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RESEARCH ARTICLE

NEUROBEHAVIOURAL AND BRAIN HISTOLOGICAL EFFECTS IN SUCKLING PUPS OF NURSING RAT DAMS ORALLY DOSED WITH MERCURIC ACETATE DURING PREGNANCY AND LACTATION PERIOD

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ABSTRACT

Thirty female albino rats and their pups daily dosed with mercuric acetate during pregnancy and lactation period, rats equally divided into three groups given 0ral doses for T1 (1mg\kgb.w), T2 group given (5mg/kg b.w). Of mercuric acetate and control group which given distilled water during a period of experiment. The neurobehavioral results were obtained in 10 randomly selected suckling pups at the end of lactation period showed significant decrease in locomotor activity and exploration, stimulation in autonomic nervous system activity (number of bolus faces), defect in vestibular function, disturbance in proper reflex, defect in degree of cognitive function and neuromuscular coordination, Microscopic examination results showed that the severity of the brain pathological lesions increased with increasing standard mercuric acetate doses received through their dam's milk or transferred through placenta during pregnancy exposure.

INTRODUCTION

Mercuric acetate (MA) is avery toxic poisonous form of mercury with the formula C4H6O4Hg which induces severe alterations in the tissues of both animals and human being (Mohboob *et al.*, 2001) it used as ingredient in dental amalgams. and it compounds are found in some over-the-counter drugs, including topical antiseptics, stimulant laxatives, diaper-rash ointment, eye drops, cosmetic products ,and nasal sprays also used in barometer ,manometers ,mercury switches fluorescent lamps thermometers (parker *et al*,2004). The prolonged contact may cause skin sensitization, have effects on the central nervous system peripheral nervous system and kidneys, resulting in ataxia, sensory and memory disturbances, tremors, muscle weakness and kidney impairment (ATSDR, 1998). Mercuric acetate cross the Blood Brain Barrier (BBB) and are distributed in the central nervous system (CNS) accumulates primarily in the kidneys, liver, spleen, bone marrow, intestine, skin and respiratory mucosa (E U, 2007). Infants and small children are particularly vulnerable because of the risk of severe injury to the developing brain (Caroline *et al.*, 2009). Mercury poisoning has been reported in human following exposure to metallic mercury and its organic and inorganic derivatives a form of poisoning is Minamata disease which is a disease of the central nervous system, caused by the consumption of fish it is not an infectious disease transferred by air or food, neither is it genetically inherited (Kelly *et al.*, 2006). Mercury poisoning incident in iraq that began in late 1971. Symptoms similar to those seen when Minamata disease affected Japan. The 1971 poisoning was the largest mercury poisoning disaster when it occurred (Al-Damluji, 1976). The objective of this study was to evaluate the mercuric acetate neurotoxic effect in suckling rat pups of dosed dams during pregnancy and lactation period. Also studying the gross and histopathological lesion in adult and pups rat brain.

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MATERIALS AND METHODS

In this study sixty (60) Albino Wistar rats of both sexes were used at weight range of (250-300) grams and their pups placed in aspecial housing room in the animal house of College of Veterinary Medicine-Baghdad University where the research was done. Housing conditions was maintained at (20-25) ºC in air conditioned room, the room was ventilated by using ventilation vacuum, The light-dark cycle was 14\10 in housing place. The litter of the cages was changed weekly (Hafes, 1970). Special rat feed pellet and water provided. Normal female rats were mated with mature males of the same strain and they were separated during pregnancy in to separate cages for lactating period, At birth dams were separated with their pups from the first day of parturition. In this experiment, rats divided equally into three groups: each with Twenty adult rats that sub divided equally for ten males and ten females orally dosed with mercuric acetate daily during pregnancy and lactation period at 1mg\kg body weight representing (T1group) .the other twenty adult rats dosed orally with fifth fold of toxic dose 5mg\kg body weight representing (T2group).the control group(C group) were given the distilled water throughout the experimental period. Ten pups from each groups were selected

randomly for neurobehavioral examination at the end of lactation period which included the following testes :-

