Neurobehavioural, neurochemical and neuromorphological effects of cadmium in male rats

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Abstract: AS Cadmium is a widespread toxic environmental and industrial pollutant. The present study was carried out to investigate the possible effect of cadmium chloride ($CdCl_2$) on memory, exploratory motor activity (EMA)and motor balance in male rats. Forty five male Wistar rats weighing (100-120 gm) were administered $CdCl_2$ in drinking water at one of three concentrations; 0 ,5 and 50 mg/ L dissolved in water for a period of 60 days. Memory retention was evaluated through open-field habituation test (non associative learning), classic maze test (associative learning) as well as working spatial memory in a Y-maze.Moreover, exploratory motor activity and motor coordination were evaluated. Brain tissue specimens, representing all treatment groups, were taken for histopathological and biochemical examination. The average body weight significantly lower in group of rats exposed to high $CdCl_2$ doses. Open field revealed marked impairment in habituation with noticed influence on both anxiety and fear in rats exposed to high $CdCl_2$ .Moreover, learning and memory assessed during classic maze test and Y-maze test showed reduced memory retention in cadmium exposed animals as compared to control group .In novelty acquisition test ,a reduced exploratory motor activity in rats exposed to high $CdCl_2$ was noticed .Additionally ,complex motor behaviour (motor coordination)was significantly impaired due to cadmium intoxication. Furthermore, ,histopathological and biochemical evaluation revealed distinct neurodegenerative changes of nerve cells especially in hippocampus , inhibition of cholinesterase activity ,as well as decrease in the antioxidant enzymes activity (GST and SOD). Overall, these results suggest that intoxication with cadmium chloride has potentially deleterious effects on brain as reflected in impairment learning and memory. Also exploratory motor activity and motor coordination were reduced. [Journal of American Science 2010; 6(5):189-202-]. (ISSN: 1545-1003).

Keywords: Cadmium intoxication; learning and memory; motor activity; hippocampus, AChE; SOD;GST;Rats.

1. Introduction

Humans and animals interact with their environments on a daily basis and as a consequence are exposed to a broad spectrum of synthesized chemicals present in the food they eat, the air they breathe and the water they drink (Wade et al., 2002). It is a widespread toxic environmental and industrial pollutant. Cadmium has been released into the environment through human activities and is routinely found as a contaminant in tissues collected from the human population throughout the world (Newsome et al., 1995).

Cadmium is unique among the other metals because of its toxicity at a very low dosage and long biologic half life (30 years in human) and its low rate of excretion from the body (Jones and Cherian, 1990). It is listed by the U.S. Environmental Protection Agency as one of 126 priority pollutants.

Acute-Cd exposure results in pulmonary edema and respiratory tract irritation, whereas chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis, and bone fractures (Friberg et al.;1986 , Goering,et al.;1995), Cd is carcinogenic for a number of tissues (Waalkes;2000) and is classified by IARC(1993) as a human carcinogen.

In laboratory animals, acute Cd poisoning produces primarily hepatic and testicular injury, whereas chronic exposure results in renal damage, anemia, and immuno- and osteotoxicity (Goering, et al.; 1995, Klaassen, et al.; 1999). Cadmium can enter into the brain parenchyma and neurons (Nishimura et al., 2006) causing neurological alterations in humans (Rose et al., 1992) and animal models (Lukawski et al., 2005) leading to lower attention, olfactory dysfunction and memory deficits. Additionally , there are studies showing the neurotoxicity of cadmium on cell culture models like neurons and glial cells (Im et al.,2006; Lo'pez et al.,2006; Nishimura et al., 2006). In contrast, there are few studies discussing the effect of cadmium on learning and memory in rats.

Regarding the locomotor activity and motor balance,decrease in distance traveled, stereotypic time and movements, ambulatory time and vertical

movements were observed in Cd-exposed rats (Ali et al .,1990).

