exposure group (38.06±3.33) (Figure 3.8: D). Control group (0.00 ± 0.00) showed no same arm repeats while 50-day exposure group (0.80 ±0.20) and 20-day exposure group (0.9 ± 0.31) more than 35-day exposure group showed spatial memory impairment. After recovery period, all recovery groups showed compromised performance as that of control group (00.00 ± 00.00). Decline in spatial memory was observed in 20-day recovery group (1.70 ± 0.3), 35-day recovery group (0.6 ± 0.22) and 50-day recovery group (1.6 ± 0.26) in comparison with their respective exposure groups (Figure 3.2 E)

(C)

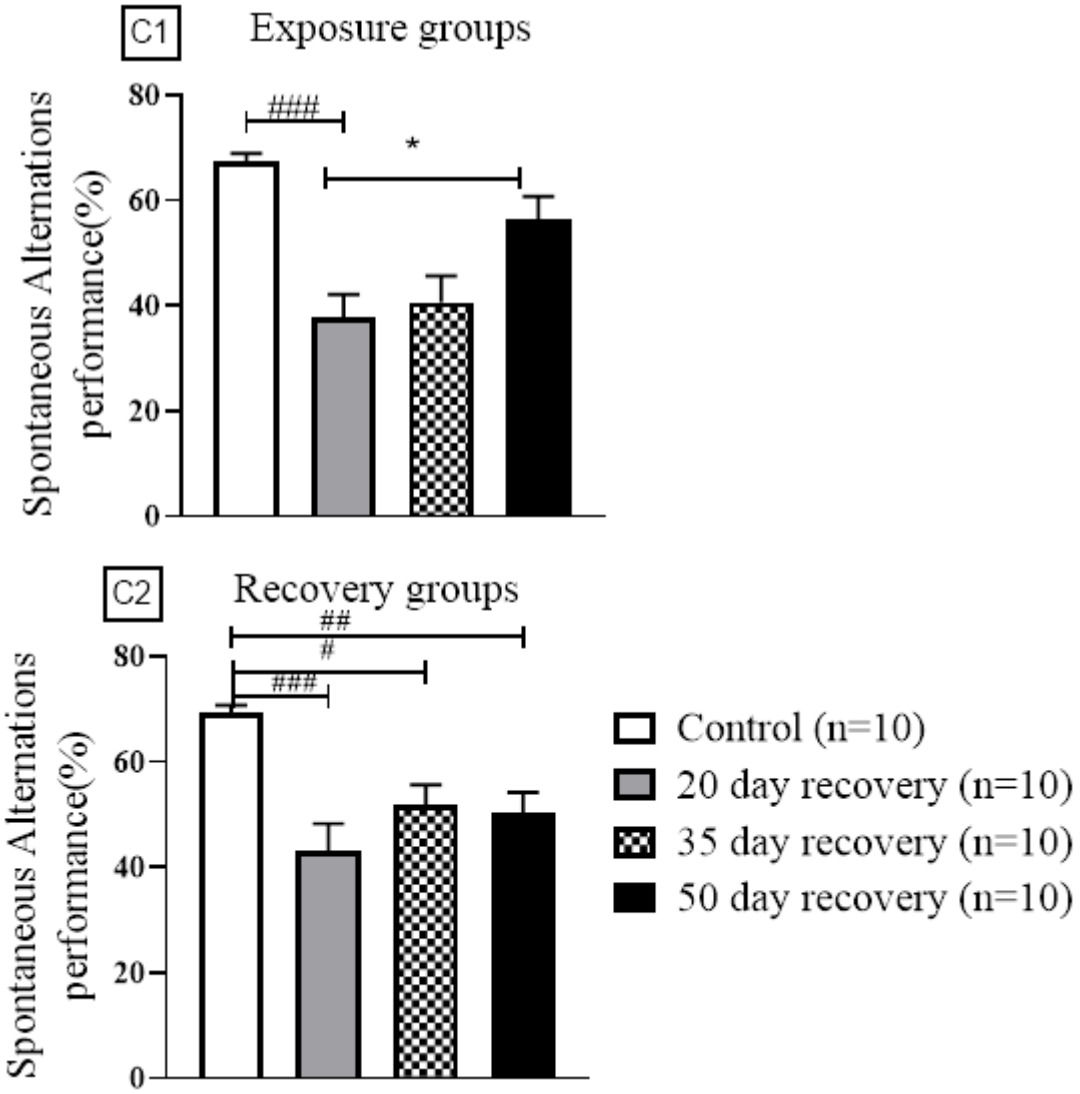


Figure 3.2 C: Effect of Arsenic on reference and working memory. Graph shows Spontaneous Alternations (%) in all groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with* = $p<0.05$, ### = $p< 0.001$ for exposure group and #=$p<0.05$ ## = $p< 0.01$, ### = $p< 0.001$ are the significance values for recovery group. s = seconds.

(D)

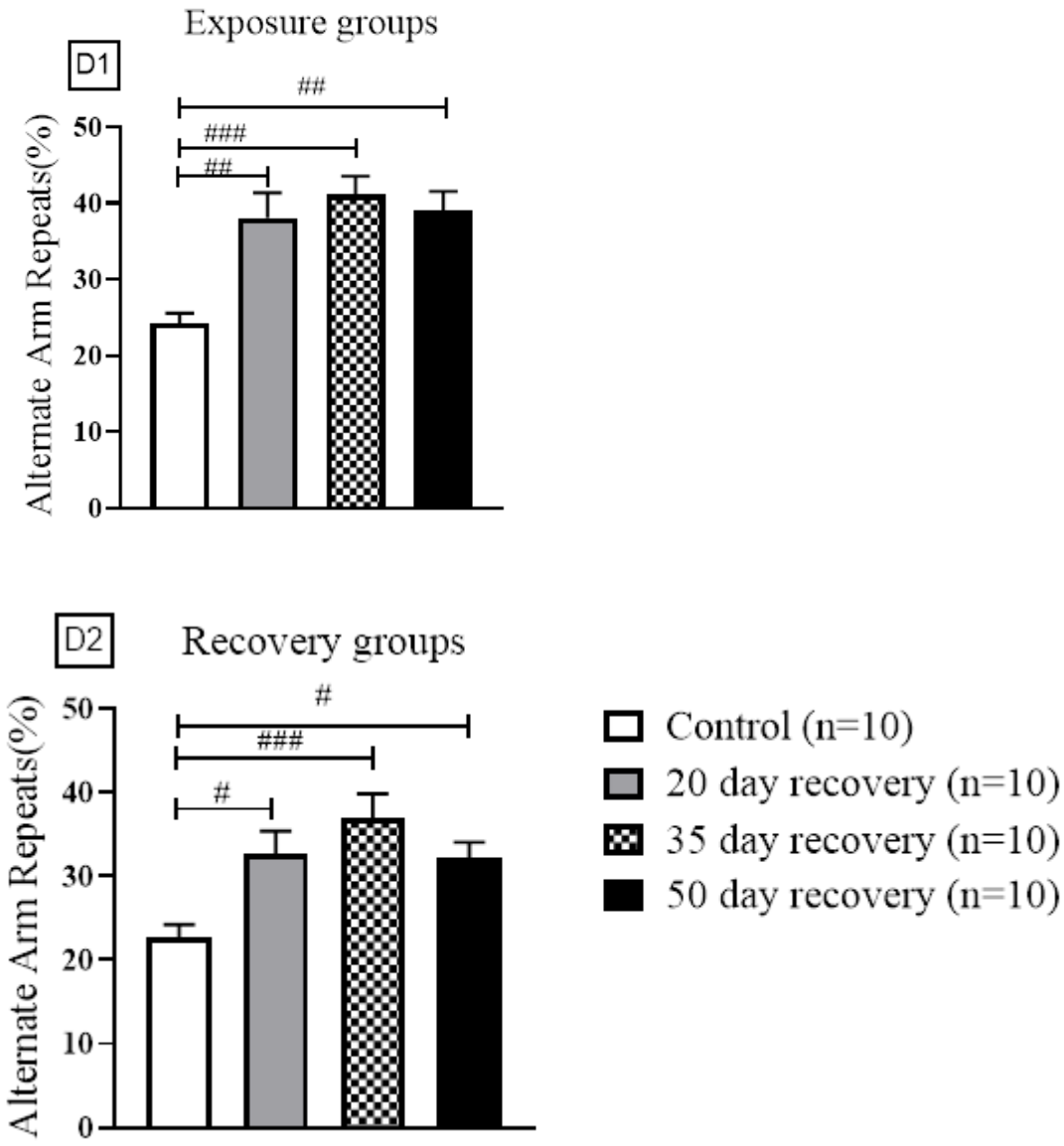


Figure 3.2 D: Effect of Arsenic on working memory. Graph shows the Alternate arm repeats (%) by all groups. Error bars are represented as mean± SEM for two-way ANOVA, followed by Bonferroni's multiple comparison test with #=p<0.05 ## = p< 0.01, ### = p< 0.001 are the significance values for both groups. s = second.

