Effect of Cadmium Pollution on Neuromorphology and Function of brain in Mice Offspring

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Abstract: Cadmium chloride ($CdCl_2$) was given to Swiss-Webster pregnant female mice at the concentrations of (50mg/L) and (100mg/L) (w/v) respectively, in their drinking water. Treatment started few days before pregnancy and it continued until delivery and weaning the offspring. After the weaning period (22 days), all male offspring were isolated and subjected to "Standard Opponent" test at the age of 25 days. The results of this test showed a significant and dose-dependent increase in the non-social behavior, such results showed a significant decline in the social behavior including naso-genital and naso-nasal contact, number of fights, rear, wall rear and displacement activities of the Cd exposed groups. Brain impairments due to the neurotoxic effect of Cd treated groups were indicated by histopathological investigation and neurochemichal analysis. The present prenatal Cd effects in the male offspring are possibly via in utero exposure and/or via mother's milk.
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1. Introduction:

Prenatal exposure to neurotoxicants may affect brain development and function. Neurotoxic agents present in the environment and especially in the food chain may reach the brain of the fetus or the newborn during critical periods of brain development. These agents may affect cerebral function and development resulting in long-lasting or permanent deficits in cerebral function that can be reflected at different ages (youngness, adulthood or elderly) as alterations in motor function or coordination and/or altered intellectual function with alterations in learning ability and/or memory. These neurological alterations would be consequence of alterations in the function of one or more [1].

Heavy metals discharged as waste products from different industries are considered a major source of environmental pollution. Cadmium (Cd) which is one of these pollutants has taken considerable attention for its great different toxic effects on living individuals. Cadmium toxicity is increasing in incidence today for several reasons. One of the primary reasons is zinc deficiency in many commonly eaten foods. Zinc, which is protective against cadmium, is becoming increasingly deficient in the soil and consequently in food. Food processing and eating of refined food further reduces zinc intake.

Exposure to cadmium is also increasing due to its use as in iron coating, steel and copper. It is also used in copper alloys, stabilizers in rubber and plastics, cigarette papers, fungicides and in many other products. Often these industries cause water, air and food pollution with this metal [2]. Cadmium used in industry finds its way into many water supplies. In addition, soft water is more dangerous since the calcium in hard water has a protective effect. Old galvanized pipes and new plastic (PVC) pipes are sources of cadmium in our drinking water. Cadmium is unique among other metals because of its toxicity at a very low dosage and long biologic half life and its low rate of excretion from the body [3]. It is listed by the U.S. Environmental Protection Agency as one of 126 priority pollutants.

Neurotoxicity appears in a variety of neurochemical and behavioral changes [4, 5]. This neurotoxicity was still not regarded to certain specific reason; Cd may exhibit several effects on neural level concerning neurochemical mediators [4, 6] Assembly of cell membrane proteins and phospholipids may also be affected under Cd toxicity [7, 8].

The aim of our present study was to asses the impact of cadmium -exposure present in the environment on some brain functions in mice off spring with special reference to neurobehavioral effects.

2. Materials and Methods

Experimental animals

Adult male and female Swiss-Webster mice were used (n=50, BW 25±5 g), the animals housed in opaque plastic cages (three females with one male in each cage) in the Animal House Unit in Faculty of Veterinary Medicine, Cairo University. Animals were

kept under reversed lighting conditions with white light on from 22.30 to 10.30 hours local time. The ambient temperature was regulated between 18 and 22 C. On pregnancy (appearance of vaginal plug was considered as day one of pregnancy), the males were removed from the cages and the females were exposed to experimental treatments. Treatment started few days before pregnancy and it continued until delivery. Food (Standard Diet) and water were available adlibitum.

Cadmium administration

Cadmium chloride (analytical grade, Riedel de Haen, Germany) was dissolved in deionized distilled water in the concentrations of (50 mg/L) and (100 mg/L) (w/v). These cadmium concentrations formed the sole drinking fluid source for the experimental group of dams during the prenatal period of the experiment. The drinking fluid containing cadmium concentrations were changed with fresh preparations every five days. The control groups were received deionized distilled water only. All pregnant mice were housed individually. Treatment of mothers was started few days before pregnancy and it continued until delivery pregnancy. After weaning at the age of 22 days, 25 male offspring from each treatment category and control group were subjected to the tests at the age of 25 days .Puberty is around day 25-30 in mice, estrogen has been shown to affect non-reproductive behaviors in humans and rodents, including anxiety, fear, and activity levels [9],therefore "Standard opponent" tests were carried out on male offspring.