A variety of neurobehavioural and biochemical effects are produced on the nervous system of rodents given repeated doses of cadmium (Murphy, 1997).It has been suggested that the mechanism of Cd toxicity involves production of reactive oxygen species and free radicals (Manca, et al.; 1994, Stohs et al.; 2001). In animals, the various toxic effects induced by cadmium may be due to increased lipid peroxidation (Manca et al., 1991; Calderoni et al., 2005). The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system (Ognjanovic et al., 1995). This defense system includes the enzymes glutathione peroxidase, thioredoxin reductase as well as the reduced glutathione (GSH), which normally protect the biological system against free radical toxicity (Sarkar et al., 1998; El-Sharaky et al., 2007). Neurotoxicity was still not regarded to certain specific reason, as Cd exhibits several effects on neural level concerning with neurochemical mediators like catecholamines, serotonin (Antonio and Leret 2003) and cholinergic transmission (De Castro *et al*., 1996). Assembly of cell membrane proteins and phospholipids may also be affected under Cadmium toxicity (Gerak-Kramberger and Sabolic 2001).

To our knowledge no literatures are available to address the effect of cadmium , on learning and memory in rats .Furthermore , measurements of both associative and non associative learning abilities as well as spatial working memory in rats are not well implemented .

So, the objective of the current study, was to evaluate the effects of Cadmium chloride solution (5 or 50mg) intake on two memory tasks in adult male rats as measured by open –field habituation (non associative learning) and classic maze (associative learning). Also, spatial working memory performance was measured in Y- maze. As the hippocampus and cholinergic system are greatly involved in the process of learning and memory, histopathological and biochemical examination were also carried out in order to detect neurodegenerative changes in brain. Additionally, exploratory motor activity (EMA) and motor coordination were evaluated as a result of neurodegenerative deficits.

2. Materials and Methods

2.1.: Animals:-

Forty five Wistar male albino rats weighing about 100-120 gm were used in this study. Animals were raised in the Animals House Unit in Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages with stainless steel wire lids; (bedded with wood shavings); on a standard laboratory feed diet. Animals were housed at constant room temperature (20-22 °C) ,60% humidity and light cycle of 12h. /day.

2.2.: Administration of Cadmium

The animals (45 male rats) were divided randomly into three groups of 15 animals each. The first group served as the control and the animals were allowed ad libitum normal tap water during the experiment without any added cadmium. The other two groups of rats (experimental), were allowed ad-libitum tap water containing 5mg cadmium chloride/L dissolved in water (low dose) and 50 mg cadmium chloride / L dissolved in water (high dose), respectively (Waalkes et al., 1999).All animals were exposed to ad-libitum supply of low doses and high doses $CdCl_2$ for 60 days in drinking water till completing all assessments of learning and memory behaviour test.

2.3. Open-field test:

Habituation , a form of non associative learning, was measured in the open –field test (Kelly, 1993; Mello e' Souza et al; 2000 and Lea et al; 2008). The open field used was a square arena (90 cm x 90 cm x 25 cm), built from wood. The wood of the apparatus is covered with plastic laminate (Formica), which prevents absorption of fluids (urine of rats). The floor was divided by black lines into 36 small squares (15x15 cm) .The rats were gently placed in the corner of the arena and left to explore for 3 minutes. Crossings of the black lines and rearings performed were counted for three consecutive days. Also,number of fecal pellets in the arena were recorded. The open field was cleaned with 10 % alcohol and water solution prior to behavioral testing to remove residues left by previously tested rats. The decrease in the number of crossings and rearings was taken as a measure of the retention of habituation (Lea et al; 2008).

2.4. Maze learning test (classic maze):

Associative learning was assessed using classic maze test. The base measure of the maze was 100x60 cm and the walls were 25 cm high. The entire maze was made of wood with a glass cover to prevent escape of animals and allow observation. Testing was carried out between 09:00 and 15:00, where all groups were randomly allowed for testing at the same day. Male rats were deprived of feed for a 23 hours period before start of testing. Rats were given their daily amount of food as a reward at the end of the maze. Animals were given one trial per day for five consecutive days. Time elapsed to locate the feed at the end and numbers of entries of blind alleys were recorded according to Staddon (1983) .

2.5. Spatial Y-maze memory:

Spontaneous alternation in a single session was assessed in a Y –maze, which is used as a measure of

short- term memory performance (Maurice et al; 1994 and Roghani et al; 2006).

Each arm of Y –maze was 40 cm long, 30 cm high and 15 cm wide (Roghani et al; 2006) and converged in an equilateral triangular central area with 15 cm at its longest axis. Rat was placed at the end of one arm and allowed to move freely through the maze for eight minutes. The sequence of each arm entry recorded manually.