(E)

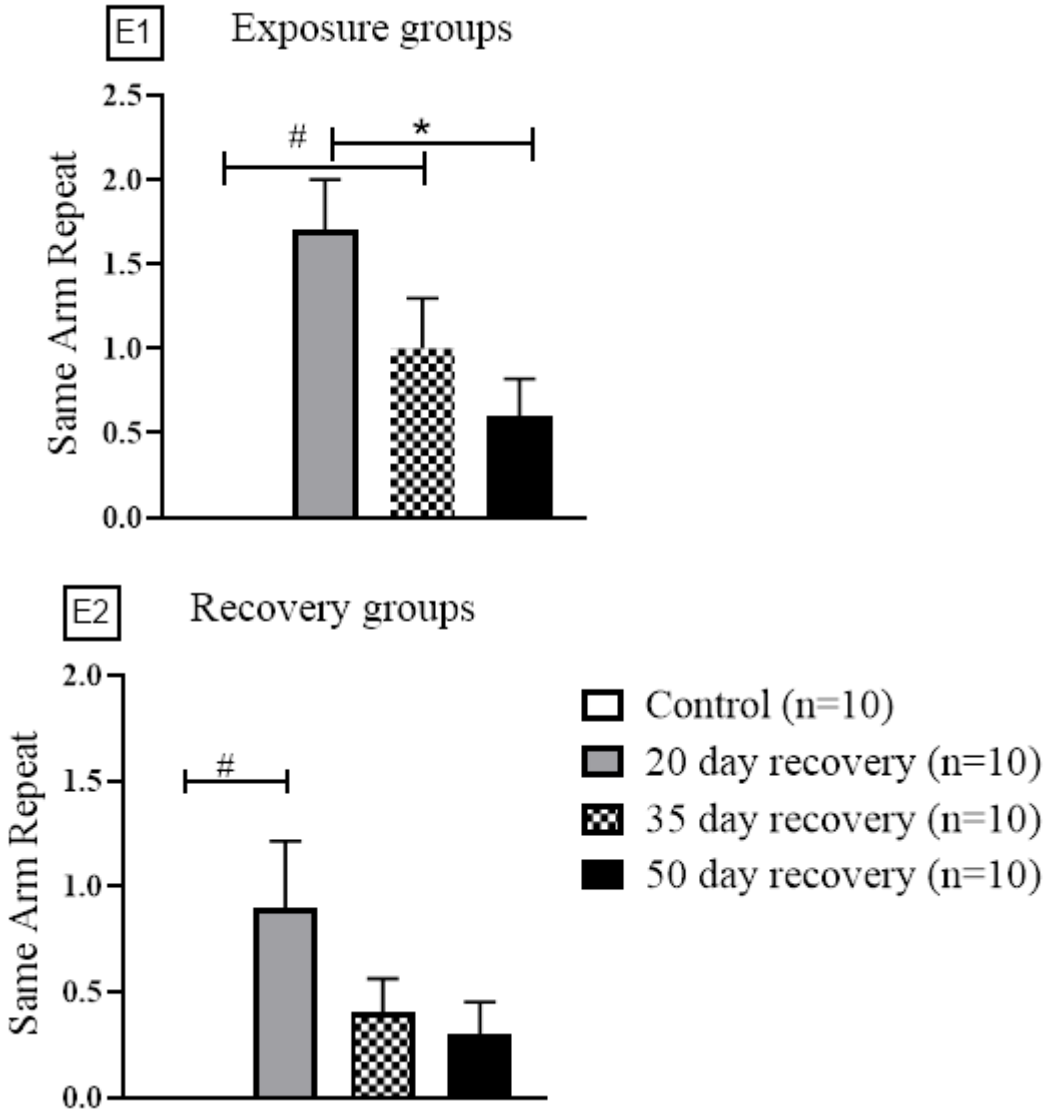
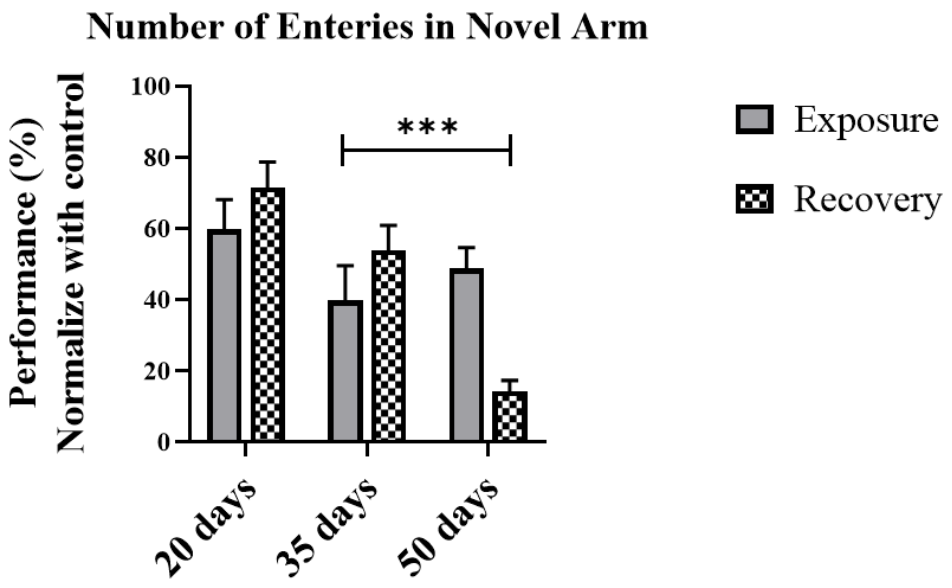


Figure 3.2 E: Same arm repeats; Y maze test. Graph shows the same arm repeats, by exposure and recovery groups. Error bars are represented as mean± SEM for two-way ANOVA, monitored by Bonferroni's multiple comparison test with *=$p<0.05$ for exposure group and #=$p<0.05$ are the significance values for recovery group. s = seconds.

(F)



(G)

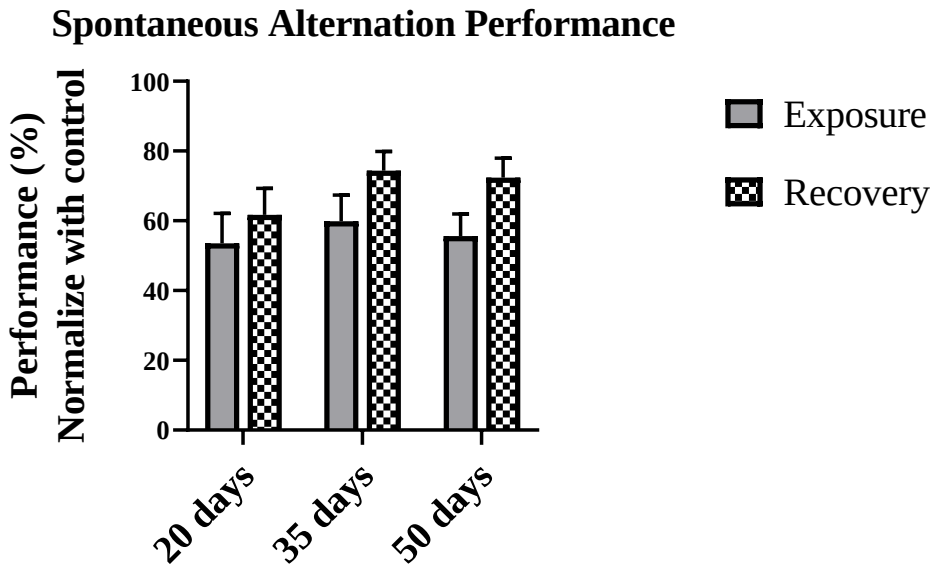


Figure 3.2(F, G) Spontaneous Alternation Performance normalize with control. # is used for significant difference between control and As treated groups. * is used for significance among As treated groups. Error bars are represented as mean± SEM for One-way ANOVA, followed by Bonferroni's multiple comparison test with *** = $p < 0.001$ as significance value. s = seconds.

3.3 Social Preference and Novelty Test

Sociability and Social preference was assessed 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, Arsenic exposed 20-day exposure group (36.3 ± 4.52), 35-day exposure group (61.20 ± 7.54) and 50-day exposure group (43.00 ± 3.61) showed low Social preference for mouse 1 as compared to control group (186.40 ± 10.68). 20-day exposure group (36.3 ± 4.52) presented least interaction time with mouse 1 as compared to other exposure groups. After recovery period, 20-day recovery group (48.20 ± 5.32) showed improved sociability in comparison with 20-day exposure group (36.3 ± 4.52). However, 35-day recovery group (56.20±4.44) and 50-day recovery group (32.10 ±4.95) exhibited no improvement instead decline in interaction (Figure 3.3 A). 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 least in 50-day exposure group (47.40 ± 5.51), 35-day exposure group (101.50 ±5.74) and 20-day exposure group (51.4 ±5.26). After recovery time, Social novelty preference was enhanced showing higher interaction with Mouse 2 in control group (153.00 ±13.58), 20-day group (93.40±4.52) and 35-day group (125.3 ± 5.96) as compared to their respective exposure groups. 50-day recovery group (75.80 ± 8.51) showed less Social novelty preference as compared to other recovery groups (Figure 3.3 B)

(A)

