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TOXICOLOGICAL EFFECTS OF PERFLUOROALKYL ACIDS ON PREGNANT FEMALE MICE

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ABSTRACT:

Perfluorinated compounds (PFCS), such as perflurooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been used for various industrial applications for over 50 years. In this study 160 pregnant dams were subjected to this study; Dams were divided into two main equal groups, PFOS and PFOA groups. Each group was subdivided into two groups, treated group (60 dams) and control group (20 dams). Both treated groups where re-divided into three equal groups. Dams in the first group were treated with PFOS in dosage of 1, 10 or 20 mg/kg b.w., while dams in the second group were treated with PFOA in dosage of 1, 5 or 10 mg/kg b.w. Control group was received an equivalent volume of deionized water. Maternal body weight, food consumption and water intake were monitored daily throughout gestation period. Ten dams of each subgroup were treated from gestation day (GD) 0 till GD17, At GD18, blood samples were collected and serum samples were obtained for determination of Lactate dehydrogenase (LDH), Gamma glutamyl transferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen, total bilirubin, total protein, albumin, globulins, calcium, inorganic phosphorus, glucose, triglycerides, phospholipids, total cholesterol, non esterified fatty acids, hydroxyl butyric acid and serum leptin concentration. Maternal liver, kidneys, lungs and brain were dissected and weighed; the organ/body weight ratio was calculated to obtain the relative organ weight and then kept for histopathological examination.. A portion of the liver was dissected and used immediately for comet assay. Results revealed significant reduction in maternal weight gain and daily feed consumption after exposure to 20 mg/kg b.w. PFOS and 10 mg/kg b.w. PFOA. Daily water intake was significantly increased after exposure to 20 mg/kg PFOS and 5 mg/kg PFOA in late gestation. There were significant increases in the absolute and relative weight of the maternal liver in a dose dependent manner associated with hypertrophy of hepatic cells after exposure to both of PFOS and PFOA, and significant increase in the relative lung and brain weight after exposure to PFOS at 20 mg/kg group. Relative kidney weight was significantly increased after exposure to PFOA. Serum lipids, protein and leptin levels were significantly decreased after exposure to PFOS and PFOA at 20 mg/kg b.w. and 10 mg/kg b.w. respectively. In addition, exposure to PFOA resulted in significant increases in serum GGT, AST, ALP activities. PFOS treatment induced DNA damage in maternal liver at 10 and 20 mg/kg groups. However, exposure to PFOA induced DNA damage at 10 mg/kg. From the previous results we can conclude that PFOS and PFOA have toxic effects on the pregnant mice and PFOA recorded the most toxic one. Further study will be carried on featuses and newnates.

INTRODUCTION:

Perfluorinated compounds (PFCs) are now ubiquitous global contaminants. These

chemicals have been detected in indoor and outdoor air, in rivers, lakes and groundwater, in wastewater treatment effluent, in landfills and in the marine environment. PFCs have also been found in the body tissues of many different living organisms throughout the world including humans (Allsopp *et al*., 2005). These compounds have been used in a wide variety of consumer products, due to their widespread use, persistence and bioaccumulative properties they are taken up by the general population from different sources (Midasch *et al*., 2007).

Perfluoroalkyl acids (PFAA) are man made fully fluorinated organic chemicals. They can be released into the environment via product manufacturing, use and disposal. Many of their degradation products have been found in the environment throughout the world, but the 8-carbon (C-8) products such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are two of the most widely detected. Because of the strong carbon- fluorine (C-F) bond associated with Perfluorinated chemicals, PFOS and PFOA are environmentally persistent (Kannan *et al*., 2004)

PFOS-related substances have been used in the packaging and paper industries in both food packaging and commercial applications to impart grease, oil and water resistance to paper, paperboard and packaging substrates. They also used for both food contact applications (plates, food containers, bags and wraps) and non-food applications (folding cartons, containers, carbonless forms and masking papers). PFOS-based products were used to improve the wetting of water-based products marketed as alkaline cleaners, floor polishes (to improve wetting and leveling), denture cleansers and shampoos (OECD, 2002).

PFOA is used in fire-fighting applications, cosmetics, grease and lubricants, paints, polishes and adhesives, herbicide and insecticide formulations (Moody and Field, 1999). PFOA is used to make Teflon (polytetrafluoroethylene PTFE), a nonstick coating used for kitchen ware (sauce pans). PFOA is used also to make Goretex (water proof fabric used for protective rain wear and insulation for wires and cables) (Renner, 2003).

Several toxicological studies have demonstrated that the liver is the primary target organ for PFOS and PFOA (Butenhoff *et al*., 2002; Kennedy *et al*., 2004 and Son *et al*., 2008). The hepatotoxicity is manifested as increased liver weights, hepatocellular hypertrophy, liver degeneration and necrosis, increases in plasma transaminases, and proliferation of smooth endoplasmic reticulum and peroxisomes in rodents. Hypolipidemia has also been reported in some rodent studies (Butenhoff *et al*., 2004). PFOS exposure increases the serum alanine aminotransferase (ALT) activity, which is a marker for hepatic damage (Seacat *et al*., 2003).

