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Original Article

A Comparison of Developmental and Maternal Toxicity of Perfluoro Octane Sulfonate (PFOS) In Mouse: Evaluation of Histopathological and Behavioral Changes

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Abstract

Perfluorooctanesulfonate (PFOS) is a widely spread environmental contaminant. It accumulates in the brain and has potential neurotoxin effects. Due to chemical properties, PFOS shows persistency in the environment and therefore has potential hazardous effect. The risk of possible intra uterine exposure to PFOS poses a health concern for developmental effects. The goal of this study was survey of histological and behavioral changes made by PFOS in pregnant mice and their fetuses using common behavioral assays and H&E staining. In the present study, doses of PFOS (1, 10, 20 mg/kg) were given orally to pregnant mouse from gestational day (GD) $_0$ to GD_{14}; then on the day 15, Behavioral experiments including (open field and passive avoidance) were used to assess toxic behavioral changes such as memory impairment and anxiety. After behavioral evaluations, fetuses were dissected on day 15 of gestation and morphological and histological studies on pregnant mouse brain and her fetus were carried out using haematoxylin-eosin staining method. Our findings showed that PFOS could induce neurotoxicity in pregnant mouse especially by induction of abnormalities in dentate gyrus of hippocamps and disruption of neurobehavioral functions .Besides in her fetus; PFOS produced significant changes in brain, liver, and thyroid gland in comparison with untreated control mouse fetus. As a conclusion, PFOS can cause both neurobehavioral and developmental toxicity in pregnant mouse and her fetus.

Keywords: PFOS, mouse fetus, developmental toxicity, neurobehavioral toxicity, isolated mitochondria.

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1. Introduction

Teratology is the study of abnormal development in embryos and the causes of congenital malformations or birth defects [1]. Teratogenicity depends upon the ability of the compound to cross the placenta. The embryo is most susceptible to teratogenic agents during periods of rapid differentiation [2].The morphological type of abnormality depends on the developmental stage at which the agent reaches the fetus. Whereas obvious anatomical disrupts result from disturbed organogenesis during early pregnancy, more subtle functional changes may occur due to intrauterine exposure after the first trimester [3]. Perfluorinated compounds (PFCs) are a classic of chemical compounds that are very persistent in the environment [4]. Perfluorooctanesulfonate (PFOS) is a member of the PFCs family that has been used in a wide range of industrial and commercial applications for the past 50 years [5]. In the humans, the main route of exposure to PFOS is believed to be through consumption of contaminated food [6].This compound accumulates in the developing brain before formation of the blood–brain barrier (BBB) also can cross mature BBB to a certain extent [7]. Previous studies showed that exposure to PFOS in experimental animals induce alterations in CNS functions, including neuron endocrine disturbances and neurodevelopment delays [8].PFOS is able to cross the placental barrier and can also be excreted with milk [9]. The study showed that in rodents, effects of PFOS on developmental toxicity consist of; reduction of fetal weight, reduced neonatal survival, defects in the peripheral nervous system, and behavioral alterations [10]. The aim of this study was to investigate the effects of exposure to PFOS on the pregnancy mice and fetus, with special focus on the tissue changes and behavioral alterations. To this end we exposed pregnancy mice to PFOS at concentrations that do not increase the mortality [11]. We analyzed the behavioral functional by open field, passive avoidance in addition, the effects histology of PFOS on the brain pregnant mice and fetus using H&E staining and also found PFOS disrupts, memory, anxiety in pregnancy mice. Altogether, our data indicate that exposure to PFOS in fetus causes reduction of fetal weight, change in size liver tissue, Umbilical hernia, and anomaly in brain fetus.