Measure of spatial memory, was defined as the entry into all three arms on consecutive choices in overlapping triplet sets. The percent of spontaneous alternation beaviour was calculated as the ratio of actual to possible alternation.

2.6. Novelty exploration test:

To investigate exploratory motor activity (EMA) in rats in the three studied groups, a "mini-holeboard" was designed that could be inserted into the base of a wooden box, with a floor (40 x 40 cm) and walls 50 cm. The mini-holeboard consisted of a dark platform (40 x 40 cm) which contained a hole (diameter 5.5, depth 5 cm) in each quadrant. A small object, which differed from in scent and texture, was placed in each hole (stimulus- rich). Exploratory behaviour of rats including numbers of rears and head-dips (to examine the interior of ,or the objects within the four holeboard holes)were counted during the 15-min. exposure period of the rats to the holeboard (Vaughan and Braunewell, 1999).

2.7. Psychomotor testing (Motor complex behaviour):

Animals of the three groups were examined with two different motor tests (rod walking and plank walking).

Rod walking: The ability of rats to balance on a stationary, horizontal rod, measures psychomotor coordination. Male rats were placed in the center of a rod (100 cm long , 26 mm in diameter , positioned 23cm above the table surface), parallel to it , and their latency to fall off the rod onto a cushion below was recorded (max . score = 60 s) .

Plank walk test : Balance and coordination were measured by exposing the rats to one trial on each of two horizontal planks (wide= 25 mm and narrow = 13 mm) , each 100 cm long , placed 34 cm above the table top .Distance traveled (in cm) and number of turns on the planks were recorded and averaged for each trial (Barbara et al , 1998).

2.8. Body weight and brain weight:

All male rats per group were weighed at the onset of treatment and weekly throughout the study. At the end of the study, five rats from each group were sacrificed by decapitation; brain of each animal was removed, cleaned and weighed.

2.9. Biochemical examination*:*

At the end of experiment five rats from each group were sacrificed by decapitation. Brain of each animal was taken on ice cold. Then homogenized in phosphate buffer with PH 8 (W/V), centrifuged at 1500 rpm for 10 min. The supernatant fluid froze at -20°C until assayed for further analysis.

- Acetylcholine esterase (AChE) activity was determined using acetylcholine iodide as a substrate according to the method of Elman *et al*., (1961).

- Estimation of lipid peroxidation. Enzymatic activity for oxidative stress were estimated including Glutathione-s-transferase (GST) and superoxide dismutase (SOD) according to methods of Habig *et al*., (1974) and Giannopolitis & Ries (1977), respectively.

- Total protein (TP) was determined by lowery's method (Lowry *et al*., 1951).

2.10. Histopathological examination:

Tissue specimens from brain of all experimental rats were collected at the end of the study and fixed in neutral buffered formalin, processed by conventional method, embedded in paraffin, sectioned at 4-5 um and stained by Haematoxylin and Eosin (Bancroft et al., 1996).

2.11. Animal Care

All animals received humane care as well as the approved ethical rules .Animal care was in compliance with applicable guidelines from Cairo University policy on Animal Care and Use.

2.12. Statistical analysis*:*

Statistical analyses were performed by using SPSS statistical software package. Data are presented as means with their standard error. Normality and homogeneity of the data were confirmed before ANOVA, differences among the experimental groups were assessed by one-way ANOVA followed by Duncan's test (SPSS,2006).

3. Results

3.1.Open field test :

In the open- field habituation (Table 1.), a significant effect of Cd regarding the number of crossed squares, number of rearings and number of faecal pellets was observed .

These parameters exhibited significant differences between high dose group (50 mg Cd) and control one. Where the group of rats treated with high dose of Cd showed a significant increase in the locomotor behaviour in the field (crossing of squares & rearings) ($p< 0.01$), the mean values were 57.90±8.26 and 19.61±3.13 when compared to the control group (26.70±2.22 and 7.02±0.82). So, over the three test sessions , impairment in habituation was markedly

seen in high $CdCl_2$ group compared to other treatments . Also, there was a significant increase in motor activity in the field ($p<0.05$) in male rats treated with low doses of cadmium .

Concerning the number of faecal pellets in the field (vegetative behaviour), there was significant differences between the high dose group and the control one, as the mean values were (5.03±0.26 and 2.22±0.31) respectively. This indicates that, with habituation impairment, fear and excitation increased in the group treated with high dose of cadmium, as rats defecated more frequently.