1- Rota Rod test: This test reflects neuromuscular coordination or ataxi (AL-Bakkoh, 2002).
2- Open field test: This test evaluate the general locomotors activity, exploration (squares crossed by four legs of animal forward and backward) rearing and also including frequency of defecation and urination during 3minutes. the apparatus cleaned after each trying (Moser, 1992; Foad, 2000).
3- Cleft avoidance test: This test useful for detection of the proper reflexes in testing rat pups (Mohammed, 1984). The rat pups put close to cleft of table with height more than 90cm, the time needed by rat pups to run away from the edge was recorded.
4- Righting reflex test: This test evaluate the motor activity of rat pups (Mohammed, 1984).
5- Swimming rank test: This test reflects the integration of brain function by monitoring each animal for 5-10 seconds for swimming in the pool (for adult rats measurement was (70x40x40cm) and the evaluation carried out by grades as following (Schapiro and Vakovich, 1970). Grade (0): when the nose is under the plane of water. Grade (1): the nose with plane or above the plane water. Grade (2): the nose and crown with or above the plane of water while the ears are under. Grade(3): as in grade 2 but the plane of water at the mid of ears. Grade(4): as in grade 3 but the plane of water under the ears.

Histological study

At the end of lactation period pups of each experimental group T1, T2 and control group sacrificed and examined grossly. Specimens were taken from target organs the brain, kept in 10% formaldehyde solution for fixation, the slides stained with hematoxylin and eosin stain and examined by using light microscope. (Luna, 1968).

Statistical analysis

Of the experimental results were conducted according to statistical package for the social sciences (SPSS) VERSION 13.00 where ANOVA was used to significance of changes between treated adult rats and their pups and control. The data were expressed as Mean ± Standard Errors (SE) and p- value <0.o5 was considered statistically significance. LSD was carried out to test the significance level among means of treatment (Joda, 2008).

RESULTS AND DISCUSSION

Open field test in rat pups of dosed rat dams

A- Number of squares crossed: The result of the number of squares crossed per 3 minutes in rat pups showed a significant decrease (P<0.05) between T1 and T2groups in dose dependant manner in comparison with control one, table (1).
B- Number of bolus faces: The result of the number of bolus faces per 3 minute in rat pups showed significant increase (P<0.05) between groups T1, T2 in dose dependant manner in comparison with control one at the end of lactation periods , table (1).
C- Number of urine frequency: The result of the number of urine frequency per 3 minute in rat pups showed no significant change (P>0.05) between treated group T1, T2 and the control one (1).

Table 1. Open field test of rat pups dosed by nursing mothers with two different doses of MA during pregnancy and lactation period

Parameter / Group	Number of squares crossed \3 minutes	Number of bolus faces\3minutes	Number of urine frequency\3minutes
Control (C)	65.71± 4.21 A	1.26 ± 0.46 A	0.20 ± 0.33 A
T1 1mg\kg	47.80 ± 6. 53 B	2.40 ± 0.22 B	0.24 ± 0.52 A
T2 5mg\kg	35.60 ± 6.32 C	2.93 ± 0.73 C	0.29 ± 0.16 A

LSD=5.33 LSD=0.43 LSD=0.26
M±SE=mean+ standard error.
N= 10 randomly selected -Different capital letters represent significant results (P<0.05) between different groups.

The results of open field tests indicated that the autonomic nervous system (mostly cholinergic) was affected during the course of experiment on pups of both treated group as recorded a change in number of bolus feces, while other tests only indicate an effect in peripheral and central nervous system at the end of lactation as noticed in result of rearing**,** number of urine frequency and number of crossed squares.

Cleft avoidance test

The results of cleft avoidance test (per 60 second) in rat pups at day14 and the of lactation period T1 and T2 group showed a significant increase in time of cleft avoidance (P< 0.05) between them and in comparison with the control group. Table (2).

Table 2. Cleft avoidance test/seconds of rat pups dosed by nursing mothers with two different doses of MA during pregnancy and lactation period

Period / Group	After 14 days of lactation Mean ± SE	End of lactation period Mean ± SE
Control (C)	18.46 ± 0.40 A a	18.40 ± 0.34 A a
T1 1mg\kg	20.25 ± 0.21 B b	22.30 ± 0.43 B b
T2 5mg\kg	35.40 ± 0.31 C c	36.72 ± 0.31 C c

M±SE=mean+ standard error. N= 10 randomly selected pups -Different small letters represent significant differences (P<0.05) between period. Different capital letters represent significant differences (P<0.05) between groups

Righting reflex test

The results of righting reflex test showed that at day14 and end of lactation T1 and T2 groups reported a significant increase in time of righting reflex (P<0.05) between them in dose

dependent manner and in comparison with the control group. Table (3).

