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Effect of Administration of Mercuric Chloride on the Social Behavior, Neuromuscular Coordination, Motor Activity, Blood Parameters and Liver Structure Alterations in Mice Offspring

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ABSTRACT

The present study was carried out to investigate the exposure of mercuric chloride on mice offsprings' social behavior, neuromuscular coordination, motor activity, blood parameters and liver structure alterations. On the first day of pregnancy, 30 pregnant mice were equally divided into three groups. Tap water was provided to the control group (Group I). Group II and Group III animals were orally received 5 and 10 ppm of $HgCl_2$.The treatment started the first day of pregnancy and continued to the day 15 after birth thearafter the dams were sitched to tap water. The behavioral results showed a significant increase in nonsocial investigation while social investigation and defense activities were decreased significantly. Results indicated that both latencies, to first threat and first attack were increased significantly, while the duration time of flight, number of fights, nasonasal, nasogenital and rears were decreased significantly. The latency to first bite was significantly increased, however the number of bites was decreased dramatically when compared with control. Motor activity and grip strength data were decreased significantly in exposed offsping. Interestingly, blood parameters like packed cell volume, red blood cell count, hemoglobin content, platelets and white blood cells were significantly reduced, while the glucose level and gamma glutamyl transferase (GGT) activity were elevated in treated animals. Liver was damaged and the liver sections showed vacuolization of the cytoplasm of the hepatocytes, necrosis of hepatocytes, polymorphism of tissue, nuclei and vessel congestions when compared with control group. The development, behavioral, biochemical and histological disorders were observed due to exposure to mercury via placenta during pregnancy and / or during lactation through weaning period. Overall, mercury chloride administration had direct influence on the social behavioural and blood parameters in the mice offsprings.

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INTRODUCTION

Mercury (Hg) is a heavy metal that has high toxicity propeties. Hg exists in three forms: elemental Hg, inorganic Hg compounds (primarily mercuric chloride, $HgCL_2$), and organic Hg compounds (primarily methyl Hg) (Dash and Das, 2012). All forms are used in thermometers, batteries, dental amalgams and skin-lightening creams. $HgCL_2$ is used as a disinfectant and pesticide and is the third most dangerous heavy metal (Zhang *et al*., 2011; Celikoglu *et al*., 2015). Methyl Hg is formed from inorganic Hg ion from methylation process and the exposure to inorganic Hg is due to working activities like spillage of mercuric compounds on clothes (Bluhm *et al*., 1992) or handling of Hg salts in the laboratories and industries (Augusti *et al*., 2007). Hg has been widely used in tooth fillings (Fung and Molvar, 1992; Al-Saleh *et al*., 2012). The safety of Hg-containing dental amalgam to the individual and their health conditions were due to the exposure in various aspects (Martin and Woods, 2006; Arokiyaraj *et al*., 2015), however, amalgam remains very popular because of its durability, relative cheapness and ease to use (Newman, 1991). It is also widely used to restore posterior teeth in pediatric dentistry (Fuks, 2002; Al-Saleh *et al*., 2012). In general, Hg enters the human body and animals through food chains. Fishes were an important source of Hg in the human body (Maqbool *et al*., 2016). Gao *et al*. (2018) reported that the poisoning of children in some Chinese provinces were recorded because of the consumption of fish contaminated with Hg.

It was previously reported that almost all forms of Hg are toxic to the organisms (Clarkson and Magos, 2006; Chehimi *et al*., 2012). Toxicity of Hg was mainly associated

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with sulfhydryl groups (–SH), forming higly stable complexes and causing structural changes of sulfhydryl group in the active site of enzymes and the inactivation of their active sites (Rooney, 2007). In all tissues, exposure to $HgCL_2$ elevated lipid peroxidation (Al-Zubaidi and Rabee, 2015). The absorbed Hg distributed widely to all tissues and caused changes in liver, brain, and other organs (Yoshida *et al*., 2005; Wadaan, 2009; Chehimi *et al*., 2012). Exposure to Hg increased the levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, alanine aminotransferase (ALT) and creatinine in mice than in control group. The other parameters such as, glutathione (GSH), cholesterol, total protein, hemoglobin (Hb), white blood cells (WBC) and red blood cells (RBC) were significantly decreased in the experimental group. Histopathlogical studies revealed that $HgCL_2$ significantly induced many modifications in kidney and liver in mice (Al-Zubaidi and Rabee, 2015).

Exposure of brain to Hg caused many diseases, including behavioral dysfunction (Chehimi *et al*., 2012). Zhang *et al*. (2013) reported that administration of $HgCL_2$ to mice during pregnancy and lactation influenced the social behavior of offspring. Hg exposure also led to elevated cytokine levels in offsprings' brains (Yoshida *et al*., 2005). The present study was aimed to investigate the maternal exposure to $HgCL_2$ during gestation and lactation period on social behavior, neuromuscular coordination, locomotor activity and histological changes in liver of mice offspring.