3.2. Maze learning Test : (classic maze)

Learning and memory assessed over five days of maze test ,showed that group of animals exposed to high concentrations of $CdCl_2$ took longer time to locate feed (Table .1) (1.68 ± 0.44 minutes , $p <0.05$) , with higher frequency for entering blind alleys (3.53±0.42).

These results demonstrating poor memory retention relative to cadmium intoxication. Regarding mean values of time elapsed and number of errors of low dose group, showed a non significant differences (1.34± 0, 29 minutes and 3.31±0.58) .

3.3. Spatial Y-maze memory:

In Table (1), the mean percent of spontaneous alternation behavior for high dose Cd, low dose Cd and control group were (36.99±3.45, 39.21±4.25and 60.70±3.36 respectively). There were significant differences in working spatial memory observed among the examined groups ($P< 0.01$ and $P< 0.05$ respectively). Furthermore, there were significant difference $(p< 0.01)$ in the mean of total number of times the animals entered arms (19.80±1.82, 19.09±1.73 and 12.13±0.63) for high dose, low dose and control group respectively.

3.4. Novelty exploration test:

A significant result in exploratory activities was found between treatments during novelty exposure. Number of both rearing and head dipping were significantly lower in high treated group (Table.1) ($p<0.05$) in comparison with the low dose Cd group and control group. Thus, a less degree of exploration was noticeably showed in rats with high doses of Cadmium intoxication in the novel environment.

3.5. Performance on psychomotor testing:

Complex motor behaviour (motor coordination), as measured by rod walk and plank walk, declined significantly in rats exposed to high concentration of Cd .(Table ,2) .

3.6. Biochemical examination :

There was a significant decrease ($p < 0.05$)in acetylcholinesterase activity in the brain of Cd treated groups (Table 3). Concerning brain oxidative state, significant decline was noted markedly in SOD , while no changes were recorded in GST level in brain of Cd intoxicated groups. .Also, there was a significant reduction ($p<0.05$) in the brain total protein of $CdCl_2$ treated groups (Table 3).

3.7. Histopathological examination:

The brain of rats treated with low dose and high dose of cadmium were macroscopically slightly congested. Microscopically, brain sections of rats treated with low dose of cadmium revealed neuronal degeneration, pyknosis of neurons (Fig.1) and neuronphagia of pyknotic neurons (Fig.2). Moreover, brain of rats treated with high dose of cadmium showed congestion of blood vessels , necrosis of neurons (Fig 3) , neuronphagia , focal gliosis (Fig .4) as well as hemorrhage in Virchow space (Fig.5) and necrosis of Purkinje cells of the cerebellum (Fig.6) .In hippocampus the pyramidal cells appeared atrophied and necrosed (Fig .7) . Meanwhile, brain of control, untreated rats, showed no histopathological changes (Fig.8).

3.8. Body weight and Brain weight:

Body weight was significantly ($p < 0.05$) low in groups of animals exposed to high concentrations of $CdCl_2$, compared to those exposed to low doses of $CdCl_2$ and in control group (Table.3).

Lower brain weight was significantly ($p<0.05$) seen in rats exposed to high and low concentration of $CdCl_2$ (1.82±0.13 gm and 1.82±0.23 gm) compared to rats in control group (2.38±0.63 gm) (Table 3) .

Table 1. Effect of exposure to different doses of $CdCl_2$ on measurements of Open-field test, Classic maze test, Y-maze and Novelty acquisition tests.

Group	Control	Low dose	High dose
Parameter			
Open –field test			
• No. of squares	26.70±2.22	36.16±1.59 [b]	57.90±8.26 [a]
• No. of rearing	7.02±0.82	9.78±1.68 [b]	19.61±3.13 [a]
• No. of pellets*	2.22±0.31	2.90±0.21	5.03±0.26 [b]
Maze test			
• time elapsed (min)	1.21±0.27	1.34 ±0.29	1.68 ±0.44 [b]
• No. of errors	3.11 ± 0.64	3.31 ± 0.58	3.53 ± 0.42
Spatial Y-maze			
• No. of arms	12.13±0.63	19.09±1.73 [a]	19.80±1.82 [a]
• % of s.alternations	60.70±3.36	39.21±4.25 [a]	36.99±3.45 [a]
Novelty exploratory test			
• No. of rearing	34.32±2.11	32.72± 2.33	27.70±2.22 [b]
• No. of head dips	18.91±1.22	18.14± 1.18	16.36±1.18 [b]

Figures in the same row with different letters are statistically significantly different (compared with the control group). [a] ($P<0.01$) and [b] ($P<0.05$), * :Fecal pellets.