Table 3. Righting reflex test/seconds of rat pups dosed by nursing mothers with two different doses of MA during pregnancy and lactation period

Period / Group	After 14 days lactation Mean ± SE	End of lactation period Mean ± SE
Control (C)	0.43±0.22	0.40±0.24
	A a	A a
T1	1.29±0.24	1.45±0.23
1mg\kg	B b	B b
T2	1.80±0.30	1.92±0.31
5mg\kg	C c	C c

LSD=0.35 M±SE=mean+ standard error. N= 10 randomly selected pups
-Different small letters represent significant differences (P<0.05) between period. Different capital letters represent significant differences (P<0.05) between groups.

Rota rod test

The result of Rota rod test (run per second) in rat pups showed significant decline (P>0.05) between groups T1, T2 in dose dependent manner after 14 days and end of lactation period in comparison with the control group Table (4).

Table 4. Rota rod test (Run /second) of rat pups dosed by nursing mothers with two different doses of MA during pregnancy and lactation period

Period / Group	After 14 days lactation Mean ± SE	End of lactation period Mean ± SE
Control (C)	6.16 ± 0.91	4.46 ± 0.89
	A a	A a
T1	3.20 ± 2.24	2.00 ± 1.73
1mg\kg	B b	B b
T2	2.80 ± 1.30	1.15 ± 2.33
5mg\kg	C c	C c

LSD= 2.17 M±SE=mean+ standard error. N= 10 randomly selected pups
-Different small letters represent significant differences (P<0.05) between period.
-Different capital letters represent significant differences (P<0.05) between groups.

This result indicates defect in neuromuscular coordination and ataxia in animal exposed to mercuric acetate during the course of experiment in dose dependent manner.

Swimming rank test

The results of swimming rank test showed that at 14 days of lactation T1 and T2 showed no significant change in swimming rank test, while T2 showed a significant decrease in swimming rank at the end of lactation in comparison with T1and control group. Table (5).

The results of neurobehavioral parameters of rat pups at the end of lactation period showed significant change in behavioral test results in both treated groups that were accordingly with their dam MA doses with the exception of swimming rank test that showed no change in its results.MA is

Table 5. Swimming rank/grade of rat pups dosed by nursing mothers with two different doses of MA during pregnancy and lactation

Period / Group	After 14 days lactation Mean ± SE	End lactation period Mean ± SE
Control (C)	3.52±0.42	3.46±0.20
	A a	A a
T1	3.36±0.24	3.15±0.23
1mg\kg	A a	A a
T2	3.20±0.30	2.13±0.62
5mg\kg	A a	B b

LSD= 0.46 M±SE=mean+ standard error N= 10 randomly selected pups
Different small letters represent significant differences (P<0.05) between period.
Different capital letters represent significant differences (P<0.05) between groups.

a potent teratogen in human, rodents, chickens and pig (Clarkson, 2003). Both teratogenic and reproductive effects have been demonstrated. MA crosses the placenta and is also transferred to newborn rats and mice via lactation (Gerhard *et al*., 1998). In the fetus, the major target is the developing central nervous system, thus MA is also considered a neurotoxic compound. our results may be attributed the reported ability of MA to penetrate blood brain barrier (BBB), This penetration occur in the time when pups BBB is still immature leading to easier pass of enough toxic amount of MA to cause extensive damage in accordance with their dam doses. It has been reported that in developing rats BBB maturation is complete around the third postnatal week (Camerino *et al*., 2002). Catecholamines, including dopamine and norepinephrine, are the principal neurotransmitters that mediate a variety of the central nervous system functions such as motor, control, cognition, emotion, memory, processing and endocrine (Grandjean and Landrigan 2006). Dopamine is a crucial for motor control and emotional learning during postnatal development while norepinephrene is required for the latent learning and long term memory formation of conditioned learning. The effect of other neurotransmitters like serotonin, Ach, GABA, glutamate abnormalities in the regulation of neurotransmitter release abnormal level of extracellular neurotransmitter concentration have remained core components of hypothesis on the neuronal foundation of behavioral disorder and the symptoms of neuropsychiatric and neurodegenerative disorders. It was showed that activation or inhibition of neurotransmitters cause a significant alteration in performance of human and laboratory animals (Villegiar *et al*., 2006). The brain histopathological results indicated that the damage in different brain areas due to MA toxic effect were in dose dependent manner also may explain the change in neurotransmitters synthesis and function that may contribute in the neurobehavioral changes seen in the treated rat pup groups. The cerebellum modulation and coordination of muscular activity are important in skilled voluntary movement as well as in the movement and posture equilibrium (Heyer *et al*., 2004).