PFOS and PFOA have also been shown to cause reductions in serum cholesterol and/or triglycerides in several animal species (Seacat *et al*., 2003 and Thibodeaux *et al*., 2003).

Many studies have demonstrated that long-term treatment with PFOA results in the development of liver tumors in rats. PFOA is potently immunosuppressive in mice. Immunosuppression can lead to increased incidence and severity of infection and tumour frequency. The exposed mice to PFOA were tested to immune response by the introduction of horse red blood cells. The number of splenocytes (cells of the immune system) that produced antibodies to the horse red blood cells was dramatically decreased in PFOA treated mice. The study concluded that PFOA causes a pronounced suppression of adaptive immunity in mice. In considering humans who have been occupationally exposed to PFCs, the study noted

that the doses of PFOA that caused immunosuppression in mice were considerably higher than levels in such exposed humans (Nilsson *et al*. 1991).

Leptin is a protein hormone produced by white adipocytes and is involved in the regulation of various neuroendocrine functions, including food intake. Leptin levels are positively correlated with body fat. The increase in serum leptin levels is believed to decrease food intake, and vice versa (Ahima, 2000).

The presented work was carried out on pregnant female mice to study the toxic effects of both PFOS and PFOA on maternal organs, biochemical parameters as well as DNA.

MATERIALS AND METHODS:

Animals:

Adult ICR male and female mice were purchased from CLEA Japan, Inc., Tokyo at 7 weeks of age and used for the experiment after one week of acclimatization. Female mice were checked for estrous cycle stage and each proestrus female was placed with an individually housed breeder male overnight, and those females with spermatozoa in a vaginal smear and/or with a copulatory plug were considered to be at GD0.

Chemicals:

PFOS: Perfluorooctane sulfonate (potassium salt 98% pure) was purchased from Fluka Chemie GmbH, Switzerland. PFOS solutions were prepared at 0.1, 1 and 2 mg/ml of 0.5% Tween-20 vehicle and administered to the pregnant mice by gavage at a volume of 10 ml/kg/day.

PFOA: Perfluoroocatnoic acid (90% pure) was purchased from Fluka Chemie GmbH, Switzerland. PFOA solutions were prepared at 0.1, 0.5 and 1 mg/ml of deionized water and administered to the pregnant mice by gavage once daily from GD0 till GD17 at a volume of 10 ml/kg.

Animal treatments:

This study protocol was approved by the Animal Research Committee, a total number of 160 pregnant dams were subjected to the study, Dams were divided into tow main groups, PFOS group and PFOA group (80 each). Each group were subdivided into two groups, treated group (60 dams) and control group (20 dams). Each treated group was divided into three equal groups. Dams in PFOS groups were exposed daily to PFOS in dosage of 1, 10 or 20 mg/kg b.w., while dams in PFOA group were exposed daily to PFOA in dosage of 1, 5 or 10 mg/kg b.w oraly. Ten dams of each subgroup were exposed daily from GD0 till GD17, at GD18 dams were euthanized and the gravid uterus were removed and fetuses kept for next study, while dams were used in this study. The other ten dams were exposed daily from GD0 till GD18 and then allowed to give birth which used in the next study. Control group were received an equivalent volume of deionized water. Maternal body weight, food consumption and water intake were calculated by obtaining the value of change on daily bases from GD0 to GD18.

Serum samples were used for the determination of blood serum levels of Lactate dehydrogenase (LDH), Gamma glutamyl transferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), total bilirubin, total protein, albumin, globulins, calcium, inorganic phosphorus, glucose, triglycerides, phospholipids, total cholesterol (T-CHOL), non esterified fatty acids (NEFA) and hydroxyl butyric acid by

using Autoanalyzer (Hitachi Autoanalyzer 7060, Hitachi Ltd. Tokyo, Japan). Serum leptin levels were determined using a commercial ELISA Kit supplied by (Ray Biotech, Inc.) and by means of micro plate reader (MPR.A4i), the assay was conducted according to the instructions of the kit.

DNA damage in maternal liver was detected using comet assay (Single Cell Gel Electrophoresis) according to the method of Sasaki *et al.* (1997) and Tsuda *et al.* (1998). Comet assay is generally quantified using comet tail length and tail moment, tail momet was calculated as tail length multiplied by the percentage of DNA in the tail (Tice *et al.*, 2000). Tail length can be used to indicate initial DNA damage, while tail moment and the percentage of DNA in the tail can be used to indicate the intensity of damage (Knopper *et al.*, 2005). Maternal liver, kidneys, lungs and brain were processed routinely for paraffin embedding technique. Tissue sections were stained with the standard Haematoxylin and Eosin method (H. & E.) according to Bancroft and Stevens (1982).

Data are presented as means and standard errors. Statistical significance was determined by the analysis of variance (ANOVA). When a significant treatment effect was detected, each treatment group was tested for difference from the control group using Dunnett's t-test. Statistically significant differences were determined at $p \leq 0.05$. Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA) according to Borenstein *et al.* (1997).

RESULTS:

Maternal weight gain was significantly reduced at PFOS group at dose of 20 mg/kg group from GD11 until the end of gestation (Fig. 1a). While there was significant reduction in weight gain in PFOA group at 10 mg/kg from GD 12 till the end of gestation (Fig. 1b).