Table 2. Effect of exposure to different doses of $CdCl_2$ on complex motor behavior (motor coordination).

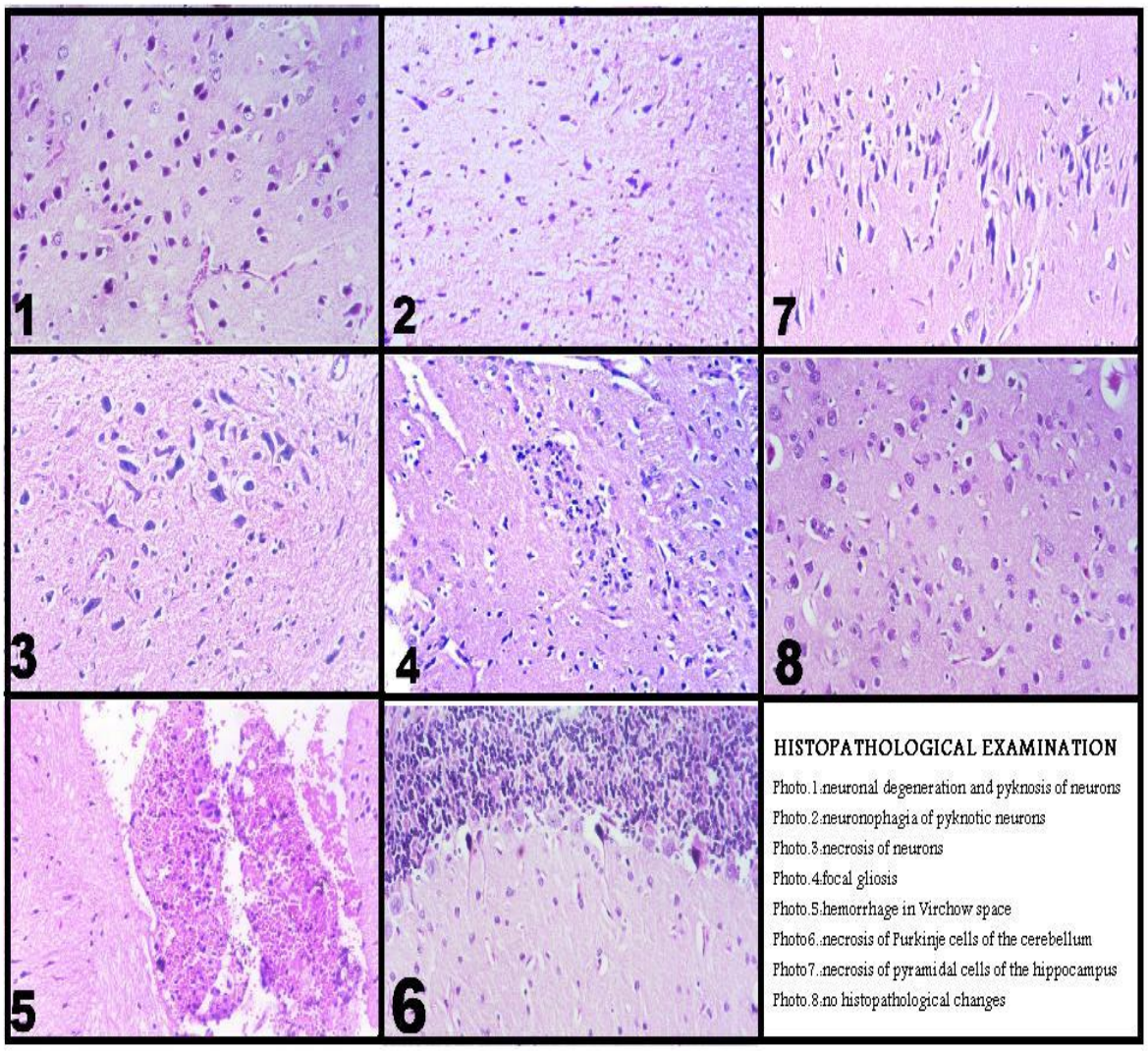
Group / Parameter	Control	Low dose	High dose
Rod walking (Latency to fall,sec)	26.58±3.77	25.61±4.55	20.15±4.58 [b]
Plank walk			
• Plank 1.3 mm			
1- No. of turns	3.50±0.27	2. 40 ±0.18 [b]	2.38 ±0.44 [b]
2- Dist.trv (cm)*	133.20±8.50	134.50±9.40	70.8±4.33 [a]
• Plank 2.5 mm			
1- No. of turns	2.90± 0.12	2.70±0.11	2.78±0.32
2- Dist.trv (cm)*	164.30± 4.22	112.14±5.44 [b]	134±6.22 [b]