Brain changes in rat pups

The Microscopically section in the rat pups brain at the end of lactation period for nursing dams orally dosed daily with 1mg\kg MA (T1group) showed Wlleran degeneration of axon

characterized by irregular bullous empty space with few microglial cells (Figure1) and increase number, size of astrocytes (astrogliosis) were seen,in addition to edema in the perivasculr space of congested blood vessels and perineural space as well as pair astrocyte Nuclei surrounded by clear space (Alzheimers, type II astrocytes) were recorded in white matter of cerebellum, also proliferation of oligodentrocytes were noticed and congestion of blood vessels in the brain parenchyma and perineural edema (Figure 2). In the section of rat pups brain for nursing mother orally dosed with 5mg\kg of mercuric acetate, the histoological examination found that the wallerian degeneration of the axon was associated with central chromatolysis which characterized by rounded pale swelling of purkinje cells, eccentric nuclei and dispersed Nissl substances (Figure 3), in addition to congested blood vessels in brain parenchyma and perineural edema, in other animal, irregular cavity in brain parenchyma surrounded by few astrocytes was reported as well as proliferation of interfascular oligodendrocytes. In other section, there were vasogenic edema characterized by widening of perivascular space and fluid filled space around the neuron cells, in addition, marked congested blood vessels with neutrophils in their lumen were recorded (Figure 4).

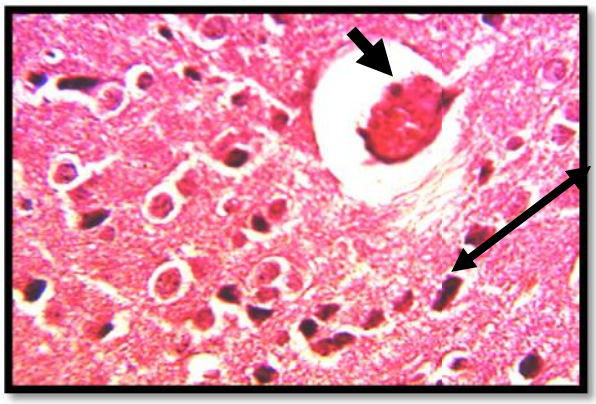

Figure 1. Section in brain of rat pups for nursing dams orally dosed daily with 1mg\kgBw of MA, showed edema in the perivascular space of congested blood vessels and proliferation of interfascular oligodendrocytes ⟷ (H&E stain 40X)

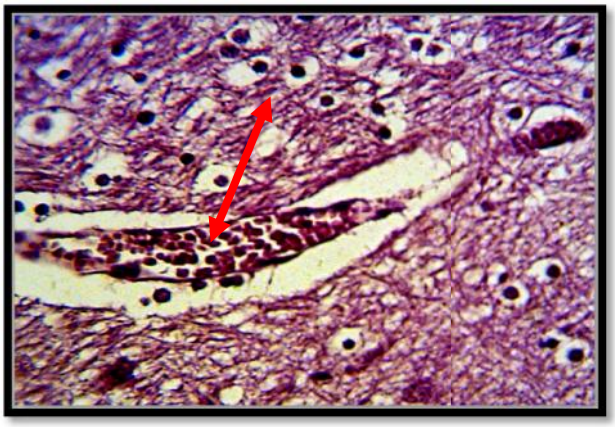

Figure 2. Section in brain of rat pups for nursing dams orally dosed daily with 5mg\kgBw of MA showed congestion of blood vessels in the brain parenchyma and perineural edema (H&E stain 40X) ⟷