MATERIALS AND METHODS

Experimental animals

About 10-12 week old Swiss–Webster strain mice (males and females) were maintained separately in an opaque cages (30×12×11 cm) at Animal Facility, Department of Zoology, King Saud University, Riyadh, Saudi Arabia. All animals were maintained under reverse lighting conditions and at an ambient temperature (20 ± 2 °C). On the first day of pregnancy of mice, the males were separated from the cages and the females were subjected to various experiments. All experimental protocols were made in accordance with the ethical guidelines for the use and care of laboratory animals. The experimental protocols were approved by the local Ethics and Care of Experimental Animals Committee.

Hg exposure and experimental design

Thirty pregnant mice were selected based on the appearance of Vaginal plug and were equally divided into three groups. Only tap water was given to Group I and considered as control. Groups II animals received 5 ppm $HgCl_2$ orally, whereas, 10 ppm of $HgCl_2$ was administered orally to Group III animals. Water and food were provided to all groups except at the time of behavioral trials. The following observations were made on experimental and control groups.

1. Group I was subjected to ''standard opponent'' test in males and the ''tube restraint test'' in females.
2. Group II was subjected to motor activity using activity meter and neuromuscular coordination using a grip strength meter.
3. Group III was subjected to liver histological analysis and analysis of various parameters in the blood.

Experiment was started from the first day of pregnancy and it was continued until post-natal day 15 (PD 15). Futher the mothers were switched to tap water. The pups of each experimental group were selected to only eight per dam on the post-natal day1 (PD 1) and were left with their mothers until post-natal day 22 (PD 22). During this time (weaning period), three pups in each litter were marked using various color to differentiate and were subjected to many behavioral experiments under dim lighting (ca 8 lux). All observations were registered on PD 21 and 36 of pup age.

Behavioral studies

Social behavior of control and experimental mice was carried out using ''standard opponent'' analysis and the ''tube restraint test'' was carried out in female experimental mice.

Standard opponent test

Ten male animals were selected from each set of control and experimental group and maintained individually in new cages for two weeks. After that, all male mice were subjected to ''standard opponent'' analysis under dim red lighting (ca. 8 lux). The docile and age-matched male individuals (standard opponents) were rendered anosmic by applying 25 µl of 4% zinc sulfate solution to the nasal tract under anesthesia for three days prior to encounters. Then anosmic ''standard opponent'' intruders were gently introduced in the cages of 'test animals' and the ''standard opponent'' test of each 'test animal' was analyzed visually for 500s.

The opponents were used to assess the selected ''elements'' of behavior as described previously (Brain *et al*., 1987; Abu-Taweel and Ajarem, 2008).

Tube restraint test

Thirty females were selected to study the 'tube

restraint test'. Ten females from each treated experimental group were selected for this analysis. The apparatus was designed as described previously (Ajarem and Ahmad, 1992; Abu-Taweel *et al*., 2006). Briefly, the apparatus consisted of a cylindrical transparent perspex tube with 13 cm in length and the internal diameter was 3.1 cm. One end of the cylindrical tube was blocked using a perforated perspex wall through which a metal target (2 cm length) was attached to a telegraph key/electronic counter arrangement. The experiment was carried out visually as suggested previously (Ajarem and Ahmad, 1994; Abu-Taweel *et al*., 2006) for 500 s under standard laboratory condition.

Neuromuscular coordination in grip strength meter

Neuromuscular coordination was evaluated using automated electronic grip strength meter (Biocompare, U.S.A). This device consists of two parts; the first square base is composed of Perspex material and is ending with rectangular column (15 cm ling), on the column cylinder fitted by another column with brass pieces (5 cm long). This is manily used to catch mouse during experiments. Maintenance of mouse was gently started in the tail and was placed in front of a copper piece. Mouse remained in the front by raising the hind limbs of the animal when measuring the power base forelimbs. Then the animal was laid at the rear base to evaluate the tensile strength of the parties Quartet. Then grip strength was calculated by kg/m after two min (Ali *et al*., 2004).

Motor activity test

Spontaneous motor activity was extensively used in rodents to evaluate the toxicological and pharmacological effects of Hg. Motor activity was elucidated using fully automated electronic activity meter as suggested by Kim *et al*. (2015) (Ugo Basile, Comerio-Varese, Italy). The vertical and horizontal motor activities were calculated by arrays of infrared beams placed above the floor of the testing place. Interruption of th beams on the "X" or "Y" axis generated an electric impulse which was recorded on a digital counter. Each animal was tested individually and the motor activity was registered for 2 min in the activity meter (Abu-Taweel *et al*., 2013a).

Analysis of blood parameters

Blood was collected aseptically from the retro-orbital plexus of the mice in heparinized vials at the end of the tests. Blood parameters such as, packed cell volume, red blood count, total white blood count, blood platelets count and hemoglobin content were measured using a automated hematology analyzer (T 450,USA).