Figures in the same row with different letters are statistically significantly different (compared with the control group). [a] ($P<0.01$) and [b] ($P<0.05$). * Dist.trv (cm):Distance traveled on the plank

Table 3. Brain, body weight and biochemical examination (acetylcholine esterase, Lipid peroxidation and total protein) of brain in rats exposed to $Cdcl_2$ through drinking water.

Group	Control	Low dose	High dose
Parameter			
Biochemical examination			
• Acetylcholine esterase (nmol/mg protein/min)	0.802±0.08	0.672±0.05 [b]	0.504±0.02 [b]
• lipid peroxidation			
- GST (unit/mg protein/min)	0.372±0.02	0.359±0.03	0.337±0.1
- SOD(unit/mg protein)	0.071±0.008	0.058±0.001 [b]	0.044±0.003 [a]
• Total protein (mg/gm brain tissue)	62.49±1.71	44.59±2.40 [b]	39.16±0.54 [b]
Brain weight(gm)	2.38±0.63	1.82±0.23 [b]	1.82±0.13 [b]
Body weight (gm)	260±6.27	238 ±4.29 [b]	226 ±4.44 [b]

SOD (superoxide dismutase enzyme) and GST (glutathione- s – transferase) were selected for measuring lipid peroxidation level in the brain (endogenous antioxidant defense). Figures in the same row with different letters are statistically significantly different (compared with the control group). [a] ($P<0.01$) and [b] ($P<0.05$).



Figures

(1): Microphotograph of brain of rat treated with low dose of cadmium showing neuronal degeneration and pyknosis of neurons (H & E stain X 200).

(2): Microphotograph of brain of rat treated with low dose of cadmium showing neuronophagia of pyknotic neurons (& E stain X 200).

(3): Microphotograph of brain of rat treated with high dose of cadmium showing necrosis of neurons (H & E stain X 200).
(4): Microphotograph of brain of rat treated with high dose of cadmium showing focal gliosis (H & E stain X 200).
(5): Microphotograph of brain of rat treated with high dose of cadmium showing hemorrhage in Virchow space (H & E stain X 200).
(6): Microphotograph of brain of rat treated with high dose of cadmium showing necrosis of Purkinje cells of the cerebellum (H & E stain X 200).
(7): Microphotograph of brain of rat treated with high dose of cadmium showing atrophy and necrosis of pyramidal cells of the hippocampus (H & E stain X 200).
(8): Microphotograph of brain of control, untreated rat showing no histopathological changes (H & E stain X 200).

4. Discussions

The main findings of the present study are learning impairment in the open – field habituation, maze learning test and spatial Y- maze memory which induced by higher cadmium chloride intake in male rats.

The results are in agreement with animal data that showed memory impairment in cadmium intoxication (Lehotzky et al; 1990). In the present study , the open field test provides simultaneous measures of both habituation and anxiety .Long term habituation to a novel environment is one of the most elementary forms of non – associative learning . In this study, where reduction in spatial exploration during test session was taken as an index for memory habituation (Montag-Sallaz et al .,1999). An impairment in the open field habituation was noticed in $CdCl_2$ treated groups. Moreover, animals treated with high cadmium were more fearful and highly anxious. Supportive evidence derived from increasing number of fecal boil. The latter was considered the most credible criteria for judging anxious animals.

In addition, associative learning in classic maze, declared that, rats with high doses of cadmium, demonstrated higher latency with increased numbers of errors in the maze reflecting a poorer memory retention relative to other treatments.