"Standard Opponent" Test

"Standard opponent" tests under dim red lighting (ca. 8 lux) as described by [10] . The docile and age-matched male "standard opponents" were rendered anosmic by applying 25µl of 4% zinc sulphate solution to the nasal tract under ether anesthesia for three days prior to encounters [11]. The anosmic 'standard opponent' intruders were introduced in the home cages of 'test animals' and the "standard opponent" test of each 'test animal' was observed visually for 500 seconds. The opponents were used only once and the selected "elements" of behavior were studied [12].

Biochemical Analysis

All the mice were weighed and sacrificed by cervical decapitation. Brains were cut into two sagittal pieces with surgical scalpel, and tissues of at least five animals were pooled to prepare enough samples for biochemical estimation. Samples were then diluted tenfold and the homogenate was spun at 10,000 rpm for 15 min and the supernatant was used for enzymatic assay. Biochemical estimation by standard methods was conducted for AchE and BchE [13], MDA [14] and ascorbic acid [15] in brain tissues of control and Cd-treated animals.

Cadmium Determination

Each sample was represented by one gram of tissues dissected from the brain of at least five animals were pooled to prepare enough samples for determination of Cd in brain tissues. The samples then placed in a clean screw-capped tube and digested according to the method described by [16]. The obtained solutions were then analyzed by using Air/ Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer) for determination of cadmium levels in examined samples.

Histopathological examination

Histopathological examination in brain of control and Cd-treated animals. Tissue specimens from brain of all experimental mice were collected and fixed in neutral buffered formalin, processed by conventional method, embedded in paraffin, sectioned at 4-5 µm and stained by Haematoxylin and Eosin [17].

Statistical Analysis

Data of "Standard opponent" tests were compared within the experimental groups by ANOVA test and Mann- Whitney U test [18].

3. Results

"**Standard Opponent**" **Test**

Results of the various treatments are shown in Tables 1 and 2. The behavioral data (mean ± SE) in the "Standard Opponent" test of male offspring are given in (Tables 1 and 2). Almost all of the elements of behavior were affected by prenatal $CdCl_2$- exposure and these effects were statistically significant in a dose-dependent manner. The non-social and social behavior of the exposed offspring were significantly and dose-dependently affected showing an increase in the former and a decrease in the later, respectively. The elements of social behavior including attack, numbers of fights, naso-nasal and naso-genital contacts, wall rears and rears were decreased significantly in a dose-dependent manner. The latencies to threat and attack were also increased significantly and dose-dependently. Overall, the results indicate that the social behavior is significantly decreased due to the prenatal $CdCl_2$-exposure in a dose- dependent manner. Conversely, the nonsocial behavior and its elements like wall rears and rears, in young male adult offspring were increased significantly and dose- dependently.

Table 1 . Effect of the prenatal cadmium exposure on the social behavior of male laboratory mice offspring, (Number of seconds allocated to behaviors like)

Parameter	Nonsocial Investigation	Social Investigation	Defense	Threat	Attack	Displacement
Group						
Control	114.7±11.02	228.3±6.44	4.2±0.9	8.4± 1.20	120.4±11.0	24.9±4.20
W/v (50mg/L)	290.31±11*	136.8±12**	5.9±0.5	10.98±1.2	35.88±3.6*	18.7±1.3
W/v (100mg/L)	364.1± **	110.4±5.2 **	3.5±1.8	13.4±2.2	4.0± .82**	6.5±0.68*

*and ** statistically significant at $P < 0.05$, and $P < 0.01$ respectively from the control by Mann-Whitney U-test. LD_{50}=110 mg/kg BW oral (cadmium chloride) WHO (1992). The average daily intake of cadmium was estimated to be 4487 µg/kg BW and 8974 for the groups receiving 50 and 100 mg/kg, respectively)

- WHO (1992) and Agency for Toxic Substances and Disease Registry (1999)

Acetylcholinestrase and Butyrylcholinesterase

Activities of AchE and BchE (Acetylcholinestrase and Butyrylcholinesterase) showed a concentration-dependent decrease in all the tissues studied (Tables 3.).

Excessive intake of Cd from $CdCl_2$ in drinking water significantly reduces AchE and BchE activity in the brain tissues. This decrease could be due to loss of neuron cell bodies in the brain, loss of synaptic structures, or inhibition of enzyme activity. These effects could be corroborated with cognitive dysfunctions observed in experimental animals.

MDA

Significant decrease in free radical scavenging enzymes and a concomitant increase in lipid peroxidation (MDA) in brain tissues studied suggests an increase in oxidative stress after excessive Cd intake. Such increase in free radicals in neuronal cell bodies could be correlated with loss of neurons in brain and synaptic structures in neuromuscular junctions.