At 10 mg/kg PFOS group; daily feed intake was significantly reduced on GD11, The 20 mg/kg PFOS group showed a significant decrease in daily feed consumption in late gestation on GD 12, 17 and 18 (Fig. 2a), while there was a significant increase in water intake in the same groups (Fig. 3a). In PFOA group, there were significant increase in food consumption on GD14, 15, 16 and 17 (Fig. 2b), and water intake in late gestation from GD 13 to GD 18 in the 5 mg/kg. In dose of 10 mg/kg, there was increase in water intake on GD10 and GD14 (Fig. 3b).

There were no apparent toxic symptoms or deaths in all PFOS and PFOA treated dams. PFOS and PFOA treatment increased the absolute and relative liver weight in a dose-dependent manner. Reducion of the absolute kidney weight was observed in PFOS group at 20 mg/kg. Incontrary there was increase in the relative kidney weight in all PFOA treated groups. On the other hand, there was significant increase in the relative weight of the lung and brain at 20 mg/kg PFOS group, while there was reduction on the absolute brain weight at the 10 mg/kg PFOA group, result were tabulated in tables (1&2).

Biochemical analysis of dam's serum revealed that administration of PFOS in a dose of 20 mg/kg resulted in significant decreases in serum total protein, globulins, calcium, triglycerides, phospholipids, total cholesterol, and non-esterified free fatty acids levels. However, there were significant decreases in serum triglycerides and free fatty acids levels at 10 mg/kg group as showed in tables (3 &4). Also it decreased serum leptin concentration in a dose dependent manner with significant reduction at 20 mg/kg group as showed in table

(3). Administration of PFOA in a dose of 10 mg/kg group resulted in significant increases in serum GGT, AST, ALP activities and A/G ratio, and significant decreases in serum total protein, albumin, globulins, phospholipids, triglycerides, total cholesterol, non-esterified fatty acids which represented in tables (5&6), and decreased the serum leptin concentration as showed in table (5).

Treatment of dams with PFOS showed DNA damage in maternal liver at doses 10 and 20 mg/kg in the form of increased DNA migration (tail length) as shown in table (7) and tail moment. While PFOA treatment cause DNA damage in maternal liver at the dose of 10 mg/kg group in the form of increased tail length and tail moment as presented in Fig. (4) and table (8).

Histopathological evaluation of maternal organs revealed hepatic hypertrophy in the 20 mg/kg PFOS group. In PFOA group histopathological examination of maternal organs revealed that the liver is the most affected organ which appeared as hepatocellular hypertrophy and increased mitosis in a dose dependent manner. On the other hand hepatocytic necrosis was found at 5 and 10 mg/kg and mild calcification of hepatocytes at 10 mg/kg (Fig. 5 a &b). Maternal kidney showed slight hypertrophy of the proximal tubule and outer medulla in the exposed groups (Fig. 6 a & b).