2. Materials and Methods

2.1. Tissue preparation

In the study, adult mice (10-12 weeks) weighting 20 g were obtained from Razi institute, Iran. The animals were fed with a standard diet with water ad labium and kept in a room with controlled light (12:12, dark: light), temperature (22±10 C), relative humidity (40-50%) and ventilation (15 air changes per hour). They were allowed to

adapting to their environment for 1 week prior to the experiments. The mice were randomly mated and for emphasis of pregnancy their vaginal plaque were assessed after mating. Then, they were separated as control (5 mice) and PFOS treated groups (in each groups 3 mice) (1, 10, 20 mg/Kg/day orally once a day for 14 days) to gestation day 14 of pregnancy [11]. Behavioral testing was held on the day 15. Then pregnant mice were sacrificed and dissected on day 15 of gestation and morphological and histological studies on the fetus were carried out [12].Measurement of fetus weight was accomplished by digital balance and Crown-Rump (C-R) lengths accomplished by coils done. Also, after tissue fixation with bouin fixative solution, fetus sections at a pre-defined thickness of 10 μm were performed. Slices were either thaw-mounted on a 1 mm thick KBr window for IR microscopy and were mounted on conventional glass slides for staining with haematoxylin (H) and eosin (E) for studying of abnormality in fetus by light microscopy. All brain tissues were fixed for 48h in 4% paraformaldehyde and embedded in paraffin. Paraffin embedded blocks was cut to 10μm slices using a microtome device (Leica, RM2035, Germany). Hippocampus sections were dewaxed and stained with haematoxylin-eosin staining method that was described previously [13] .The dentate gyrus was observed under light microscopy with (×40) magnification.

2.2. Behavioral Assessments

2.2.1. Locomotors activity

The locomotors activity test was carried out in cages made of transparent Plexiglas (40×40×40cm) as described by Onishchenko et al., (2011). After administering PFOS for14 days, on the day 15 mice were individually placed in the new cage and the Movement was recorded with a camera that was placed above the cage for 10min.Total distance movement, peripheral, and central zone spent time for each animal were registered using video tracking software EthoVision® XT (Version 8, Noldus, Netherlands) and the data were used for the statistical analysis [14].

2.2.2. Passive Avoidance

Passive avoidance test is believed to evaluate the long term memory in mice. The apparatus [15] was formed from bright and a dark compartment (20×20×20cm each) that was separated by a wall with guillotine door. The dark compartment was equipped with an electric grid floor. The Training and testing was performed on two consecutive days; on the training day the mice were placed in the bright compartment and allowed to explore for 30s, after that the guillotine door was opened and the mice were allowed to step into the shock compartment freely. When the mice entered the shock compartment, the door was closed and an electrical foot shock (0.5mA, 2s) was given to the mice and then was gently carried to their cages. The mice which did not enter the shock compartment in 60s were excluded from the experiment. 24 hours later the mice were placed in the bright compartment and the guillotine door was raised after 30s.Latency to enter the dark part

was recorded; the cut-off time was 300s. In all experiments mice were divided randomly into experimental groups (n = 10) [16], [17].

2.2.3. Statistics

The data from the fetus weight and the fetus C-R length were analyzed by using Graph pad Prism© software. The statistical results of the PFOS treated fetus weight with the control fetus weight were compared with Students t-test ($p<0.01$). Also, the results were analyzed by one-way ANOVA followed by Tukey's test. Data are presented as mean ± SEM. Values of $p< 0.05$ were considered significant.

3. Results and Discussion

3.1. Behavioral studies

3.1.1. Open-field Test

The open-field test was performed to evaluate activity and behavioral response of pregnant mice following the exposure to PFOS. As shown in (Figure 1A,) PFOS treatment for 14 days in pregnant mice significantly increased total distance movement compared to the untreated control group which received only vehicle. Besides the pregnant mice in our PFOS receiving test groups, spent lower time in central zone compared to the untreated control group (Figure.1B).

3.1.2. Passive Avoidance Test

Passive avoidance test is believed to evaluate the long term memory in pregnant mice. (Figure 2) shows the avoidance latency in mice of test groups. The avoidance latency in the PFOS treated pregnant mice is significantly lower than that of the untreated pregnant control mice. In addition, our results showed a dose-dependent response in avoidance latency reduction in comparison to the untreated pregnant control group.

3.1.3. Histological Analysis

3.1.3.1. Effect of PFOS Treatment on C-R Length and Weight

In this study, the weight of PFOS treated fetus was more than that of normal fetus and there was a significant difference between them (control group weight mean, 0.254±0.018 and treated group weight mean, 0.409±0.029) ($p<0. 01$) (Figure3 (A)). The C-R length PFOS treated fetus was more than the normal fetus, and also there was significant difference between them (control group C-R mean, 11.54±0.76 and treated group C-R mean, 14.53±0.66) ($p<0.01$) (Figure 3 (B)).