The impairments due to the neurotoxic effect of Cd in brain of mice treated with high dose (100 mg /L) were indicated by histopathological investigation. The impairments included congestion of blood vessels, necrosis of neurons (Fig.A), neuronphagia (Fig.B) and focal gliosis (Fig.C) as well as haemorrhage in Virchow space. Moreover, in hippocampus the pyramidal cells appeared atrophied and necrosed (Fig. D).

Ascorbic acid

A parallel increase in ascorbic acid content (Table 3.), an important antioxidant in brain after Cd intake, is difficult to explain. An increase in ascorbic acid content suggests its role in amelioration of stress.

Concentrations of cadmium in the brain tissues

The concentrations of cadmium in the brain tissues after 52 days of treatment were 10 µg/g and 65 µg/g of (50mg/L) and (100mg/L) (w/v) respectively, in their drinking water (< 0.05 vs. control). While, the mean concentration of Cd in the brain tissues of control group was 2 µg/g.

Table 2. Effect of the prenatal cadmium exposure on the number of various acts and postures of social behavior of male mouse offspring in a 'standard opponent test'. [Mean number and latency (mean±SE) of acts and postures]

Parameter / Group	Latency to Threat (sec)	Latency to Attack(sec)	Number of fights	Number of Naso-Nasal	Number of Naso-Genital	Wall contact	Rears contact
Control	20	96	20	28	29	23	11
W/v (50mg/L)	90*	140*	8 *	19 *	18*	10 *	7*
W/v % (100mg/L)	260**	280**	2 **	10**	8**	4**	2**

* and ** statistically significant at $P < 0.05$, and $P < 0.01$ respectively from the control by Mann-Whitney U-test.

Table 3. Effects of Cd in drinking water on various parameters in the brain tissues of Mice

Group / Parameter	Control	50 mg/L (A)	100 mg/L (B)
AchE (ΔOD)	0.10± 0.01	0.059±0.02*	0.024±0.006**
BchE (ΔOD)	0.09± 0.007	0.018±0.013*	0.028±0.026**
MDA (nmoles/mL)	1.95± 0.42	1.31±0.38*	4.87±0.66**
Ascorbic acid (mg/100mL)	0.89± 0.32	1.73±0.19*	2.44±0.26**

P<0.01;. Comparison between: Control with A and **P<0.05; *Data represented as mean ±S.E. control with group B. AchE (ΔOD): Acetylcholinesterase Absorbance/min · mg protein. BchE Δ(ΔOD).

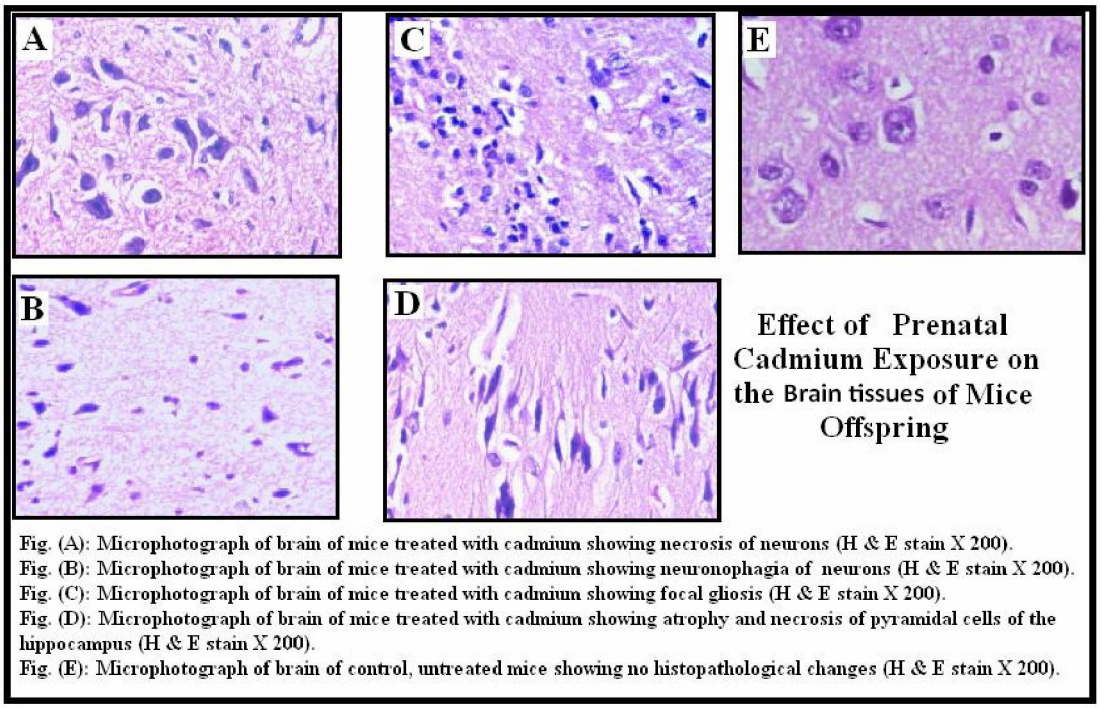

Fig. (A): Microphotograph of brain of mice treated with cadmium showing necrosis of neurons (H & E stain X 200).