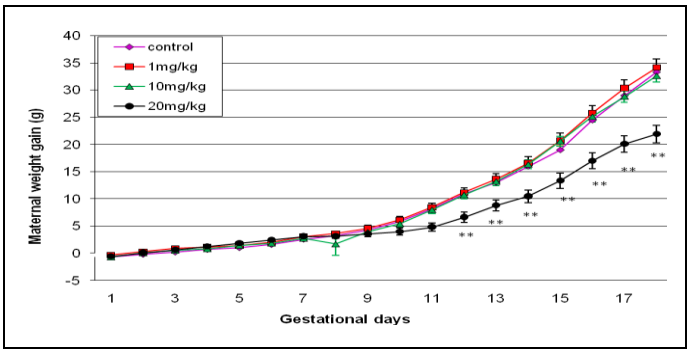


Fig. (1a) : Effect of PFOS on maternal weight gain

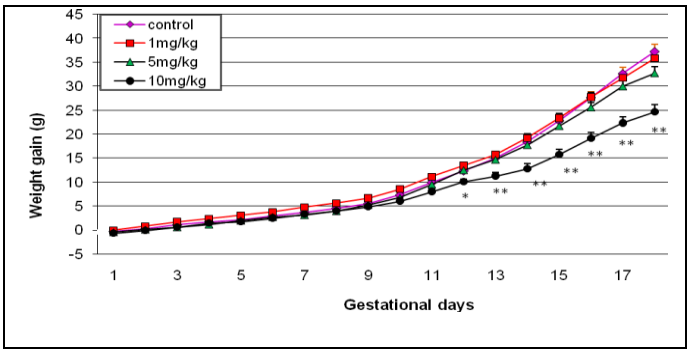


Fig. (1b): Effect of PFOA on maternal weight gain

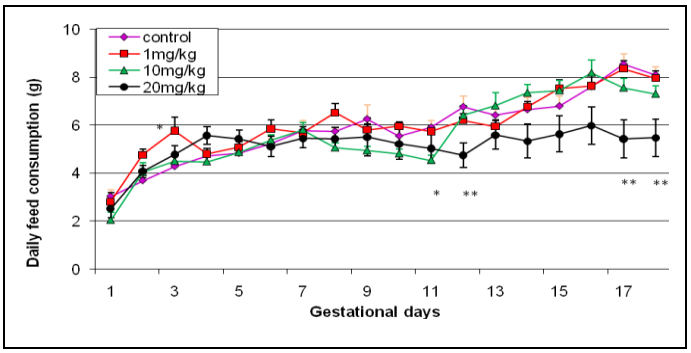


Fig. (2a): Effect of PFOS on daily feed consumption

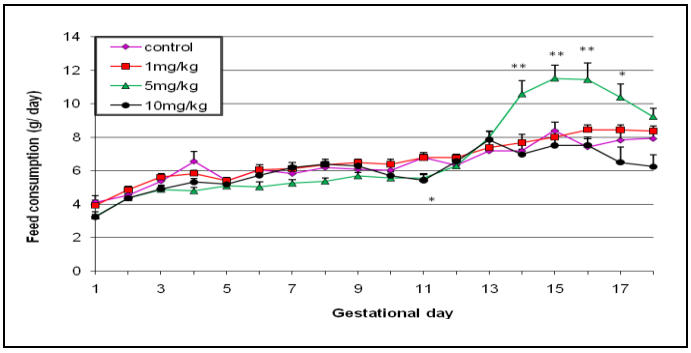


Fig. (2b) :Effect of PFOA on daily feed consumption

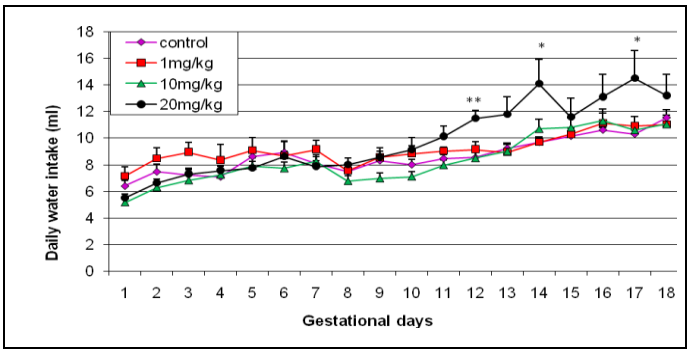


Fig. (3a):Effect of PFOS on daily water intake

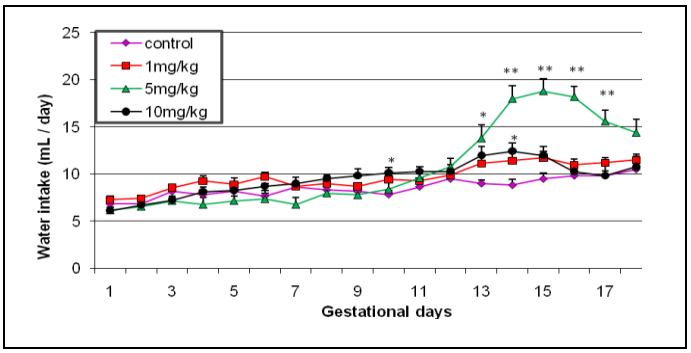


Fig. (3b): Effect of PFOA on daily water intake

Table (1): Effects of PFOS and PFOA on organs weight of exposed dams at GD18

Substance	Organs				
	Dose	Liver (g)	Kidney (g)	Lung (g)	Brain(g)
PFOS	Control	2.70 ± 0.10	0.45 ± 0.01	0.21± 0.01	0.46 ± 0.01
	1 mg/kg	2.92 ± 0.11	0.45 ± 0.02	0.21 ± 0.01	0.46 ± 0.01
	10 mg/kg	4.05 ± 0.19**	0.42 ± 0.01	0.20 ± 0.01	0.46 ± 0.01
	20 mg/kg	**4.40 ± 0.11	0.40 ± 0.01*	0.19 ± 0.00	0.45 ± 0.01
PFOA	Control	2.77 ± 0.05	0.43 ± 0.05	0.21 ± 0.01	0.48 ± 0.01
	1 mg/kg	3.58 ± 0.30	0.47 ± 0.02	0.21 ± 0.01	0.47 ± 0.01
	5 mg/kg	5.17 ± 0.23**	0.43 ± 0.02	0.19 ± 0.01	0.47 ± 0.01
	10 mg/kg	6.39 ± 0.24**	0.45 ± 0.02	0.19 ± 0.01	0.45 ± 0.01**

Data represent mean ± SE. Significant difference (*p <0.05, **p<0.01) between control and treated groups.

Table (2): Effects of PFOS and PFOA on the relative organ weight of exposed dams at GD18

Substance	Organs				
	Dose	Liver (%)	Kidney (%)	Lung (%)	Brain (%)
PFOS	Control	4.02 ± 0.12	0.67 ± 0.01	0.32 ± 0.01	0.68 ± 0.02
	1 mg/kg	4.45 ± 0.15	0.68 ± 0.02	0.33 ± 0.01	0.71± 0.02
	10 mg/kg	6.58 ± 0.18**	0.68 ± 0.01	0.33 ± 0.01	0.75 ± 0.02
	20 mg/kg	8.14 ± 0.22**	0.74 ± 0.02	0.36 ± 0.01*	0.84 ± 0.02**
PFOA	Control	4.01± 0.06	0.62 ± 0.01	0.30 ± 0.01	0.69 ± 0.02
	1 mg/kg	5.37± 0.25*	0.72 ± 0.02**	0.031± 0.01	0.73 ± 0.02
	5 mg/kg	8.59 ± 0.29**	0.71 ± 0.01*	0.031 ± 0.01	0.78 ± 0.02
	10 mg/kg	11.42 ± 0.60**	0.79 ± 0.01**	0.34 ± 0.01	0.80 ± 0.04

Data represent mean ± SE. Significant difference (*p <0.05, **p<0.01) between control and treated groups.