3.1.3.2. Effect of PFOS Treatment on Fetus

Figure 4 shows the H & E stained sections of a normal and PFOS treated mice fetus brain. Obvious necrosis in lateral ventricles of brain

B A

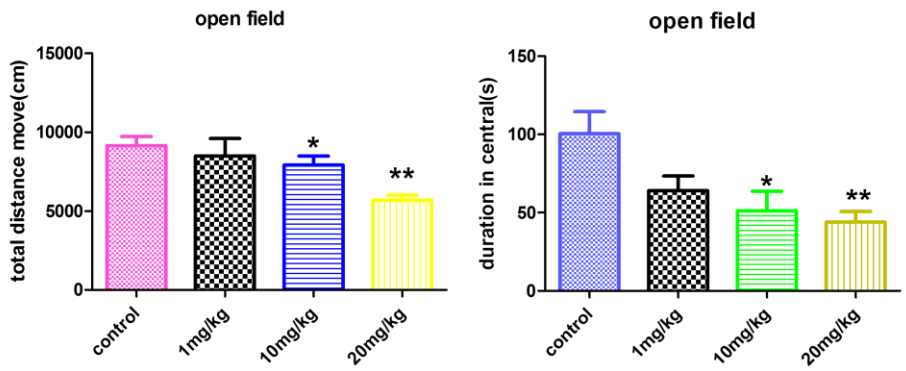

Figure1. Effects of PFOS on total distance movement in open field test (A). Effects of PFOS on duration of time spent in the central zone (B). The results were analyzed by one-way ANOVA followed by Tukey's post hoc. Data are presented as mean ± SD. * and ** represent $p<0.05$, $p<0.01$ of significance respectively in comparison with the untreated control group.

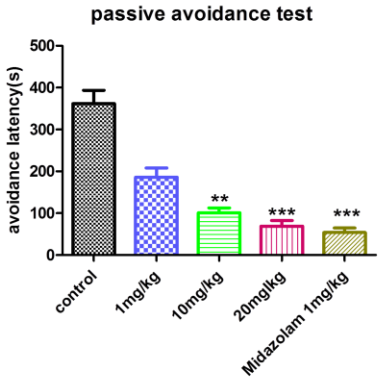

Figure 2. Effects of PFOS on avoidance latency in PFOS receiving pregnant mice. Midazolam was used to create a positive control group. The results were analyzed by one-way ANOVA followed by Tukey's post test. Data are presented as mean ± SD. *** represents $p<0.001$ of significance in comparison to the untreated pregnant control group.

A B

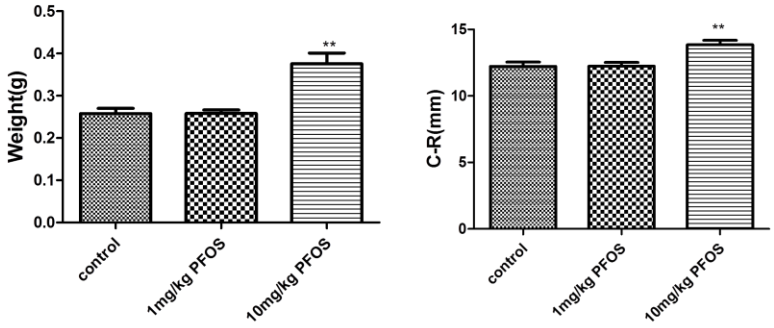

Figure 3. The weight of mice fetus (A), (control group weight mean, 0.254±0.018and treated group weight mean, 0.409±0.029), ($p< 0.01$).The C-R length of mice fetus (B)(control group C-R mean, 11.54±0.76 and treated group C-R mean, 14.53±0.66), ($p< 0.01$).

as seen in PFOS treated fetus compared to

normal fetus (Figure 4 (A)).Umbilical hernia and increased size of liver were observed in PFOS treated fetus (Figure 4 (B).At higher dose of 20 mg/kg, PFOS caused anomaly in fetus brain in compared to untreated control group(Figure 4 (C)). (40×magnification).