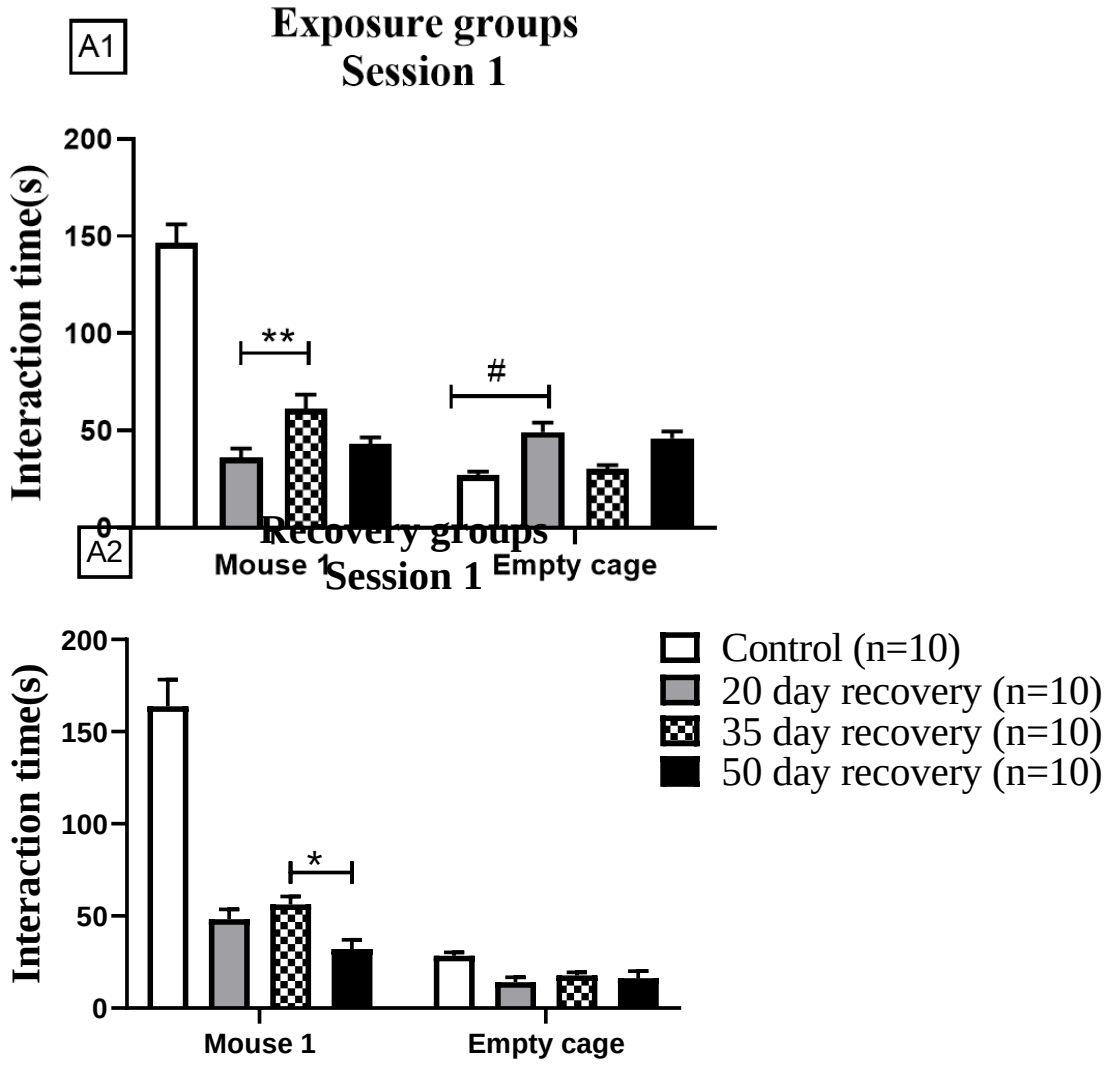


Figure 3.3 A: Effect of Temporal exposure of Arsenic on Sociability behavior (Session-I): Graph shows interaction time during session I (s) by the Control, 20-day exposure, 35-day exposure, 50-day exposure and their respective recovery groups. # =p<0.0001, show significance between control and As treated groups and * = p< 0.01 among As treated groups.

(B)

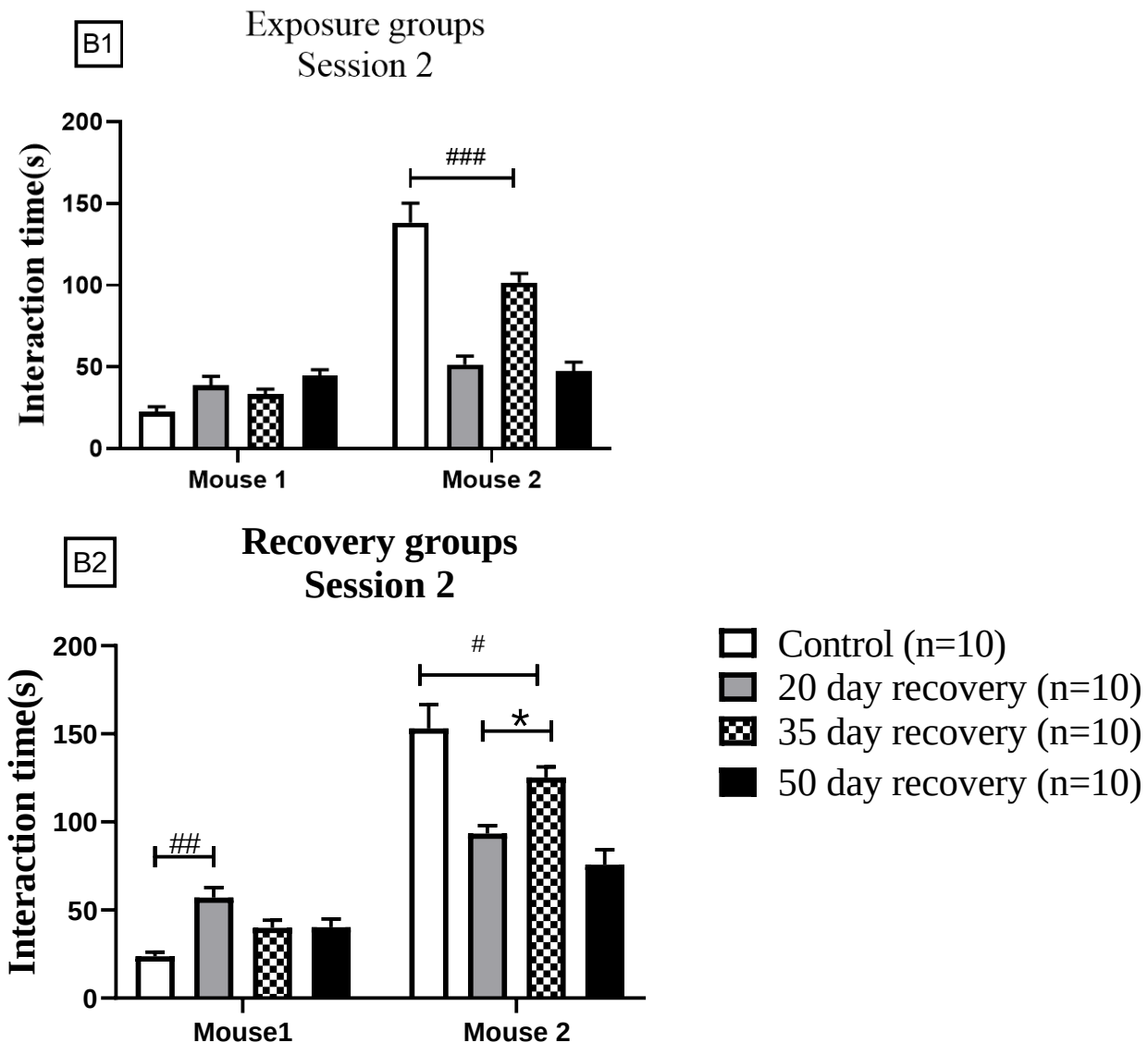


Figure 3.3 B: Effect of Temporal exposure of Arsenic on Sociability behavior (Session II): Graph shows interaction time during session II (s) by the Control, 20-day exposure, 35-day exposure, 50-day exposure and their respective recovery groups. *=$p<0.05$, show significance betweencontrol and As treated groups, # = $p< 0.05$, ## = $p<0.01$.

Trends 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 (182.13 ± 13.43), 35-day exposure group (278.70 ± 19.04) and 50-day exposure group (200.30 ± 8.46) spent higher time in Mouse 1 chamber as compared to center and empty cage chamber. 20-day exposure group (271.20 ± 13.14) spent least time with mouse 1 as compared to Control group (343.4 ± 11.28). After recovery period, 35-day recovery group (338.71 ±23.56) showed improved sociability as compared to 35-day exposure group (278.70 ± 19.04), 20-day recovery group (162.30 ± 12.59) and 50-day recovery group (169 ± 14.12) showed deficit in sociability after recovery (Figure 3.3 C). In session II control group (337.60 ± 15.27), 50-day exposure group (228.6 ± 31.88) and 30-day exposure group (294.40 ±23.23) showed higher Social novelty preference than 20-days exposure group (212.7 ± 16.43). In comparison with control group, All Arsenic exposed group showed less Social novelty preference. After recoveryperiod, it was observed that 50-day recovery group (247.8 ± 30.84) and 35-day recovery group (326.8 ± 12.89) spent more time with mouse 2 as compared to their respective exposure groups.Though, 20-day recovery group (237.6 ± 22.79) showed less Social novelty preference as compared to other recovery groups by spending less time in mouse 2 chambers (Figure 3.3 D). Percentage discrimination index clearly shows that all Arsenic exposure groups, 20-day exposure group (57.65 ± 4.18), 35-day exposure group (74.75 ± 2.41) and 50-day exposure group (50.62 ± 4.39) interacted less with novel mouse (mouse 2) as compared to control group (85.23 ± 2.30). 50-day exposure group showed least preference for novelty. After recovery, moderate improvement in performance was observed in 50-day recovery group (64.55 ± 3.32) and 20-day recovery group (62.58 ± 2.78). Little regression was observed in 30-day recovery group (75.9 ± 2.48) (Figure 3.3 E).