Random glucose level and GGT activity

The random glucose level and GGT activity were estimated in the blood using an automatic analyzer (Hitachi automatic analyzer-902, Roche Diagnostic,USA to estimate random glucose level and (Reflotron Plus System, Roche, Germany) to estimate GGT.

Histological tests

For the histological slide preparation, liver was collected from sacrificed animals fixed in Bouin's fixative. Sections (4–5 µm) were prepared with a microtome, de-waxed, hydrated and stained in Mayer's haemalum solution for 3 min. The sections were stained with hematoxylin and Eosin for one min, washed in tap water and dehydrated in ethanol as described by (Ebaid *et al*., 2012).

Statistical analysis

The experimental data of tube restraint tests and standard opponent were analyzed within the experimental groups by using analysis of variance (ANOVA) and subsequently analyzed using Mann-Whitney U tests. Locomotry behavior data, blood parameters, neuromuscular activities in grip strength meter and biochemical analysis were compared within the experimental animals by using analysis of variance (ANOVA) by Minitab computer program. Further analysis was made using Student"s t-test. The significance levels were defined at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$ (Yamane, 1973).

RESULTS

Social behavior in standard opponent test

Table I displayed the prenatal exposure of Hg induced social behavior deficits. Non-social investigation and threat were increased ($P<0.001$, $P<0.05$) respectively, while social investigation and defense were decreased at $P<0.01$ and $P<0.05$ level respectively when compared with control. Both latencies, to first threat and first attack were increased significantly ($P<0.001$) while the amount of fight ($P<0.01$), nasonasal, nasogenital ($P<0.05$) and rears were decreased significantly ($P<0.05$, $P<0.01$) when compared with control mice (Table I). Table II shows that exposure to $HgCL_2$ induced defense behavior in female offspring. The latency to first bite was increased ($P<0.05$), while the number of bites was decreased ($P<0.01$, $P<0.001$) when compared with control group.

Neuromuscular coordination analysis

Exposure to Hg influenced on neuromuscular coordination in experimental mice. Hg exposure reduced neuromuscular coordination significantly in treated animals in both doses ($p<0.05$ and $p<0.01$), respectively, when compared with control animals (Fig. 1). Results

indicated that the neuromuscular coordination was reduced with respect to the increase in the concentration of the administration of the medrcuric chloride. At 5 ppm concentration the grip strength was 750 kg/M and at 10 ppm it was 490 kg/M respectively. These results clearly evidenced that the activity was inversely proportional to the concentration of the $HgCL_2$.

Table I. Effects of Hg perinatal exposure on social behavior acts and postures in male mice offspring. The time allocated to behavior is in seconds (with ranges).

Group	Control	5 ppm	10 ppm
Nonsocial investigation	145.20 (120.30-170.1)	302.40 *** (286.3-315.5)	327.90 *** (295.7-360.2)
Social investigation	223.30 (214.10-232.5)	99.70 ** (98.00-101.4)	94.00 ** (88.00-100)
Defense	20.40 (20.10-20.70)	15.60 (10.80-20.40)	1.55 * (00.00-3.10)
Threat	21.70 (20.10–23.30)	25.20 (18.00-32.40	52.30 * (26.60-78.00)
Attack	60.30 (40.40-80.20)	23.25 *** (20.00-26.50)	8.50 *** (0.00-17.00)
Displacement	29.30 (26.50-32.10)	33.85 (18.60-49.10)	13.20 * (4.40 -22.00)
Latency to threat	7.50 (5.00-10.00)	105.00 *** (90.00-120.00)	309.50 *** (220.00-399)
Latency to attack	40.00 (20.00-60.00)	170.00 *** (120 - 220.00)	405.50 *** (390.00-421)
No of fights	19.00 (18.00-20.00)	4.00 ** (2.00-6.00)	1.50 ** (1.00-2.00)
No. of naso-nasal contacts	26.00 (25.00-27.00)	19.00 (17.00-21.00)	10.50 * (8.00-13.00)
No. of naso-genital contacts	18.00 (14.00-22.00)	7.50 (5.00-10.00)	2.50 * (2.00 –3.00)
Wall rears	13.00 (10.00-16.00)	11.50 (9.00-14.00)	7.00 (6.00-8.00)
Rears	16.00 (11.00– 1.00)	5.50 * (14.00-25.00)	3.00 ** (2.00-4.00)

*, ** and *** significantly different ($p<0.05$, $p<0.01$ and $p<0.001$) respectively from the control by using Mann-Whitney U test and ANOVA.

Motor activity

$HgCL_2$ induced motor activity in experimental mice (Fig. 2). Vertical and horizontal activities were significantly reduced at $p<0.001$ level in both doses compared to control group. Results proved that the vertical activity was higly reduced when compared to the horizontal activity. Especially, the vertical activity of control, 5 ppm and 10 ppm concentrations were noted as less than 10 cm respectively, whereas the horizontal activity was recorded above 100 cm for both 5 and 10 ppm concentration of the $HgCL_2$ supplementation.