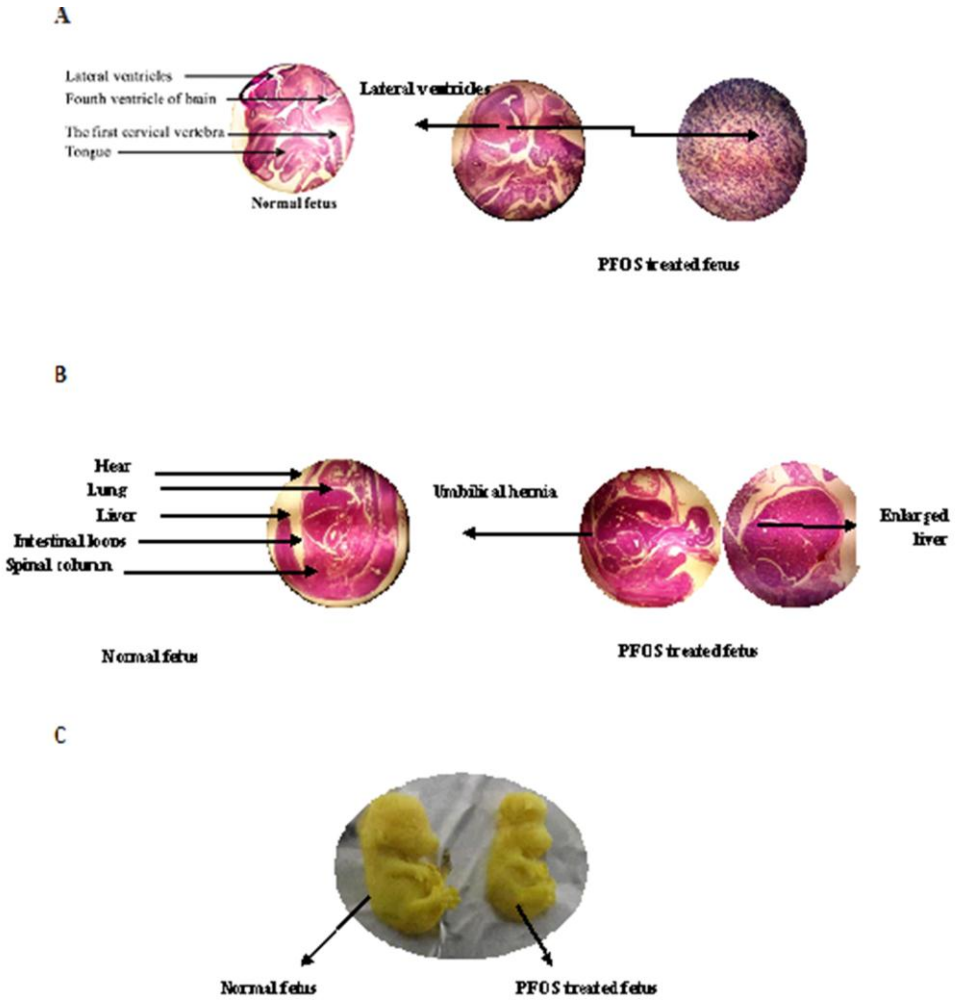

Figure 4. H & E stained sections of both normal and PFOS treated mouse fetus(40×magnification). Brain necrosis (A), Umbilical hernia and liver enlargement (B) And brain anomaly (C) were clearly observed in PFOS treated groups.

3.1.3.3. Effect of PFOS Treatment on Pregnant Mouse Brain

Figure (5. A and B) shows the haematoxylin-eosin stained dentate gyrus cells in hippocampus of the untreated control and PFOS treated (20 mg/kg/day) pregnant mice. A significant nucleus size reduction along with significant decrease in nuclei number were seen in PFOS treated (20 mg/kg/day) maternal dentate gyrus cells compared to those of untreated control pregnant mice(40×magnification).