(C)

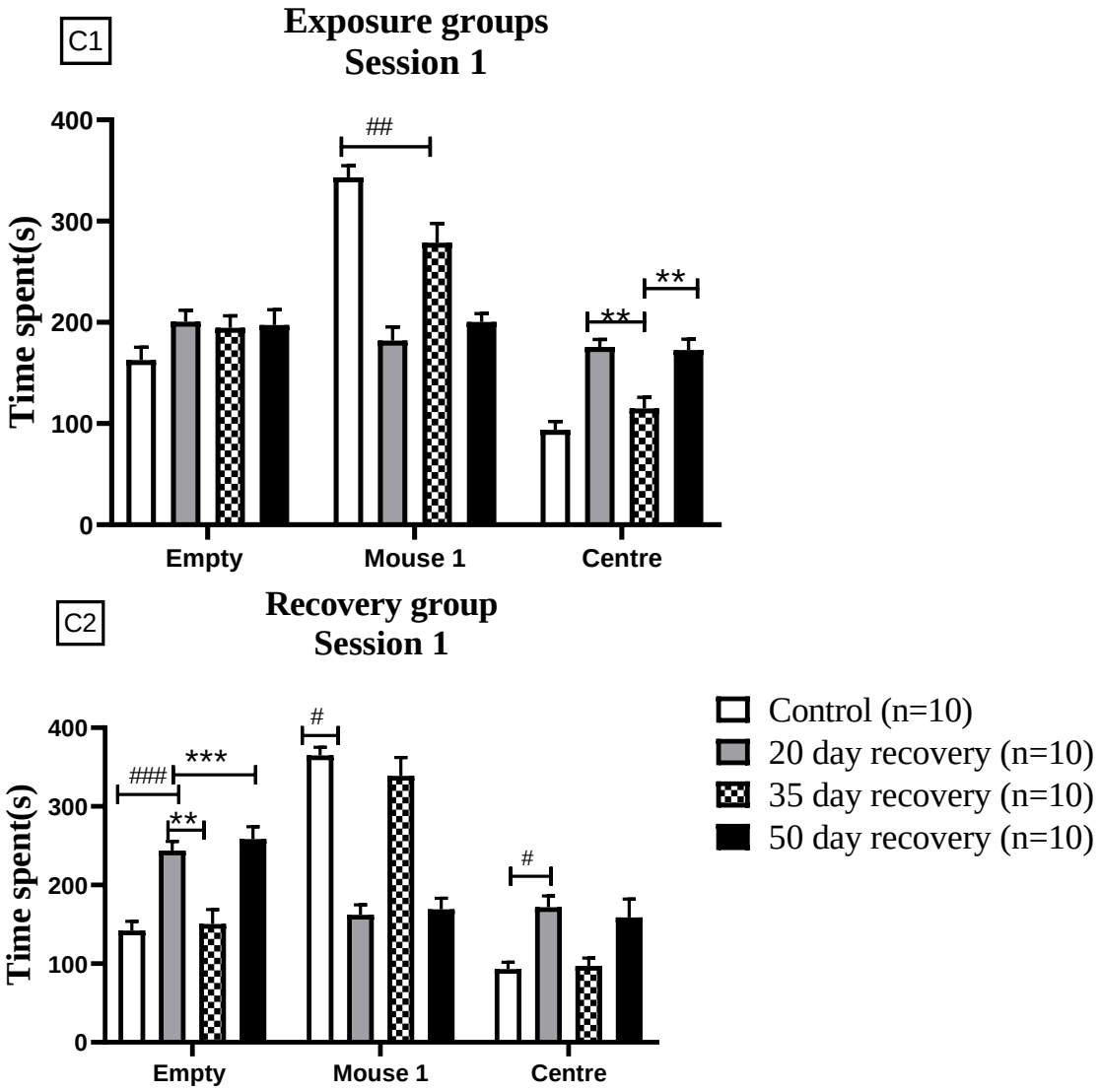


Figure 3.3 C: Social Novelty preference (Session-I): Graph shows time spent (s) in each chamber during session I All groups. # = p< 0.01 ## =p<0.05 ###= p< 0.005 show significance between control and As treatedgroups and * = p< 0.01 **= p< 0.001 *** = p< 0.0001 are the significance values among As treated groups.

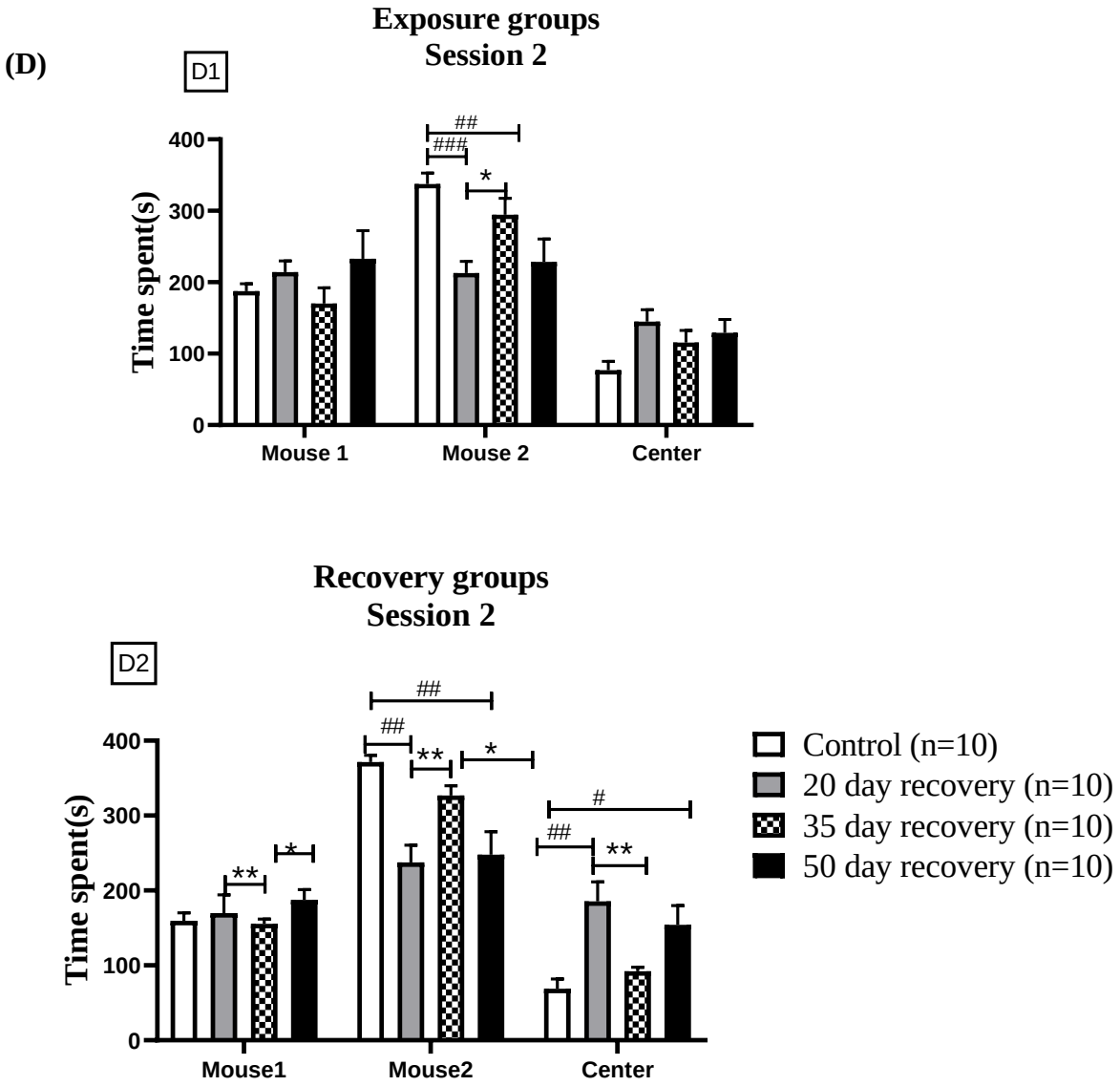


Figure 3.3 D: Social Novelty preference (Session-II): Graph shows time spent (s) in each chamberduring session II by the Control, 20-day exposure, 35-day exposure, 50-day exposure and recovery group*=p<0.05 ** =p<0.001 show significance between As treated groups, # = p< 0.01, ## = p< 0.001 ###= p< 0.001 are thesignificance values among As treated groups and control.

(E)