Table II. Effects of Hg perinatal exposure on defense behavior in female mice offspring.

Group	Measures (Median values with ranges)	
	Number of bites	Latency to first bite (sec)
Control	70.0 (45.0-96.0)	15.0 (10.0-40.0)
5 ppm	36.0 ** (0.00-41.0)	77.0 (0.00-100.0)
10 ppm	10.0 *** (0.0-35.0)	132.0 * (0.0-180.0)

*, ** and *** significantly different ($p<0.05$, $p<0.01$ and $p<0.001$) compared with control group by ANOVA and Mann-Whitney U test.

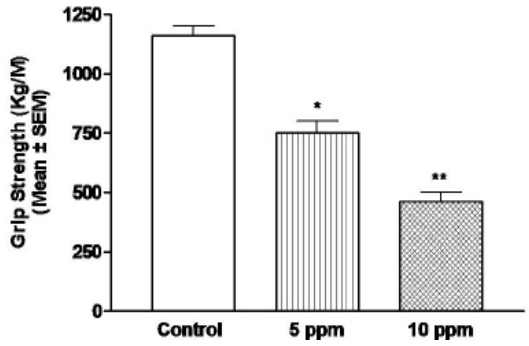

Fig. 1. Effect of perinatal exposure to Hg on neuromuscular coordination in mice offspring.* and ** showed significant variation at $P < 0.05$ and $P < 0.01$ level respectively compared with control group by ANOVA and student's t-test.

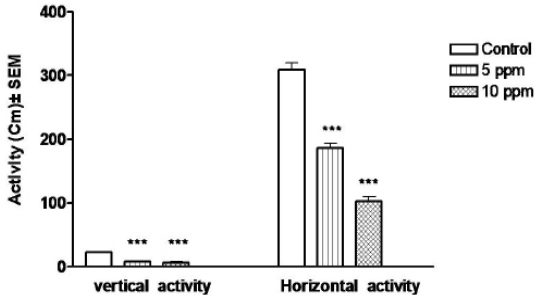

Fig. 2. Perinatal Hg exposure effects on locomotor activity behavior of mice offspring.*** shows statistically significant variation at $P < 0.001$ level than control group by ANOVA and student's t-test.