Table (3): Biochemical parameters in dams at GD 18 after exposure to PFOS

Groups / Parameters	Control	Concentration (mg/kg b.w.)		
		1 mg	10 mg	20 mg
LDH (U/l)	1245.1 ± 236.1	1570.7 ± 326.1	1664.7 ± 175.4	1577.8 ± 282.7
GGT (U/L)	0.09 ± 0.07	0	0.04 ± 0.04	0.02 ± 0.01
AST (U/L)	113.17 ± 14.15	102.21 ± 15.19	111.46 ± 12.58	197.17 ± 37.16
ALT (U/L)	24.86 ± 6.60	24.33 ± 5.53	40.30 ± 12.92	47.01 ± 14.85
ALP (U/L)	195.80 ± 27.44	229.71 ± 33.63	185.40 ± 31.76	227.30 ± 24.53
Creatinine (mg/dl)	0.15 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.11 ± 0.02
BUN (mg/dl)	24.68 ± 1.73	22.15 ± 1.46	26.35 ± 1.19	23.55 ± 1.36
Total bilirubin (mg/dl)	0.01 ± 0.01	0.01 ± 0.00	0	0.02 ± 0.07
Total protein (g/dl)	4.11 ± 0.13	4.19 ± 0.26	4.02 ± 0.23	3.35 ± 0.16*
Albumin (g/dl)	2.52 ± 0.09	2.64 ± 0.10	2.52 ± 0.14	2.14 ± 0.10
Globulins (g/dl)	1.58 ± 0.05	1.54 ± 0.10	1.49 ± 0.11	1.21 ± 0.08*
A/G ratio	1.59 ± 0.05	1.72 ± 0.05	1.72 ± 0.09	1.74 ± 0.18
Leptin (ng/ml)	313.22 ± 40.77	216. 64 ± 46.63	188.45 ± 33.75	129. 19 ± 52.15*

Data represent mean ± SE. Significant difference (*p <0.05) between control and treated groups.

Table (4): Serum minerals and lipid parameters in dams at GD 18 after exposure to PFOS

Parameters / Groups	Control	Concentration (mg/kg b.w.)		
		1mg	10 mg	20 mg
Phosphorus (mg/dl)	8.11 ± 0.91	8.42 ± 0.57	9.70 ± 0.38	9.17 ± 0.48
Calcium (mg/dl)	10.07 ± 0.26	10.01 ± 0.42	9.34 ± 0.68	7.94 ± 0.55*
Glucose(mg/dl)	105.54 ± 20.21	97.15 ± 17.6	118.93 ±16.48	72.68 ± 9.06
Triglyceride (mg/dl)	157.80 ± 23.53	147.04 ± 34.4	65.15 ± 7.93**	37.40 ± 5.16**
Phospholipids (mg/dl)	95.83 ± 12.08	78.05 ± 8.59	77.43 ± 5.90	60.21 ± 3.19**
T-CHOL (mg/dl)	54.62 ± 5.21	52.45 ± 5.05	52.88 ± 3.70	40.34 ± 2.27*
NEFA (mmol/l)	1248.1 ± 146.6	1441.8 ± 159.4	798.0 ±78.24*	478.50 ± 29.9**
Hydroxy butyric acid (µmol/l)	195.24 ± 42.03	265.30 ± 52.83	252.04 ± 37.28	249.08 ± 27.47

Data represent mean ± SE. Significant difference (*p <0.05, **p<0.01) between control and treated groups.