3.2. Discussion

It is estimated that approximately 10-15% of congenital structural anomalies are the result of the adverse effect of environmental

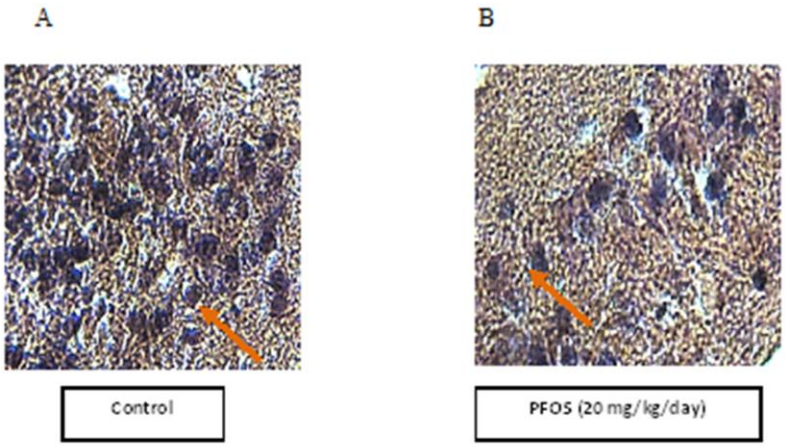

Figure (5.A and B). Effects of PFOS on the dentate gyrus cells (40×magnification) in the hippocampus of the control (A) and PFOS (20mg/kg/day) treated pregnant mice (B). Significant reduction in nuclei number and nuclear size are demonstrated in the dentate gyrus of the PFOS treated pregnant mice.

factors on prenatal development [18].A teratogen is defined as any environmental factor that can produce a permanent abnormality in structure or function, restriction of growth, or death of the embryo or fetus [19].Perfluoro octane sulfonate has been manufactured for several decades. Because of its widespread use in industrial fields and consumer products, ubiquitously in the environment and animal tissues PFOS was assessed by previous studies [20]. Our behavioral findings showed that dietary exposure of PFOS to pregnant mice caused significant reduction in total distance movement and time spent in central zone in the open field assay compared to the untreated control group in a dose-dependent manner. Onishchenko et al., 2011 showed that PFOS could cause decreased locomotion in a novel environment after oral exposure in mice. The results of this investigation support our findings, that PFOS may disrupt locomotion and increase anxiety by inducing neurobehavioral toxicity in pregnant mice [14]. Our results on hippocampal dentate gyrus' by haematoxylin-eosin staining showed that PFOS could induce some abnormalities in dentate gyrus cells which is consistent with the memory dysfunction in the passive avoidance test results. The dentate gyrus (DG) is a cortical region that is an integral portion of hippocampal formation [21] and also has an important role in hippocampus-dependent learning and memory [22, 23]. Hence, DG is thought to contribute to new memories as well as other functional roles [24]. Therefore, damage to DG provides enough justification for the observed impairment in memory and also anxiety in the PFOS treated group. Our results in H & E stained sections of mouse fetal tissues showed long-term exposure of PFOS to pregnant mice that may cause anomalies in their fetuses. For example, increased size of the liver and umbilical hernia

was observed in the PFOS treated fetus. As per a survey conducted by Wang at al., 2014 in rats, it was reported that PFOS can cause changes in size of liver and also liver related biochemical factors which again is quite consistent with our findings [25]. previous studies have shown that PFOS could affect action potential and L-type ca^{+2} current of cell membrane in rats, suggesting that this chemical has some effect on the nervous system, which may be related with our observed necrotic changes in the lateral ventricles of fetal brain [20].In addition to, H & E staining of brain sections showed exposure to high doses of PFOS could induce anomaly in brain fetus due to high neurotoxicity of this compound.

4. Conclusion

In our behavioral, morphological, and histological studies, we got some findings which is quite novel including;

1) Open field test in pregnant mouse, increased total distance movement and reduced time spent in the central zone in a dose-dependent manner observed in the PFOS treated pregnant group compared to the untreated control group.

2) Passive avoidance test in pregnant mouse, significant reduction in avoidance latency time in the PFOS treated maternal group compared to untreated maternal control group.

3) Brain histological assay in pregnant mouse, dentate gyrus cells in the PFOS treated (20 mg/kg) maternal group were different in size and number of neuronal nuclei compared to the untreated maternal control group.

5) Morphological assay in mouse fetus, increased size of the liver and also umbilical hernia observed in PFOS treated mouse fetal group compared to untreated fetal control group.

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