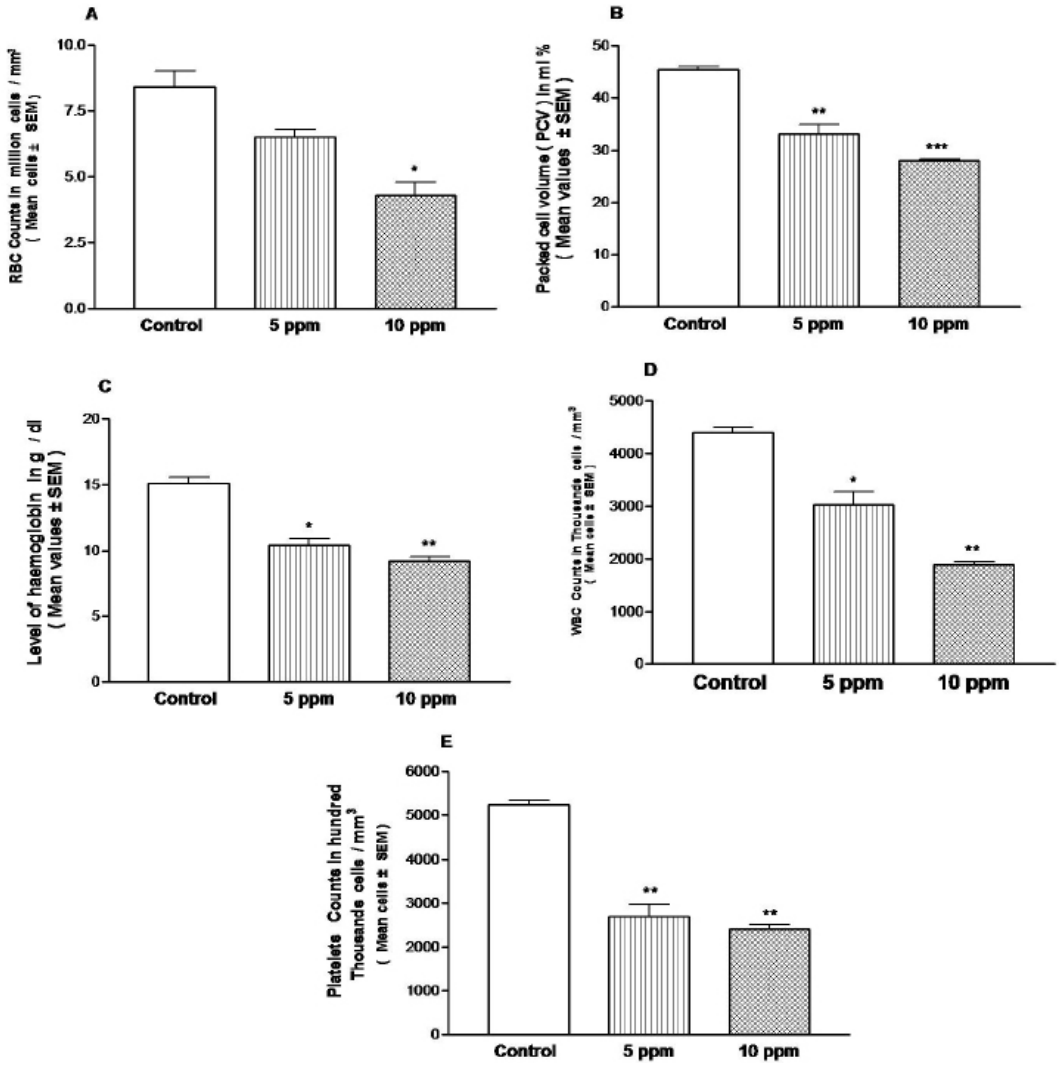

Fig. 3. Effect of Hg toxicity on red blood cells (A), volume of packed red cells (B), hemoglobin content of mice (C), white blood cells (D), platelets (E) of mice offspring. *, ** and *** implied significant different at $p<0.05$, $p<0.01$ and $p<0.001$ level respectively from the control group by ANOVA.

Analysis of blood parameters

$HgCL_2$ exposure significantly depleted red blood cell count, white blood cell count, the packed cell volume, hemoglobin content and platelets count (Fig. 3). At 5 and 10 ppm cocnetration the values of RBC countes were less than 7.5 million cells/mm^3 and 5 million cells/mm^3 respectively, whereas the control experiment noted above 7.5 million cells/mm^3 clearly indicating the direct effect of $HgCL_2$ on the RBC counts. Interestingly, the supplementation of $HgCL_2$ was inversely proportional to the concentration of the packed cell volume. Therefore, it is concluded that the supplementation of the mercurric chloride has direct influence on the alterations of the blood paramenters. Simmilarly, the RBC counts and platelets counts were inversely proportial to the concentrations of $HgCL_2$.

Random glucose level and GGT activity

Figure 4 showed a significant increase in random glucose level ($p<0.05$, $p<0.01$) respectively in the doses of Hg and ($p<0.001$) in GGT activity due to Hg exposure as compared to control. The level of total glucose concentrations and the gamma glutamyl transferase ranges were directly proportional to the concentrations of the administration of the $HgCL_2$. Compared to the control, the level of the totlal glucoe and the enzyme levels were significantly increased with increase (5 ppm and 10 ppm) in the concentrations of the $HgCL_2$.

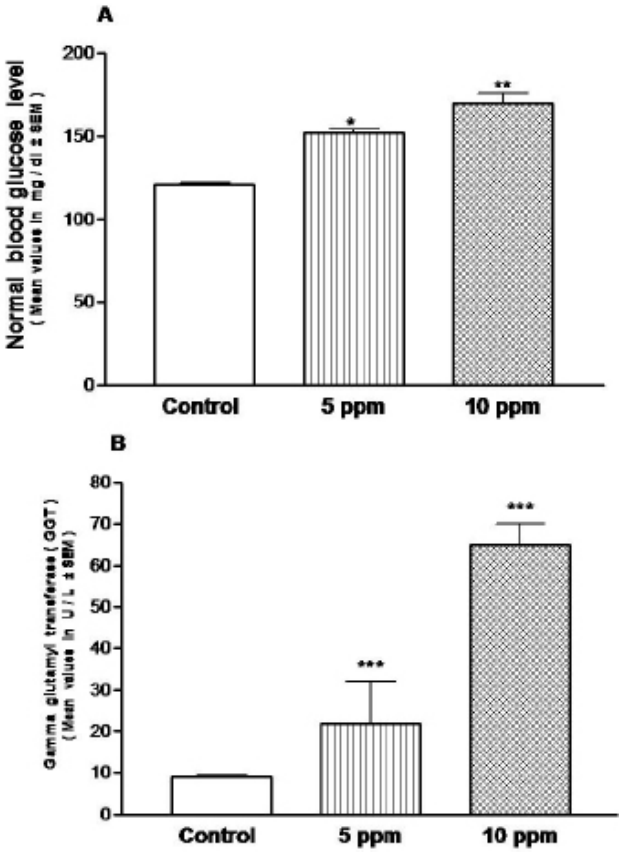

Fig. 4. Effect of Hg on random glucose level (A), gamma glutamyl transferase (B). *, ** and *** significantly different at ($p<0.05$, $p<0.01$ and $p<0.001$) respectively from the control group by ANOVA student's t-test.