Table (5): Biochemical parameters in dams at GD 18 after exposure to PFOA

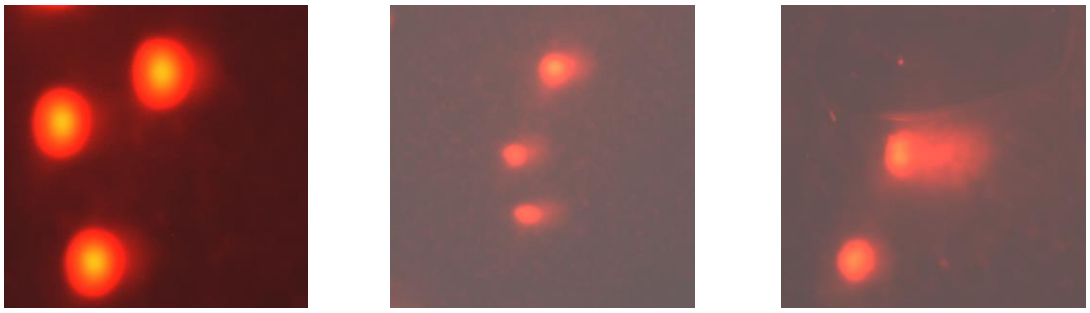
Parameters / Groups	Control	Concentration (mg/kg b.w.)		
		1 mg	5 mg	10 mg
LDH (U/l)	1802.0 ± 263.7	1476.2 ± 276.2	1307.7 ± 204.9	2810.5 ± 618.39
GGT (U/L)	0	0	0	2.50 ± 1.11*
AST (U/L)	103.81±10.21	104.6 ± 9.65	427.80 ± 79.64	1179.9 ± 282.8**
ALT (U/L)	24.60 ± 8.35	15.78 ± 6.24	61.62 ± 13.78	223.06 ± 110.39
ALP (U/L)	195.02 ± 23.5	217.40 ± 19.52	256.70 ± 31.72	772.5 ± 157.6**
Creatinine (mg/dl)	0.15 ± 0.01	0.21 ± 0.04	0.14 ± 0.05	0.15 ± 0.02
BUN (mg/dl)	22.61 ± 1.42	27.80 ± 1.30*	25.44 ±1.68	20.45 ± 0.97
Total bilirubin (mg/dl)	0	0	0	0.08 ± 0.05
Total protein (g/dl)	3.95 ± 0.15	3.94 ± 0.17	3.35 ± 0.16*	3.09 ± 0.09**
Albumin (g/dl)	2.36 ± 0.18	2.45 ± 0.13	2.11 ± 0.09	2.00 ± 0.07*
Globulins (g/dl)	1.58 ± 0.02	1.49 ± 0.04	1.24 ± 0.06**	1.08 ± 0.04**
A/G ratio	1.49 ± 0.05	1.60 ± 0.05	1.70 ± 0.04	1.86 ± 0.07**
Leptin (ng/ml)	460.68 ± 52.04	565.38 ± 29.14	413.82 ± 90.7	82.10 ± 13.67**

Data represent mean ± SE. Significant difference (*p <0.05, **p<0.01) between control and treated groups.

Table (6): Serum minerals and lipid parameters in dams at GD 18 after exposure to PFOA

Parameters / Groups	Control	Concentration (mg/kg b.w.)		
		1 mg	5 mg	10 mg
Phosphorus (mg/dl)	6.05 ± 0.91	9.26 ± 0.62*	8.80 ± 0.86*	7.96 ± 0.51
Calcium (mg/dl)	8.96 ± 0.67	9.34 ± 0.51	9.26 ± 0.44	8.05 ± 0.40
Glucose(mg/dl)	111.70 ±18.68	129.76 ± 8.11	104.70 ± 8.57	69.96 ± 9.70
Triglyceride (mg/dl)	175.28 ± 29.34	131.96 ± 31.45	91.66 ± 21.06	31.55 ± 7.68**
Phospholipids (mg/dl)	76.05 ± 4.55	84.54 ± 10.52	68.20 ± 7.55	50.76 ± 4.69*
T-CHOL (mg/dl)	50.02 ± 2.56	54.44 ± 7.79	45.80 ± 5.61	34.20 ± 3.65*
NEFA (mmol/l)	1266.14 ± 143.52	1237.20 ± 238.03	832.40 ± 156.24	703.87 ± 98.84*
Hydroxy butyric acid (µmol/l)	191.44 ± 27.34	415.52 ±155.20	286.96 ± 64.29	178.10 ± 25.66

Mean ± SE. Significant difference (*p <0.05, **p<0.01)



Control **A** **B**

Fig. (4): Image of an alkaline comet (hepatocytes) stained with ethidium bromide showing undamaged nucleus (control) and damaged nucleus, A) Partially damaged, B) Severely damaged

Table (7): Effects of PFOS exposure on maternal liver DNA migration and tail moment

Parameters	Control	Concentration (mg/kg b.w.)		
		1 mg	10 mg	20 mg
DNA migration (µm)	15.97 ± 0.94	13.11±0.75	22.45 ±1.02**	26.98 ± 0.91**
Tail moment (µm)	1.10 ± 0.14	1.03 ± 0.15	2.03 ±0.19**	2.25 ± 0.20**

Mean ± SE. Significant difference (p<0.01)**

Table (8): Effects of PFOA exposure on maternal liver DNA migration and tail moment

Parameters	Control	Concentration (mg/kg b.w.)		
		1 mg	5 mg	10 mg
DNA migration (µm)	20.88 ± 1.01	18.67 ± 1.24	24.51 ± 1.21	30.59 ± 1.94**
Tail moment (µm)	1.27 ± 0.11	1.32 ± 0.14	2.31 ± 0.22	4.77 ± 0.69**