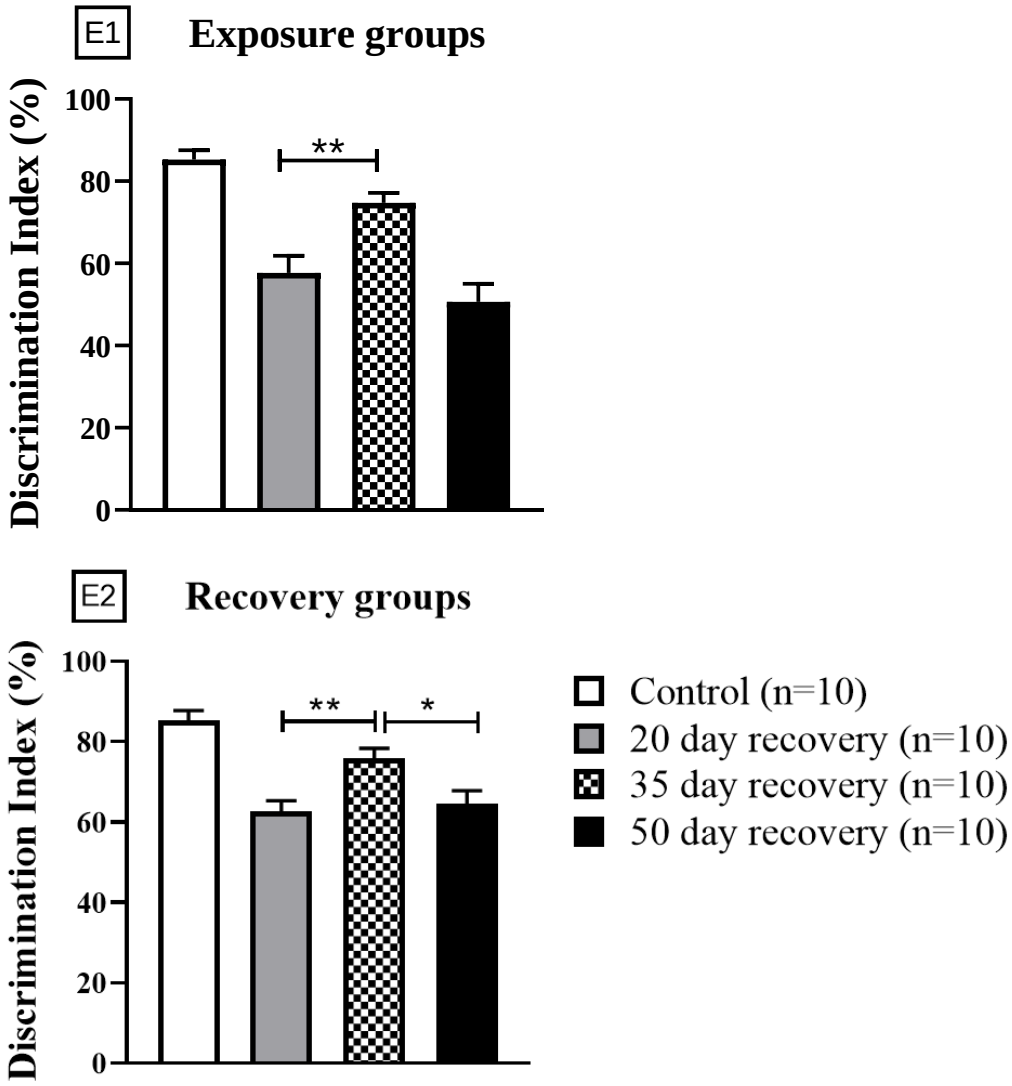
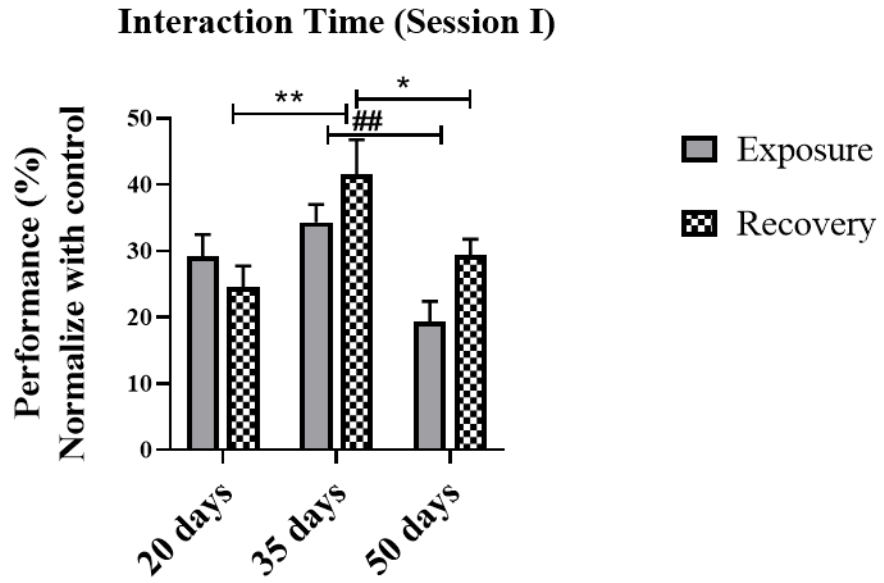


Figure 3.3 E: Outcome of Arsenic on social novelty test: Graph present percentage Discrimination Index during session II by the Control, 20-day exposure, 35-day exposure, 50-day exposure and recovery groups. *=p<0.05, ** = p< 0.01 show significance among As treated groups through One-way ANOVA and post hoc Bonferroni's test.

(F)



(G)

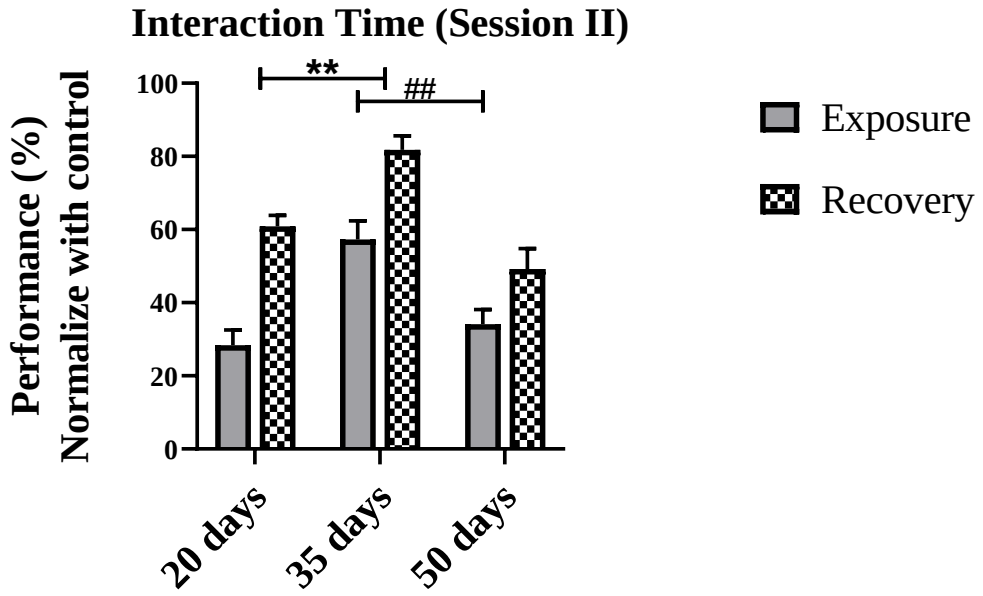


Figure 3.3 F, G: Outcome of Arsenic on social novelty test normalized with control: Graph present the interaction time in session II by 20-day, 35-day, 50-day exposure and recovery groups. *=p<0.05, ** = p< 0.01 show significance among As treated groups through One-way ANOVA and post hoc Bonferroni's test.

(H)

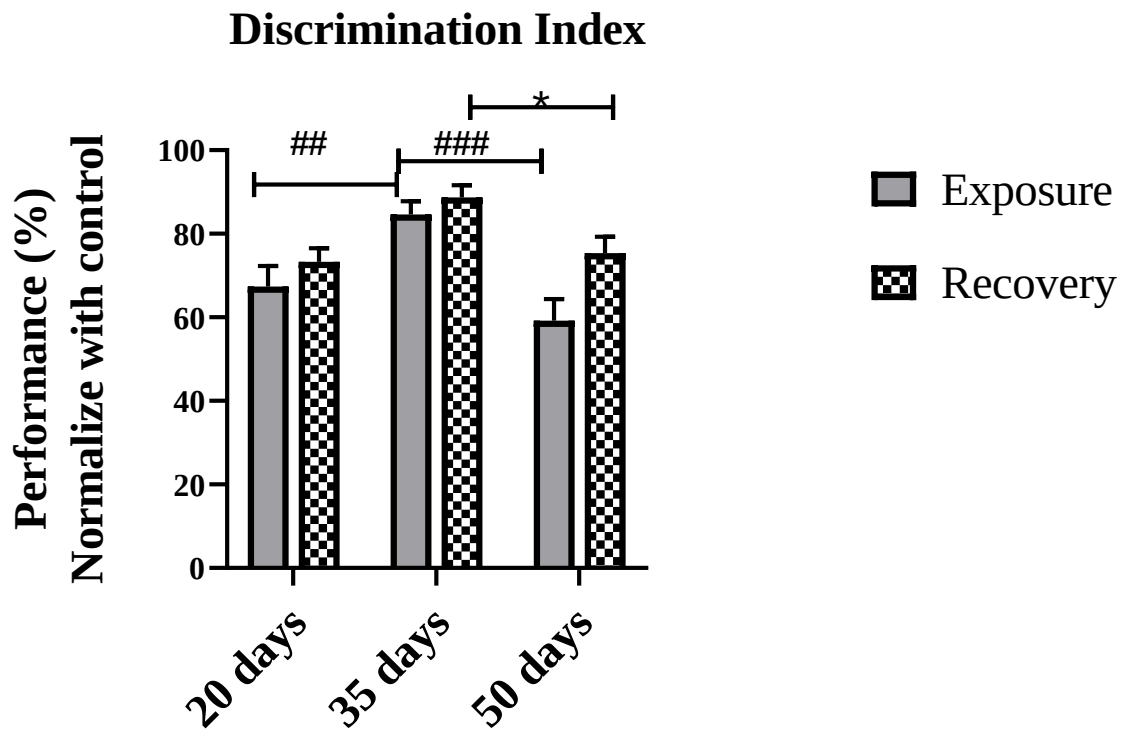


Figure 3.3 (H): Outcome of Arsenic on social novelty test normalized with control: Graph present the discrimination index by 20-day, 35-day, 50-day exposure and recovery groups. *=p<0.05, ** = p< 0.01 show significance among As treated groups through One-way ANOVA and post hoc Bonferroni's test.