Liver histology

Figure 5 shows a section from normal and Hg-Treated livers. B and C sections of Hg-Treated livers showed a major damage in liver. The liver sections revealed necrosis of hepatocytes, polymorphism of nuclei, tissues, vacuolization of the cytoplasm of the hepatocytes and vessel congestions.

DISCUSSION

Hg is a heavy metal that has high toxicity. It exists in three forms: elemental Hg, inorganic Hg compounds (primarily $HgCL_2$), and organic Hg compounds (primarily methyl Hg) (Dash and Das, 2012). Natural ones exist through soil or water. It also enters industrially through human activities (Wadaan, 2009). Hg enters the bodies of organisms in different ways and accumulates in the tissues. The target tissue of Hg accumulation is the brain (Al-Saleh, 2012). Toxicity of Hg results in behavioral, biochemical, blood parameters and organs structural changes (Clarkson and Magos, 2006; Chehimi *et al*., 2012).

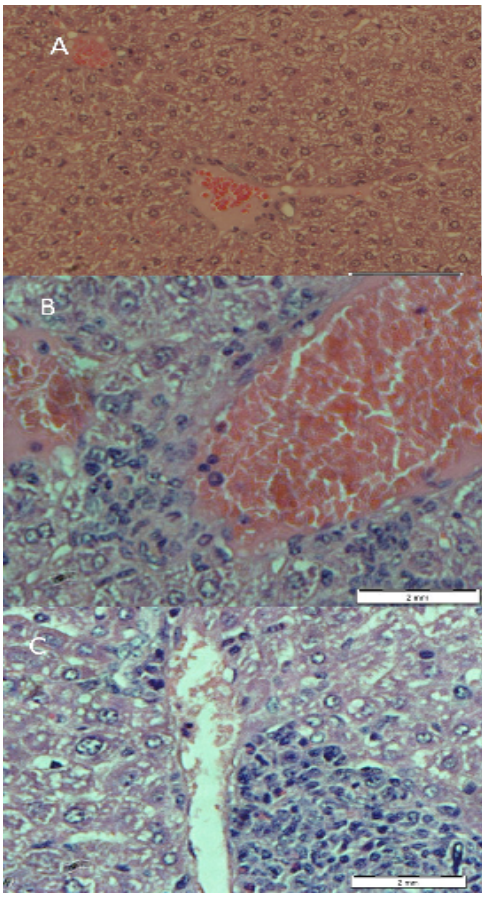

Fig. 5. Effect of Hg on liver histology x400. A, Liver plate (control) showed: normal tissue. B, liver (5 ppm) showed: drug induced mild mixed mononuclear inflammatory cells with little esinophiles, congested capillaries and hydropic degenerations.C,liver (10 ppm) showed: drug induced mixed mononuclear inflammatory cells with esinophiles and dilated capillaries and hydropic degenerations.

The present study showed that the peri`natal exposure to $HgCL_2$ induced social behavior deficits. Nonsocial investigation and threat were increased while social investigation and defense were decreased as compared to control group. Both latencies, to first threat and first attack were increased significantly while the amount of fight, nasonasal, nasogenital and rears were decreased significantly as compared to control group. The latency to first bite was also increased, while the number of bites was decreased as compared to their control. The results were in agreement with Zhang *et al*. (2013).

Zhang *et al*. (2013) attributed social behavior disorders to autoimmunity, which was induced by Hg

perinatal exposure. Hei *et al*. (2011) detected IgG antibrain antibodies in the sera of mice and human with impaired social behaviors and aberrant behaviors were induced in animals after exposure to IgG from mothers (Singer *et al*., 2009; Zhang *et al*., 2013). Induction of maternal auto-antibody by Hg was very critical and IgG can entered fetal brains because blood-brain barrier was less intact or absent in the developing central nervous system (Zhang *et al*., 2013).

The high amounts of IgG in the Hg-exposed experimental mice are significantly related to the ability of Hg to enhance the activation of lymphocyte (Badou *et al*., 1997). Zitzmann (2006) and Lynn (2008) showed a close relationship between testosterone and different behavioral changes, especially aggression in mice. Animal studies (Abu-Taweel *et al*., 2013b; Abu-Taweel, 2016) indicated a strong evidence of change, serotonergic neurotransmission, being associated with change in aggression. It was previously reported that the dopaminergic and serotonergic system was modulated by aluminum (Al), cadmium, LPS, MSG and ASP decreased the aggression rate in adult mice (Abu-Taweel *et al*., 2011; Ebaid *et al*., 2012; Abu-Tawee *et al*., 2013a; Abu-Tawee *et al*., 2014; Abu-Taweel, 2016). Thus, Hg may affect the testosterone level with some other neurotransmitters such as acetylcholine, serotonin and dopamine. Exposure of Hg in mice critically altered the social behavior by affecting olfactory nerve of the nervous system, which critically control the sense of smell in animals (Park *et al*., 2000). Inorganic Hg exposure significantly influenced the biochemical parameters in Wistar rat (Merzoug *et al*., 2009). Exposure of chronic $HgCl_2$ in rats induced functional changes, including motor deficits, long- and short–term memory impairments (Teixeira *et al*., 2014).