In Y- maze test, the treated groups of rats showed significant decrease in alternation behaviour scores in comparison with the control group and there was a significant difference in total number of times the animals entered the arms. Where groups of animals exposed to high concentrations of cadmium, showed higher frequency for entering arms. A proof that there was impairment in working spatial memory. These results confirmed that cadmium intoxication impair learning and memory. In a cadmium toxicity study for Baranski et al , (1983) , a decreased acquisition of avoidance behaviour and alterations in behaviour in open field in adult rats was noticed .

The neurotransmitters in the central nervous system have important roles in normal functioning and behaviour of the adult individual. They interact with each other in complex networks in the process of learning and memory, in which acetylcholine is proposed to have a central role (Decker and McGaugh ;1991). Acetylcholinestrase (AChE) is an enzyme that responsible for hydrolyzing and so deactivating acetylcholine in the body. It is a good indicator of sublethal toxicity by heavy metals (Forget *et al.*, 1999). Brain contains 2 forms of AChE, membrane bound forms constitute 90% of the enzyme and soluble form represents the rest 10% (Atack *et al*., 1986 and Mortensen *et al*., 1998). Level of the soluble form considered a simple and sufficient indicator of relative change of AChE in the brain (Muller *et al*., 1985 and Zakut *et al*., 1985) which measures the turnover of ACh activity (Sastry *et al*., 1983). Alterations in this enzyme level are indicative to impairment of cholinergic function (Slechta and Pokora, 1995). Results in this study revealed significant inhibitory effect on AChE activity in brain tissue which is in accordance with previous investigations of Gupta *et al*., (1993) as well as Antonio *et al*., (2003). Additionally, Murphy (1997) reported that exposure to cadmium generally impairs enzymes involved in the synthesis of neurotransmitters. Our results confirm the presence of an association between the cholinergic innervations and memory. Similar data reported by Flicker et al . (1983), where impairment of learning was evidenced by decreased cholinergic activity in brain .

Oxidative stress caused by different metals may damage certain tissues and liberate various transaminases into the plasma (Jackim et al ., 1970).Cadmium posses the ability to affect the activation of various signaling pathways and produce reactive radicals, which lead to oxidative stress state, resulting in DNA damage and lipid & protein oxidation (Ognjanovic et al ; 2008 ,Valko et al,2005). Also, Cadmium may be associated with the production of reactive oxygen species (ROS) (Szuster- Ciesielska et al, 2000; Liu et al 2002) .As lipid peroxidation was involved in the memory impairment , SOD and GST were selected for measuring lipid peroxidation level in the brain (endogenous antioxidant defense). In the present study, significant decrease in SOD enzymatic activities in brain tissues of rats administered $CdCl_2$ (high dose and low doses), which evidenced oxidative damage of brain tissues . The oxidative damage mechanism caused by Cd intoxication might be related to it's displacement to iron ((Fe^{+2}) and copper (Cu^{+2}) from cytoplasmic and cell membrane proteins with consequent elevation in their ions inside the cell leading to free radical generation . These like hydroxyl radicals , superoxide anions , nitric oxide and H 2O 2 (Koizumi et al .,1996, Casalino et al.; 1997, Ognjanovic et al .,1995 and Waisberg et al ., 2003). Those deplete the endogenous antioxidant defense (GST ,SOD,GSH , Peroxidase and Catalase) resulted in increased lipid peroxidation and DNA damage (Ognjanovic et al.,2003). Therefore, a significant oxidative stress caused by cadmium intoxication ,may be related to impaired learning ability .

Since. De novo protein synthesis and neurotransmitter system are critical event in memory formation (Davis and Squire ,1984; Milner et al; 1998; schafe et al ;1999; Wang et al ;2008), total protein content (TP)of brain tissues were measured in the three treated groups . Results revealed a significant decrease in total protein level in both low and high

doses of Cd. treated groups. Similar finding was recorded for rat's liver and kidney tissues in the study of Jadhav *et al*., (2007). This reduction in TP might be regarded to decreased protein synthesis due to hepatic dysfunction under heavy metal exposure (Ayensu and Tchounwou 2006, Goswami *et al*., 2005 and Mousa 2004). Also chronic renal diseases associated with heavy metal toxicity resulted in excessive loss of protein (Barbier *et al*., 2005, Madden and Fowler 2000). Moreover Cd binds to sulfhydryl group (SH) of many enzymes and inhibit the protein synthesis resulted in inhibiting of many enzymatic activities (Shaikh *et al*., 1999 and Waisberg *et al*., 2003).