3.4 Hole Board Test:

Hole board test was used in order to assess the consequence of Arsenic exposure on long haul memory and transient memory in different temporal groups. Locomotion activity of mice was also assessed by calculating Activity/ min in hole board, 20-day exposure group (4.84 ± 0.42), 30-day exposure group (4.5 ± 0.29) and 50-day exposure group (3.56 ±0.37) showed less locomotion than control group (5.84 ± 0.56). On day 4 similar trends was observed but overall locomotion activity was decreased in in control group (2.58 ± 0.59), 20-day exposure group (3.18 ± 0.17), 35-day exposure group (2.52 ± 1.86) and 50-day exposure group (2.37 ± 0.27). Least locomotion activity was witnessed in 50-day exposure group from day 1 to day 4. However, after recovery period 50-day recovery group (4.07 ±0.28) showed increased locomotion than 50-day exposure group but least than 20-day recovery group (5.28±0.23) and 35-day recovery group (5.64 ± 0.21) from day 1 to day 4 (Figure 3.4 A).Latency to visit the first hole (baited or un baited) was calculated to evacuated 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 (23.22 ± 1.54), 35-day exposure group (24.56 ±1.52) and 50-day exposure group (26.14 ± 2.76). Highest level of anxiety was observed in 50-day exposure group (26.14 ± 2.76). After recovery period performance of 35-day recovery group (13.8±1.39) was improved. While 20-day recovery group (32.57 ±5.86) and 50-day recovery group (32.4 ±4.72) showed higher anxiety level as compared to rest of the groups. Similar trends were seen on day 4 with control group (6.17 ± 0.42) showing least anxiety level as compared to 20-day exposure group (14.03 ± 1.73), 35-day exposure group (7.26 ± 0.58) and 50-day exposure group (11.33 ± 1.34). After recovery period performance on day 4 was improved in all the groups with anxiety level lower in 30-day recovery group (7.26 ± 0.58). (Figure 3.4 B)

(A)

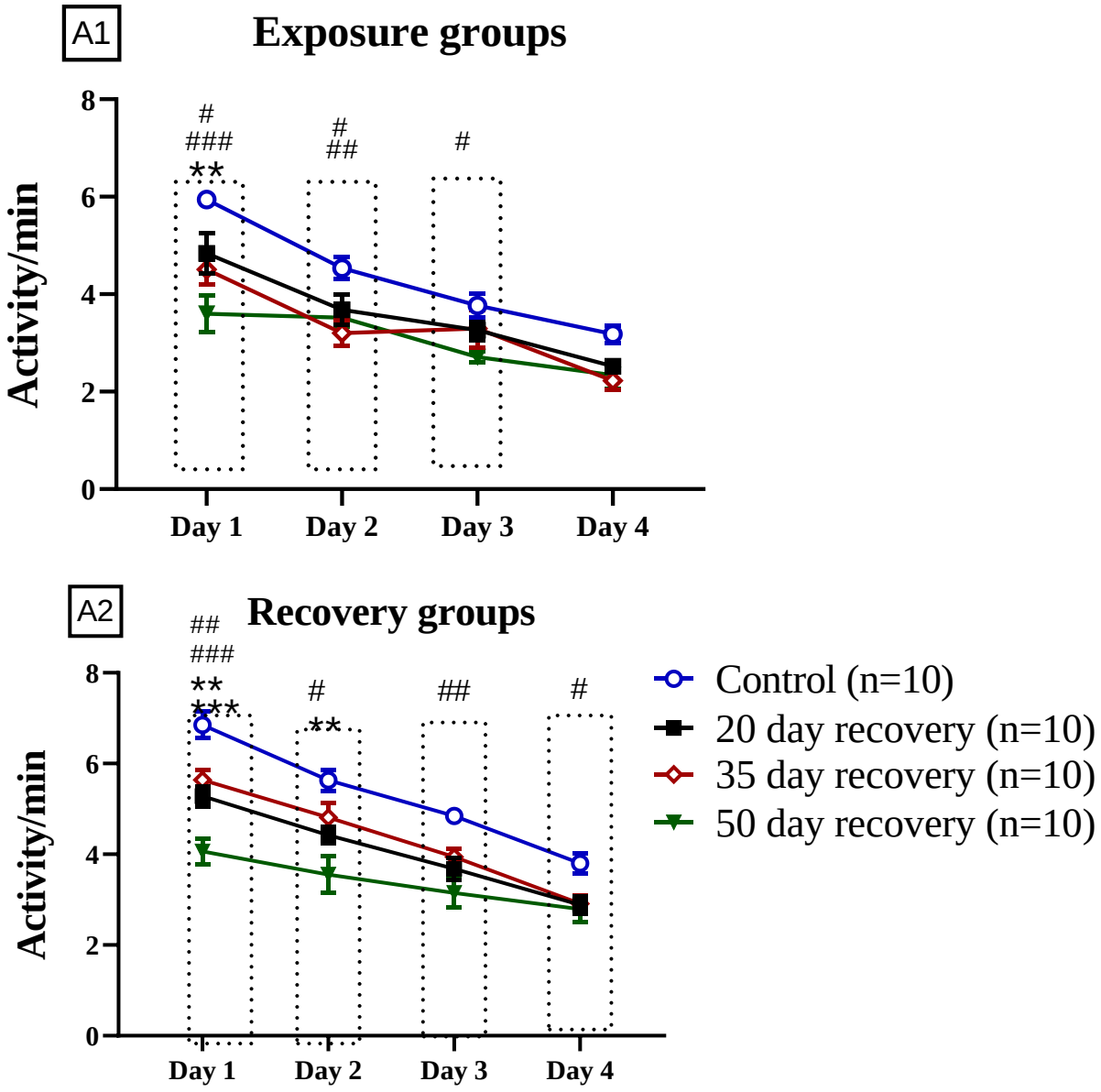


Figure 3.4 A: Effect of Arsenic exposure on locomotion activity. Graph shows activity per minute Control, 20-day exposure, 35-day exposure and 50-day exposure and their respective recovery groups. # = $p < 0.01$, ## = $p < 0.001$, ### = $p < 0.0001$ is the significant value between Control and Arsenic treated groups. ** = $p < 0.001$ ***= $p < 0.0001$ Error bars are represented as mean± SEM by 2 way ANOVA test. s= seconds.

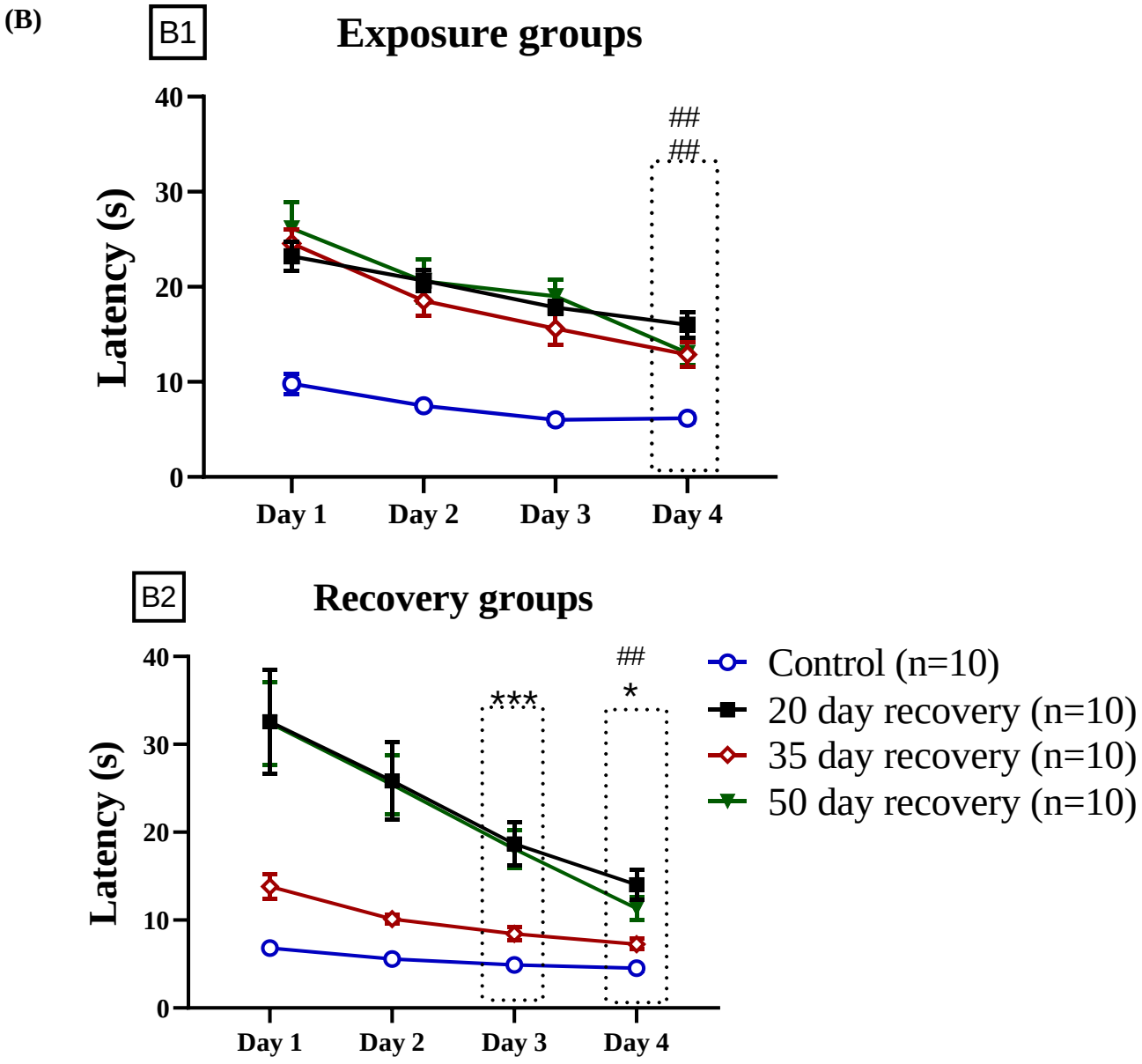


Figure 3.4 B: Effect of Arsenic exposure on anxiety levels. Graph shows the latency (s) for visit to first hole, by the groups Control, 20-day exposure, 35-day exposure and 50-day exposure and their respective recovery groups.## = $p < 0.001$, is the significant value between Control and Arsenic treated groups.* = $p < 0.01$ ***= $p < 0.0001$ 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 (21.12 ± 0.91), 35-day exposure group (17.0 ±0.99) and 50-day exposure group (22.78 ± 0.85) 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 (22.78 ± 0.85). 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 (19.50 ± 1.27) and 35-day recovery group (14.83 ± 1.62) have improved reference memory as compared to 50-day recovery group (20.80 ± 0.80). Similar trend was observed at day 4, 50-day recovery group (12.75 ± 1.34) showed higher number of reference memory error as compared to control (2.07 ± 0.32), 20-day recovery group (10.95 ± 0.57) and 35-day recovery group (8.35 ± 0.79) (Figure 3.4 C). 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 to20-day exposure group (8.09 ± 0.44), 35-day exposure group (6.55 ± 0.38) and 50-day exposure group (10.40 ± 0.48). Highest impairment of short term memory was observed in 50-day exposure group (10.40 ± 0.48). 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 (4.67 ±0.36) at day 4. After recovery period working memory (short term memory) on day 1 was improved in 20-day recovery group (8.63 ± 0.69) and 35-day recovery group (5.88 ± 0.29). 50-day recovery group (10.35 ± 0.52) did not show any improvement as compared to 50-day exposure group (10.40 ± 0.48). On day 4 slight improvements was observed in 20-day recovery group (3.72 ± 0.37), 35-day recovery group (2.47 ± 0.19) and 50-day recovery group (4.625 ± 0.26) as compared to their

respective exposure groups (Figure 3.4 D).