Many studies showed that inorganic Hg exposure critically caused changes in organ weight, body weight, decrease in renal δ-aminolevulinic acid dehydratase activity, renal histopathological damages and increase in serum creatinine and urea levels (Favero *et al*., 2014). The effect of Hg at perinatal exposure on neuromuscular coordination in mice offspring was visible. Experimental animals at both concentrations affected neuromuscular coordination. Vertical and horizontal activities were reduced in the experimental mice significantly. These results were in agreement with earlier findings (Day *et al*., 2005; Vezer *et al*., 2005; Yoshida *et al*., 2005; Zimmermann *et al*., 2014; Kirkpatrick *et al*., 2015). Some research groups attributed the low levels of motor activity in Hg-treated animals to the imbalance caused by this element in brain function (Yoshida *et al*., 2005). The results of this study indicated that weight loss might have affected the structure of the brain and its biological functions, especially the astrocytes were highly important in the process of nerve conduction. The defect may be due to the effect of Hg on the molecular bond between these cells or channels of communication with gaps between them or to interfere in the structure or function of structural proteins supported by the structure of these cells. Prolonged Hg exposure weakens or delays the arrival of orders to the muscles, decreases the speed of the nerve impulses, resulting in a very slow response (Cooper and Kusnecov, 2007). The decreases in motor activity in treated animals may be due to muscle weakness due to the imbalance caused by Hg in the important calcium component in regulating muscle contraction (Ramırez-Bajo *et al*., 2014; Teixeora *et al*., 2014). Our results (data not shown) showed anxiety disorders in exposed animals with Hg. The lack of motor activity may be attributed to a lack of energy due to the imbalance caused by Hg in glucose metabolism. Ramırez-Bajo *et al*. (2014) reported that exposure to Hg effected skeletal muscle glycolysis in mice.

$HgCL_2$ exposure led to a significant depletion in some of the observed blood parameters such as red blood cell count, packed cell volume, hemoglobin content, white blood cell count and platelets count.These results are in agreement with previous results (Shaw *et al*., 1991; Al-Zubaidi *et al*., 2015). Some studies attributed a decrease in the number of blood cells to the changes caused by Hg in the bone marrow of those cells. Hg accumulated in the bone instead of calcium, causing a malfunction in the bone structure and thus reducing its ability to snythsize blood cells (Rasmussen *et al*., 2013). Shaw *et al*. (1991) reported that anemia may arise due to the effect of Hg in red blood cells. Most of the changes that occur in blood when exposed to Hg are due to the reduction of the life span of the red blood cells or increase in their degradation. Osinska *et al*. (2004) pointed out that Al changes the properties and shape of red blood cells, thus changing membrane liquidity. Exposure to Al increases the production of organic free radicals that oxidize lipids containing unsaturated fatty acids such as malonedialdehyde (MDA), which reacts with amino acids in the membrane of the cells, changes the membrane's liquidity and reduces its ability to restore its original form during passage cells in the capillaries. Some studies proved that the anemia is caused by the ability of Al to produce cells that are different from the normal, such as being small in size or larger than normal, with a lack of natural pigment or increased pigmentation of others (Farina *et al*., 2002, 2005). Chmielnicka *et al*. (1996) believes that the cause of anemia is due to imbalance in the biothensysis of the iron element. Some researchers report a decrease in the amount of iron due to the interferometry of Al in iron biothensysis by inhibiting some important enzymes in its metabolic pathway, such as the alpha-aminolevulinic acid

dehydratase (δ-ALA-D) enzyme because the element ion (Al^{3+}) is related to the sulfur group in the enzyme (Vieira *et al*., 2000). The study correlated the depletion of white cells with the high effect of Al ions on some types of white cells, especially lymphocytes (Gomez *et al*., 1986). The immunity of people with iron-deficiency disorders is less. White cells lose their ability to function because the blood cells in their various processes need the iron component, which is transported by the transferrin proteins, which are occupied by Al instead of iron (Osinska *et al*., 2004; Zhang *et al*., 2019). The effect of Al on the number of white cells causes changes in its cellular membrane (Shenker *et al*., 2000). The mechanical effect of Hg on blood parameters may be similar to that of Al (Su *et al*., 2008).