Fig. (B): Microphotograph of brain of mice treated with cadmium showing neuronophagia of pyknotic neurons (H & E stain X 200).

Fig. (C): Microphotograph of brain of mice treated with cadmium showing focal gliosis (H & E stain X 200).

Fig. (D): Microphotograph of brain of mice treated with cadmium showing atrophy and necrosis of pyramidal cells of the hippocampus (H & E stain X 200).

Fig. (E): Microphotograph of brain of control, untreated mice showing no histopathological changes (H & E stain X 200

4. Discussion:

The present results clearly suggest that after the weaning period, various behavioural indices in young male offspring were affected in the "Standard Opponent" test. Thus, these results clearly emphasize that pre and postnatal cadmium exposure is extremely dangerous. A strong correlation exists between maternal and umbilical cord blood cadmium levels indicating prenatal transfer of cadmium from mother to developing fetus in utero [19]. Cadmium can enter into the brain parenchyma and neurons during the critical point of development [20] causing neurological alterations [21, 22]. [23] described the toxic result of cadmium exposure in the form of morphological development, sensory motor reflexes, biochemical and behavioral outcomes in rodents. It is now well documented that significant quantities of compounds that are given to mothers during late pregnancies and during postnatal period, may be transmitted to the offspring in utero and /or via mother's milk during lactation [24-28,19]. It is known that a major portion of brain cells (70%) of the closely related rats are formed after birth [29]. Furthermore, it has been established that the hippocampus in the brain may be most vulnerable to the neurotoxicity of neurotoxic materials in the very rapid growth period. The hippocampus and the cerebral cortex are the key structures of memory formation [30-33], because the hippocampus is especially indispensable in the integration of spatial information.

There were significant differences in hippocampus and the Opponent" test, in mice which received high dose of Cd (100 mg / L) and untreated group (control). The Opponent" test has been associated with hippocampal activity and cholinergic activity neurotransmission (learning ability and motor activities) Many investigators have reported that hippocampus is one of the most vulnerable regions in the AD brain [34] and hippocampal lesions in general produce changes in rat's activity levels [35] and impairment in spatial memory [36]. So, we could be stated that, hippocambus is the main target of CNS due to Cd exposure. The impairments due to the

neurotoxic effect of Cd in brain of mice treated with high dose (100 mg /L) were indicated by histopathological investigation (Fig. A-E) showed congestion of blood vessels, necrosis of neurons), neuronphagia, focal gliosis as well as the pyramidal cells appeared atrophied and necrosed especially in hippocampus (Fig.D).

Histological changes were observed in rat organs exposed to 8 mixtures of metals [37]. Cadmium causes hemorrhages in the autonomic ganglia with cell necroses and damage to nerve cells and nerve fibers. [4] observed changes of drinking behavior in rats exposed to acute Cd intoxication. There were significant decreases *(P< 0.05)* in the activity of acetylcholinestrase. (AchE) enzyme in the brain of Cd administered groups (Table 3). [6] observed enzymatic changes in rats exposed to lead intoxication.

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 [38]. Brain contains 2 forms of AchE, membrane bound forms constitute 90% of the enzyme and soluble form represents the rest 10% [39, 40]. Level of the soluble form considered a simple and sufficient indicator of relative change of AchE in the brain [41, 42] which measures the turnover of Ach activity [43]. Alterations in this enzyme level are indicative to impairment of cholinergic function [44]. Our results revealed significant inhibitory effect on AchE activity in brain tissue of mice offspring which is in accordance with previous investigations of [45- 47] in rats.

Cadmium inhibits release of acetylcholine, probably by interfering with calcium metabolism [37]. Cadmium can enter into the brain parenchyma and neurons [20] causing neurological alterations in human [21] and animal models [22].

This study suggested that, exposure to cadmium present in the environment and especially in the food chain may reach the brain of the fetus or the newborn during critical periods of brain development and could be produced cumulative developmental abnormalities in the brain prenatally, which is expressed in the young adult male offspring in disturbed form of long lasting social behavioral outcomes and learning ability. It seems that the impairments of behaviors in relation to learning and memory are due to the disturbance of the hippocampal circuit and its vast connections through cortical and subcortical pathway.

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