(C)

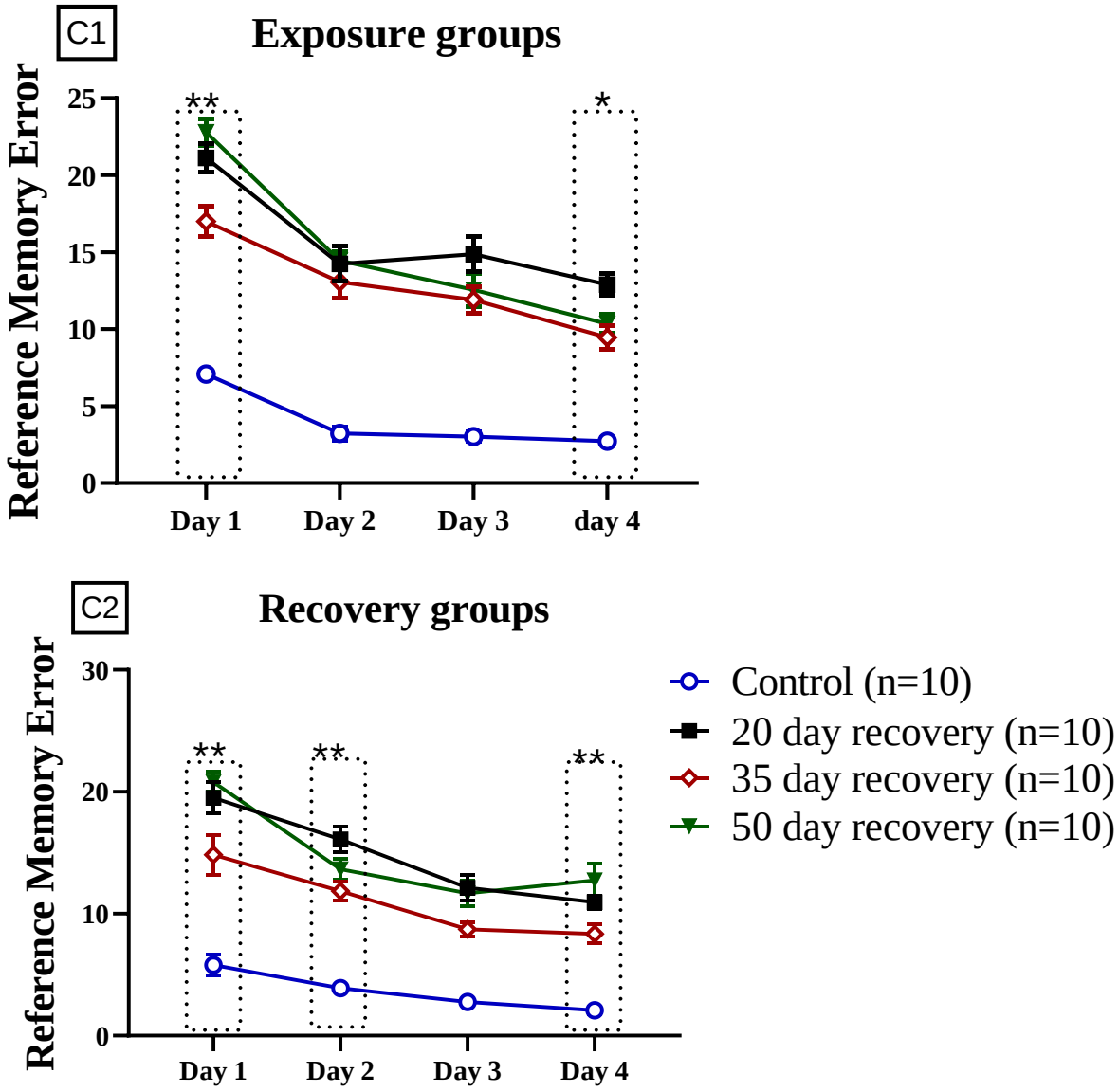


Figure 3.4 C: Effect of Arsenic temporal exposure on reference memory. Graph shows reference memory errors in Control, 20-day exposure, 35-day exposure and 50-day exposure and their respective recovery groups. *= $p < 0.05$, **= $p < 0.01$, is the significantvalue among Arsenic treated groups. Error bars are represented as mean± SEM by 2-way ANOVA test.

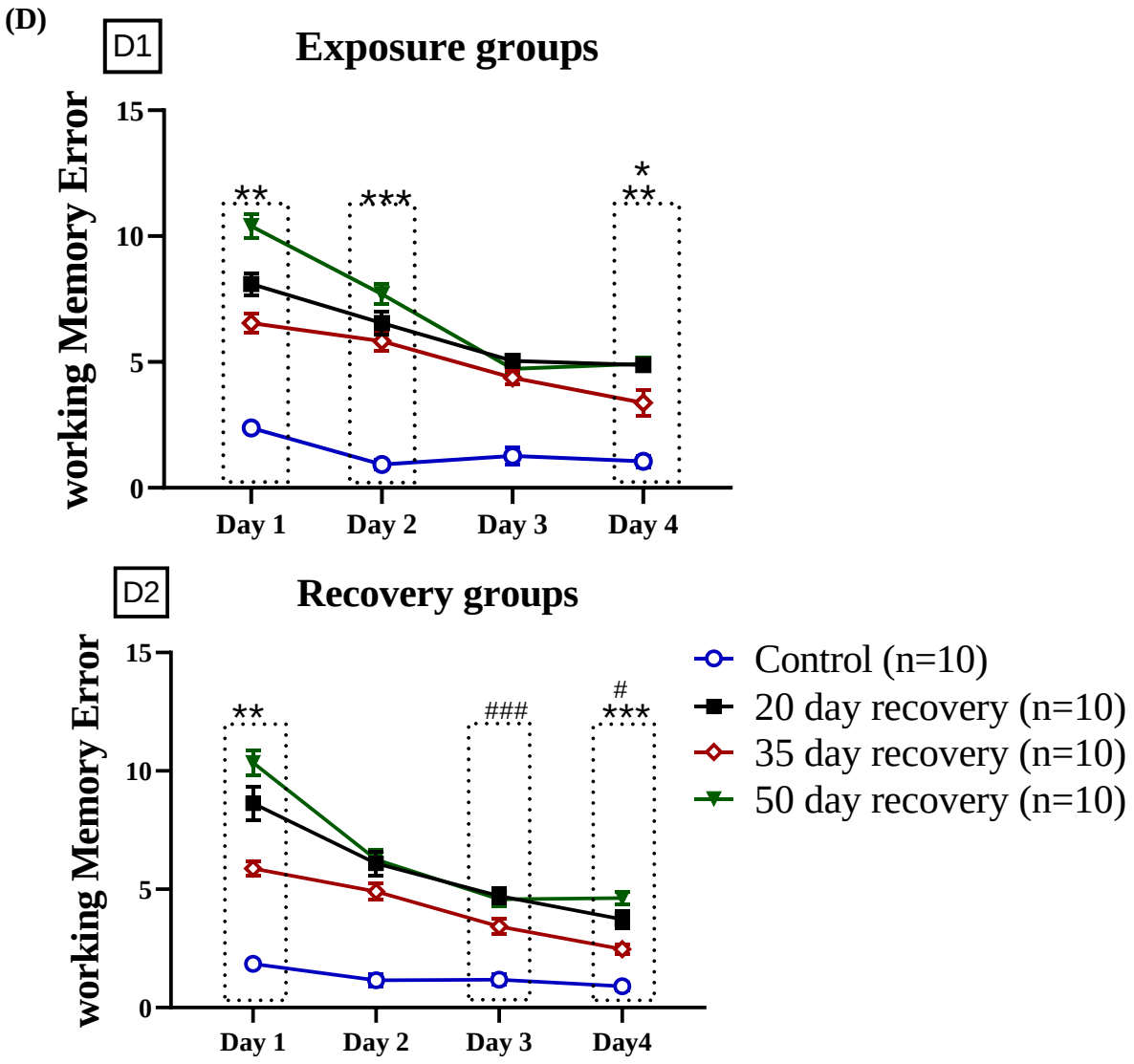


Figure 3.4 D: Effect of Arsenic temporal exposure on working memory. Graph shows working memory errors in Control, 20-day exposure, 35-day exposure and 50-day exposure and their respective recovery groups.# = p < 0.01, ## = p < 0.001, ### = p <0.0001 is the significant value between Control and Arsenic treated groups and *= p <0.01, **= p <0.001, ***= p <0.001 among Arsenic treated groups. Error bars are represented as mean± SEM by 2-way ANOVA test.

CHAPTER 4
DISCUSSION

Discussion

Cognitive impairment has been extensively studied in different studies with different types of metal exposures (Liu et al., 2017). In this review we assessed the intellectual capacity debilitation by openness of arsenic and its post openness recuperation has been reported. It is also reported that arsenic can induce different changes in adult and neonatal brain cells in vivo (Chattopadhyayet al., 2002). It is reported in a recent study that arsenic can induce neurotoxicity in developing rats' brain mainly by dysfunction in dopaminergic and cholinergic system (Chandravanshi et al., 2919).