The present study recorded the increase of the random glucose level in treating animals with Hg. Dufault *et al*. (2015) revealed that glucose levels were higher in human with blood inorganic Hg. Their results indicated that organic Hg exposure affected insulin levels, but not glucose levels, whereas chronic Hg exposure affected blood glucose levels. Maqbool *et al*. (2016) reported that hyperinsulinemia represented high risk of type 2 diabetes mellitus and this could be due the the resistance of insulin. Jeppesen *et al*. (2015) reported a significant association between diabetes mellitus (DM) risk and total blood Hg concentrations. Roya *et al*. (2017) concluded that there was a strong relationship between Hg exposure and diabetes mellitus. Previous reports showed that organic form of Hg could be a major cause of insulin resistance and type 2 diabetes mellitus (Chang *et al*., 2011). DNA methylation also plays a key role in the regulation of genes involved in glucose homeostasis. GLUT4 gene plays a major role in regulating whole body glucose homeostasis.The role of Hg in affecting LH levels has been studied. LH is among the known regulators of insulin release in pancreatic cells. Reports have linked chronic Hg exposure with reduced levels of LH (Dufault *et al*., 2015). Al and Hg are strong oxidizing agents that affect living cells and their functions. It is possible that the mechanisms of their effect on these cells may be similar. Yousef (2004) attributed the increase in sugar in the rabbit blood due to Al due to the imbalance caused by the element in carbohydrate metabolism to increase the glycogenolysis form the liver as a result of the oversecretion of alpha cells in the islands of Langerhans in glucagon hormone. El-Demerdash (2004) confirmed the conclusion of the previous study, but attributed the increase in sugar to the excessive secretion of adrenocorticotrophic hormone, which stimulated adrenal cortex hormones such as glucocorticoids, especially cortisol, which stimulated glucose production of non-carbohydrate substances.

The results revealed that the increase in GGT activity was due to the Hg exposure as compared to control. This finding was similar to previous reports (Wadaan, 2009; Lee *et al*., 2017; Choi *et al*., 2017). The biological mechanism of associations between Hg exposure and liver dysfunction is mainly explained by oxidative stress, cell death, and impairment of metabolism (Choi *et al*., 2017). Some studies have suggested an inverse relationship between GGT enzyme activity and some antioxidants like a-carotene, lycopene and vitamin C. These studies suggested that GGT might be considered an early oxidative stress marker (Lim *et al*., 2004). Wadaan (2009) observed that Hg exposure to male rats elevated the levels of liver enzymes such as, GGT, ALT, AST and necrotic changes were found in most of the liver tissues.

The liver sections showed necrosis of hepatocytes, vacuolization of the cytoplasm of the hepatocytes, polymorphism of nuclei and tissue and vessel congestions. These findings were in agreement with previous studies (Al-Attar, 2011; Burger *et al*., 2011; Quirino *et al*., 2012; Ibegbu *et al*., 2014). Also, $HgCL_2$ exposure to experimental rats showed congestion of hepatoportal blood vessels, edema in the portal tract, congestion of central vein indicating critical toxic effect of $HgCL_2$ (Ibegbu *et al*., 2014). Because of exposure to $HgCL_2$, the metabolic pathway of Kupffer cells and liver hepatocytes were affected and implied chronic illness of liver (Kumar *et al*., 2010; Sujatha *et al*., 2011).

Liver toxicity is the condition of critical liver damage and at this stage some cells get infiltrated very close to the damaged hepatocytes and critically play an important role in the development of fibrosis (Agarwal *et al*., 2010; Ibegbu *et al*., 2013). This fibrosis leads to Liver cirrhosis which is highly irreversible (Junqueira and Caneiro, 2005; Hesse, 2007; Quirino *et al*., 2012). Liver damage critically decreased the synthesis of proteins and these finding implied degradation of liver cells (Trebucobich *et al*., 2014). $HgCL_2$ toxicity induced the formation of reactive oxygen species (ROS) and critically promoted damages in liver tissues, where liver cells were damaged due to the toxic effect of Hg as observed in the present investigation. Hence, an increased level of ROS formation by the exposure to $HgCL_2$ stimulated not only the alternation of functional properties and biochemical changes but also caused liver cell damage (Bharathi *et al*., 2014).

CONCLUSION

In summary, the administration of $HgCL_2$ to mice offspring directly affected the social behavior, neuromuscular coordination, motor activity and blood parameters. Results confirmed that the administration of different concentrations of $HgCL_2$ direcly affected the behavioural studies such as social behavior, neuromuscular coordination and vertical and horizontal portion of the

motor region of spinal cord. The results indicated that the different cocnetrations of the $HgCL_2$ were inversely proportional to the activity of the behavioural aspects. Hg toxicity induced oxidative stress in the experimental mice and this was mainly due to the formation of toxic Hg metal in the form highly stable complexes with the sulfhydryl groups of various enzymes and proteins. Interestingly, blood parameters suh as packed cell volume, red blood cell count, hemoglobin content, platelets and white blood cells were significantly reduced, while the glucose level and gamma glutamyl transferase (GGT) activity were elevated in treated animals. The present finding showed the risk of Hg exposure on neonates, fetuses and the possibility of transport simply through milk and/or placenta. Further studies are required to investigate the effect of behavior changes and medication and/or antioxidant which may reduce the effect of toxic elements.

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Statement of conflict of interest

Authors declare no conflict of interest.

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