The hippocampus and the cerebral cortex are the key structures of memory formation (Shirai and Suzuki; 2004), because the hippocampus is especially indispensable in the integration of spatial information. Since cadmium is classified as neurotoxic substance, our histopathological examination of the brain confirmed that hippocampus is the most affected region due to cadmium intoxication, as well as significant reduction in wet brain weight. Results showed congestion of blood vessels , neuronal degeneration , necrosis of neurons and neuronphagia (Fig.1,2,3) ,focal gliosis (Fig,4) as well as hemorrhage in Virchow space (Fig, 5) and necrosis of Purkinje cells of cerebellum (Fig,6).Moreover , the pyramidal cells appeared atrophied and necrosed (Fig,7). Jadhav et al (2007) , observed dose-dependent vascular , degenerative and necrotic changes in the brain of male rats exposed via drinking water to a mixture of metals (arsenic , cadmium ,lead, mercury ,chromium ,manganese , iron and nickel).The impairments of behaviours in relation to learning and memory may be due to the disturbance of the hippocampal circuit and its vast connections through cortical and subcortical pathways (Skutella and Nitsch,2001 **).** Also Deacon et al (2002) ,. has accounted that hippocampal lesions in general produce impairment in spatial memory.

Holland et al ;(1999) recorded that hippocampal lesions in general produce changes in rat's activity levels. In novelty acquisition during exploration, our results revealed a significant reduction in exploratory motor activity (EMA)in high $CdCl_2$ treated animals .This can be interpreted on the basis of increase emotionality in high concentration animals . Moreover, the animals in the novel environment were highly anxious and fearful .In Cadmium toxicity for Nation et al. (1990), a decreased movement and increased rest time was noticed. Also, Hans, (2006) observed skeletal deformations and flaccidity of muscles produced by cadmium in rats. The Agency for Toxic Substances and Disease Registry (2008) reported that acute oral exposure of cadmium in rats and mice resulted in weakness and muscle atrophy . This could be attributed to the symptoms of fatigue and disturbance of sensory motor function in individuals exposed to cadmium (Murphy, 1997). Desi et al. (1999) related the decrease of exploratory activity and a significantly lower exploration frequency of the open field centre in rats, to cadmium, which affects the bioelectrical and higher order functions of the nervous system .

In the present study, complex motor behaviour (motor balance)as measured by rod-walking and plank walking were significantly impaired in rats exposed to high concentration of Cd . Since these behavioural tests require the execution of complex coordinated movements, balance and strength, so this impairment may be attributed to the effect of cadmium on sensory motor capability. Supportive results derived from Viaene et al., (2000), who recorded that, workers suffered from peripheral neuropathy and complains about equilibrium in chronic occupational exposure to cadmium. Also, Ali et al. (1990) observed significant decrease in distance traveled, stereotypic time and movements, ambulatory time and vertical movements in Cd-exposed rats. Intermediate – duration oral exposure to cadmium caused weakness and muscle atrophy and significant decrease in motor activity .In addition , Murphy (1997) reported that individuals exposed to cadmium, showed increased symptoms as fatigue and disturbance of sensory and motor function. Since, Cadmium (Cd) is a neurotoxic metal, which induces oxidative stress and membrane disturbances in nerve system. . Claudia and Maria,(2005) confirmed that, Cadmium chloride increases oxidative stress in the skeletal muscle cell line c2 c12 and production of reactive oxygen species (ROS) in tissues and inhibits the activity of some enzymes of the antioxidative defense system (Sikic et al, 1997).

In the final, lower body weight was observed in our study in rats exposed to high daily doses of cadmium. Similar results derived from other studies with Cd treated animals (Smith et al .1985, and Gupta et al, 1993).

In conclusion, where, developing brain is greatly targeted to damage by toxic agents. Along with evidence derived from our study where exposure to cadmium constitutes a great threat being associated with neural injurious effects. Hence, concern should be directed to limit the inadvertent incorporation of cadmium in human – consumed products.

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