Mean ± SE. Significant difference (p<0.01)**

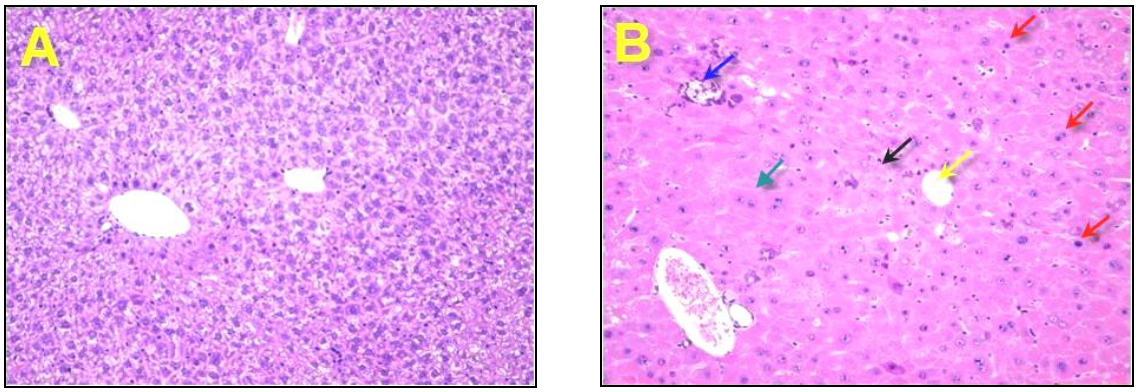


Fig. (5): Photomicrograph showing liver of dam A) Control liver, B) PFOA group received 10 mg/k g, showing necrobiotic changes in hepatocytes (black arrow), increase mitotic figures (red arrow), dilatation of central vein (yellow arrow) and blood sinusoids, hypertrophy of hepatocytes (green arrow) and discrete foci of calcification (blue arrow) (400X).

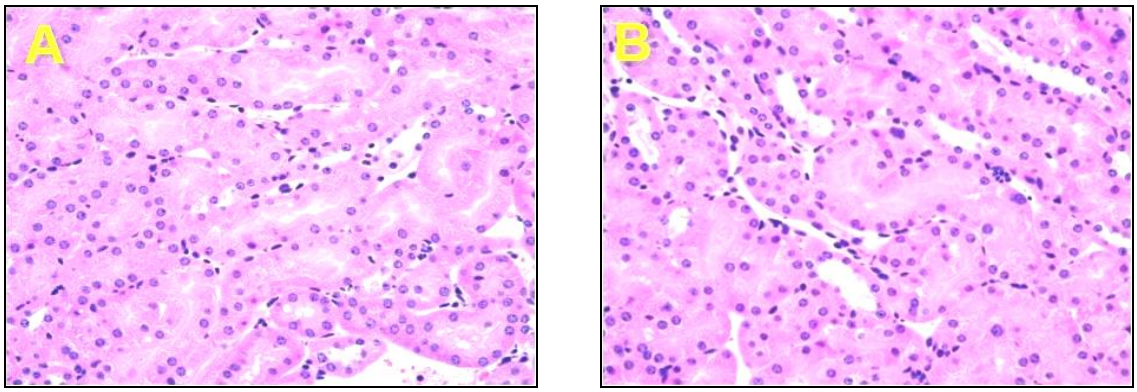


Fig. (6): Photomicrograph showing kidney of dam, A) Control kidney, B) Kidney from PFOA group received 10 mg/k g, showing hypertrophy of the outer medulla cells

DISCUSSION:

Perfluorinated compounds (PFCs) have been used in a variety of commercial and industrial applications for over 50 years. They have recently received attention due to their widespread contamination in the environment, wildlife and human (Calafat *et al*., 2007). PFOS and PFOA are the most widely detected and studied compounds in this class. The levels of PFOS and PFOA have been increasing more and more in global oceans. Due to their use and toxicological importance.

In the current study, decrease in maternal weight gain was observed after exposure to 20 mg/kg PFOS and 10 mg/kg PFOA starting from GD11 and GD12 respectively and till the end of gestation period. PFOS and PFOA were reported to cause a decrease in maternal weight gain (Lau *et al*., 2006). The decrease in the maternal weight gain in this study may be attributed to the decrease in feed consumption. While Thibodeaux *et al*. (2003) said that the adverse effect on maternal weight gain may be a common feature of toxicity for the perflourochemicals. The present maternal effects of PFOS and PFOA exposure to pregnant mice including inhibitions of maternal weight gain and food consumption, same results were obtained in mice and rats by Case *et al*.,

(2001) and Grasty *et al.*, (2005), except for increased water intake after exposure to 20 mg/kg PFOS. The reason for this difference may be attributed to reduction in serum leptin concentration which results in increased water intake as represented in figures (1a & b, 2a & b and 3a & b).

Several toxicological studies have demonstrated that the liver is the primary target organ for PFOS and PFOA (Son *et al.*, 2008). PFOS is accumulated in the serum and liver (Seacat *et al.*, 2003). PFOA distributes predominantly to the liver and plasma, and to a lesser extent to the kidney and lungs (Vanden *et al.*, 1991). Exposure to PFOS and PFOA in the present study showed significant increase in absolute and relative liver weights in a dose dependant manner as shown in tables (1 & 2). This increase in liver weights may be attributed to proliferation of smooth endoplasmic reticulum and peroxisomes (Son *et al.*, 2008). Exposure to PFOS and PFOA in this study resulted in hepatocellular hypertrophy. Administration of PFOA caused hepatic degeneration, necrosis, increased mitosis and mild calcification as shown in figure (5).