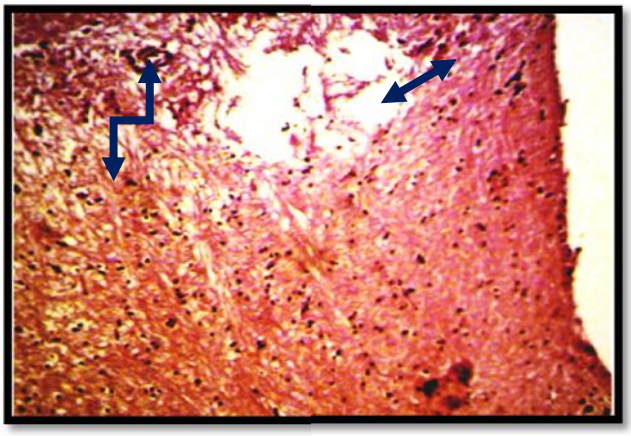

Figure 3. section in brain of rat pups for nursing dams orally dosed daily with 5mg\kgBw of MA showed irregular cavity in brain parenchyma surrounded by few astrocytes congestion blood vessels and proliferation of interfascular oligodendrogli (H&E stain 40X)

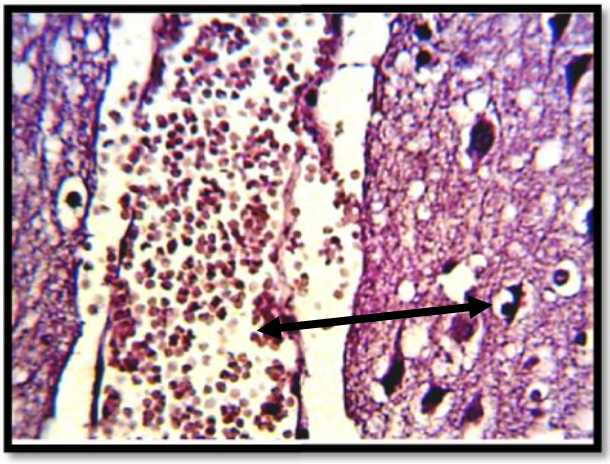

Figure 4. Section in brain of rat pups for nursing dams orally dosed daily with 5mg\kgBw of MA, showed congested blood vessels with neutrophils in their lumen and perineural edema (H&E stain 40X) ⟷

The results of this study showed that brain of rat offspring prenatally exposed to MA by dosed dam mother contained more effect, These results may be attributed to the ability of MA to penetrate BBB of neonate easier than adult one. This penetration occurs at the time when BBB is still immature during lactation period and because of the presence of high enough concentration MA in milk that able to be transferred to the relatively small sized pups groups and then through their brain in dose dependent manner leading to induce proportional degree of damage in different important brain tissues including cerebellum and hippocampus, responsible for cognitive, memory and also may interfere with their release and function to important CNS neurotransmitters. The neurological damage occur without continuous or heavy exposure (Yoshida 2002). The recent study in rat indicated that MA has neurotoxic effects reflected in its ability to penetrate and damage BBB system (Crinnion 2000). The direct effect of MA on dopamine and other CNS neurotransmitters that may affect the brain function responsible for the tested neurobevioural activity. It

has been reported that in developing rats BBB maturation is complete around the third postnatal week (Daisuke *et al*., 2003). The histopathological results indicated that damage in the different brain areas, due to MA toxic effect was in dose dependent manner supported such assumptions also may explain the change in neurotransmitters synthesis and function that may contribute in the neurobehavioral changes seen in the both T1 , T2 rat pups groups (Satoh. 2003). In the current study it was expected that it MA easily penetrates BBB and cause damage in brain tissue in dose dependent manner. At the end of this study one would say that a cortical structural abnormality in subjects maternally exposed to mercury compounds, which could explain the neurobehavioral abnormality associated with them, let us include the possibility of transfer of MA from dosed pregnant rat dams to the growing fetuses during pregnancy and then to the pups brain that may contribute in increasing deposition of MA in brain of pup groups coming from dosed dam milk in dosed dependent manner.

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