Study showed that arsenic exposure which is present in drinking eat can increase the risk of neurodegenerative diseases (Samad, et al., 2019). Also, environmental arsenic exposure can increase the risk of Alzheimer's disease (Rahman et al., 2021). Groundwater is one of our planet's most significant customary assets. It is widely utilized throughout many places of the world, with substantial increases in extraction in late a very long time because of the wide openness of new and more affordable penetrating and siphoning innovation (Barbier., 2019).

Ongoing openness to arsenic through drinking water can possibly make inconveniences during pregnancy (Milton et al., 2005). Arsenic (As)contamination is becoming a major source of worry in current history due to its high toxicity to people (Polya et al., 2019). Aquifer's health can also be harmed by arsenic pollutants in groundwater.

Arsenic is present in minute quantities in rock materials and certain areas drinking water, it can cause very adverse health effect and can lead to different abnormalities. That's why this issue is of great interest to be addressed and strategies should be made to eliminate the arsenic from drinking water and environment and reduce the burden of arsenic. Presence of arsenic in groundwater can affect millions of individuals globally (Podgorski and Berg., 2020). Arsenic pollution in groundwater has been found in over 108 countries throughout the world. Approximately more than 230 million individuals are at risk worldwide, including 180 million Asians. Long haul admission of arsenic-tainted groundwater can cause genuine medical issues (Shaji et al., 2021).

Contamination of arsenic is broadly remembered quite possibly the most weighty ecological pollutant. Remediation and toxicity of arsenic is of prime concern of different research groups, because of the issues it can create worldwide in the form of different diseases, different techniques and technologies are proposed for the said purpose (Alka et al., 2021).

The Morris Water Labyrinth test (MWM) was utilized to decide what Arsenic meant for long haul memory and spatial memory in particular worldly gatherings. The consequences of the test preliminary test were assessed for section in the objective quadrant and the quantity of stage intersections. Study results were correlated to Luo et al., 2009 which showed decreased spatial learning on openness to arsenic in drinking water more than 90 days at a portion level of 68 mg/L more time taken foe platform acquisition. In our study on first day all the three exposure groups

have shown significant deficit in spatial learning as compared to control, so was supported by Jing et al., 2012 study which subjected 15mg/L dose of arsenic administered for 3 months. Highest deficit was seen in 50-day exposure group and 20-day exposure group. Control group, 20-day exposure and 35-day exposure group have displayed almost similar learning behavior over the training period. Whereas, 50-day exposure group, 20-day exposure group has shown decreased learning as compared to control group Kumar et al.,2013 also suggested prominineni decrease in acusition of spatial learnig and descreased locomotor activity, increased escape latency on administeration of arsenic. Recovery groups have shown significant learning as compared to exposure groups. 50-day recovery group has shown least learning as compared to control, 20-day recovery and 35-day recovery group. Evaluation between exposure groups and recovery groups depict enhanced learning and memory after recovery period in all the recovery groups. Over All poor spatial learning behavior was observed in 50-day exposure group and 20-day exposure group which did not improved much after recovery period.

The Y-labyrinth test was performed to recognize creature regular exploratory conduct. The hippocampus-dependent reference memory of the animals was also examined. Jing et al., 2012 found a substantial loss in spatial memory after 3 months of 15mg/L treatment. Novel arm was preferred by all four groups, as seen by the increased number of submissions in Novel arm. When contrasted with the benchmark group, the 50-day openness bunch showed the least inclination. Following the recuperation time frame, the 20-day recuperation bunch showed an increment in the

quantity of passages in the Original arm, but the 35-day and 50-day recovery groups showed a reduction in spatial memory. The trend witnessed while assessing time spent in Novel arm exhibited extreme decline in 50-day exposure group presented minimum inclination to novel arm as compared to other groups. After recovery period augmentation in spatial memory was witnessed in recovery groups 20-day recovery group and 35-day recovery group, 50-day recovery group as compared to respective exposure groups. Spontaneous Alternations performance and Alternate Arm Repeats (%) were calculated to assess deficiency in spatial memory. Spontaneous Alternations performance showed memory deficit in Arsenic treated groups. Highest impairment was shown by 50-day exposure group and 35-day exposure group. After recovery time all the groups showed minor improvement in spatial memory except 50-day recovery group. Short term memory impairment was observed by calculating Alternate arm repeats (AAR) and same arm repeats (SAR). 20-day exposure group, 35-day exposure group and 50-day exposure group showed higher arm repeats Accordingly, contrasted with the benchmark group, there is a larger memory deficit. When comparing the 35-day exposure group to the control group, the greatest loss was seen. All groups improved when the recuperation time ended. Highest improvement in spatial memory was seen in 20-day recovery group as comparedto 20-day exposure group. Same arm repeats exhibited impairment in all the exposure group whereas 20-day exposure group and 50-day exposure group more than 35-day exposure group showed spatial memory impairment. After recovery period, all recovery groups showed compromised performance as that of control group. Decline in spatial memory was observed in 20-day recovery group, 35-day recovery group and 50-

day recovery group in contrast with their respective exposure groups.

Three chamber sociability assay was performed to assess sociability in three phases. Htway et al 2019 states a visible decrease social behavior when exposed to arsenic 85mg/L examined at 74 weeks in three chamber test in control mice, time spent for Stranger 1 was out and out more essential than that for void enclosure. Curiously, mice with developmental arsenic receptiveness didn't contribute energy particularly for Stranger 1 and void enclosure, exhibiting that they had decreased amiability. For social conduct, the arsenic-uncovered mice showed the tendency to Stranger 2, at this point it was not critical while the control mice contributed basically more opportunity for Stranger 2. In the current study Three chamber sociability test assessed the animal social behavior results provided insight about session I, All the groups displayed greater preference for mouse 1 as compared to Empty cage. Conversely, Arsenic exposed groups showed low Social preference for mouse 1 as compared to control group. 20-day exposure group presented least interaction time with mouse 1 as compared to other exposure groups. After recovery period, 20-day recovery group presented enhanced sociability. However, 35-day recovery group and 50-day recovery group exhibited no enhancement instead decline in interaction. 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 Social novelty preference was least in 50-day exposure group. After recovery time, Social novelty preference was heightened showing higher interaction with Mouse 2 in all groups as compared to their respective exposure groups.

50-day recovery group showed less Social novelty preference as compared to other recovery groups. Percentage discrimination index presented that all Arsenic exposure groups interacted less with novel mouse (mouse 2) as compared to control group. 50-day exposure group showed least preference for novelty. After recovery, moderate improvement in performance was observed in 50-day recovery group and 20-day recovery group. Little regression was observed in 30-day recovery group.

Hole board test was used to assess the spatial long term and short term memory with the assessment of anxiety levels. Locomotion activity of mice was also assessed by calculating Activity/ min in hole board from day 1 to day 4. Saritha et al., 2019 suggested a visible decrease in the locomotor activity of test subjects on exposure of sodium arsenite of dose 30mg/kg along with the low number of head dips and less duration of the head dips concurring with our study results on day 1, all exposure groups showed less locomotion than control group. On day 4 similar trends was witnessed but overall locomotion activity was decreased in all groups. Least locomotion activity was witnessed in 50-day exposure group from day 1 to day 4. However, after recovery period 50-day recovery group showed increased locomotion than 50-day exposure group but least than 20-day recovery group and 35-day recovery group from day 1 to day 4. Latency was calculated to evacuated anxiety level in mice. On day 1 control group showed least latency thus less anxiety as compared to As exposure groups. Highest level of anxiety was observed in 50-day exposure group. After recovery period performance of 35-day recovery group was improved. 20-day recovery group and 50-day recovery group displayed higher anxiety level as compared to rest of the

groups. Similar trends were comprehended on day 4 with control group showing least anxiety level as compared to exposure groups. So was suggested by the Kumar et al., 2018 and Kumar et al., 2013 a significant deficit in the exploratory behavior of the mice on arsenic exposure. After recovery period performance on day 4 was improved in all the groups with anxiety level lower in 30-day recovery group. To evaluate reference memory throughout 4 days, Reference memory error (RME) was calculated. On day 1, exposure groups showed higher impairment in referential memory as compared to control group. Highest reference memory errors were observed in 50-day exposure group. 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, 20day recovery group and 35day recovery group have improved reference memory as compared to 50-day recovery group. Similarly, on day 4, 50day recovery group showed higher number of reference memory error as compared to control groups. 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 showed least working memory error as compared toexposure groups. Highest impairment of short term memory was observed in 50-day exposure group. WME were reduced in all the groups from day 1 to day 4. Highest numberof working memory errors were seen in 50-day exposure group at day 4. After recovery period working memory (short term memory) on day 1 was improved in 20-dayrecovery group and 35-day recovery group. 50-day recovery group did not show any improvement as compared to 50-day exposure group. On day 4 slight improvements was observed

in 20-day recovery group, 35-day recovery group and 50-day recovery group as compared to their respective exposure group.

Conclusion

Learning and memory have been affected as a result of increased exposure to arsenic-contaminated drinking water. This research shows how distinct time-dependent exposures to the same total dosage of arsenic can result in diverse impairments in hippocampal-dependent learning and memory, which can be reversible on their own. The study's findings show that both long-term low-dose Arsenic exposure and short-term high-dose Arsenic exposure can produce learning and memory impairment that is irreversible without therapy. Arsenic exposure at a modest dose for a reasonable amount of time, on the other hand, causes a little learning and memory impairment that may be partially corrected on its own. More study is needed to determine the precise mechanism of Arsenic's effect on higher cognitive processes when exposed to it over time.

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