PFOA treatment resulted in a significant increase in the relative kidney weight in all treated groups as in table 2. Histopathological examination of the kidney revealed hypertrophy of the cells of the proximal tubule and outer medulla (Fig. 6). This result is in agreement with that obtained by Butenhoff *et al.* (2004) who found that treatment of rats with PFOA increased the relative kidney weight. On the other hand Son *et al.* (2008) found that oral treatment of mice with PFOA for 21 days did not affect the kidney.

Biochemical analysis of dam's serum revealed that administration of PFOS in a dose of 20 mg/kg and PFOA in a dose of 10 mg/kg resulted in hypolipidemia and hypoproteinemia. A significant decrease in serum triglycerides, phospholipids, total cholesterol, and non-esterified fatty acids was recorded in this study, total protein and globulins showed significant decrease as well. Decrease in serum calcium concentration in the 20 mg/kg PFOS group was recorded and decrease in serum albumin in the 10 mg/kg PFOA was obtained. Significant increases in serum GGT, AST, ALP activities were registered after exposure to 10 mg/kg PFOA.

PFOS and PFOA produced the same effect on the parameters of serum lipids measured in the current study; these compounds have also been shown to cause reduction in serum cholesterol and/or triglycerides in several animal studies including rats and mice (Thibodeaux *et al.*, 2003) and Cynomolgus monkeys (Seacat *et al.*, 2003). Reduction in phospholipids, triglycerides, cholesterol and free fatty acids indicated that PFOA had hypolipidemic effect as Xie *et al.* (2003) reported that treatment of mice with PFOA caused severe adipose tissue atrophy and hypolipidemic effect. Furthermore, exposure to PFOA was reported to inhibit the secretion of very low-density lipoprotein (VLDL), cholesterol and triglycerides from the liver (Kennedy *et al.*, 2004). These results explain the hypolipidaemic effect of exposure to PFAA in the current study.

PFOS treatment resulted in hypoproteinaemia and hypoglobulinaemia. However, exposure to PFOA resulted in reduction in all protein parameters (total protein, albumin and globulins) ($p<0.01$). The reduction in serum globulin level may be attributed to immunotoxicity; previous studies reported that both of PFOS (Peden-Adams *et al.*, 2008) and PFOA (DeWitt *et al.*, 2008) have an immunotoxic effect. It was stated that the production of the immunoglobulin M (IgM) antibody decreased in mice exposed to PFOS

(Peden-Adams *et al.*, 2008) and PFOA (DeWitt *et al.*, 2008). Another study, evaluated the immune systems of mouse pups whose mothers were exposed to the compound during pregnancy, showed that natural killer cell function was decreased in male pups. The authors added that PFOS targeted antibody production and that males appeared more sensitive than females to the effects of PFOS (Keil *et al.*, 2008). Another cause for hypoproteinemia after exposure to PFOA is the reduction in serum albumin level, which may be attributed to the severe hepatic dysfunction (Murray, 2003).

Serum enzyme activities were significantly increased after exposure to PFOA. However, no significant changes were observed after exposure to PFOS. Enzymes measured in the present study reflect mainly the status of the liver (Bogin *et al.*, 1986). The significant increases in serum AST ($p<0.01$), GGT ($p<0.05$) and ALP ($p<0.01$) confirm the gross and histopathological findings recorded in the present study and indicated that the hepatotoxic effect of PFOA was more severe than PFOS. The same results were reported by Kennedy *et al.* (2004).

Leptin is a protein hormone produced by white adipocytes and is involved in the regulation of various neuroendocrine functions, including food intake. Leptin levels are positively correlated with body fat. The increase in serum leptin levels is believed to decrease food intake, and vice versa (Ahima, 2000).

Measurement of serum leptin concentration in this study, revealed a significant decrease after exposure to PFOS and PFOA. This decrease was dose dependent and significant changes were observed only at 20 mg/kg (129.19 ± 52.15) for PFOS and at 10 mg/kg (82.10 ± 13.67) for PFOA. Similar results were reported by Austin *et al.* (2003) who found that treatment of rats with 10 mg/kg PFOS for 2 weeks reduced the serum leptin concentration. The low leptin levels observed after exposure to PFOS and PFOA may be related to the reduced body fat stores, as treatment of mice with PFOA was reported to cause severe adipose tissue atrophy (Xie *et al.*, 2003). However, this reduction in leptin levels failed to stimulate food intake in the treated animals, suggesting a possible derailment of the neurotransmitter activity that regulates feeding behavior. The inhibitory effect of PFAA exposure on food intake was therefore independent of their effect on serum leptin levels.

DNA damage using comet assay is generally quantified using comet tail length and tail moment. Our results indicated that PFOS and PFOA had genotoxic effects on hepatic cells because these compounds induced remarkable DNA damage in hepatic cells. Significant increase in tail length (DNA migration) and tail moment was recorded in maternal hepatic cells exposed to PFOS at dose of 10 and 20 mg/kg. Meanwhile maternal exposure to PFOA increased the tail length and tail moment of maternal hepatic cells at groups 10 mg/kg only.

From the obtained result we conclude that PFOS and PFOA are toxic to pregnant mice with several degrees and PFOA has the most toxic effect. The toxic effect of both combounds was tested for fetuses in next study.

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التأثيرات السامة لأحماض البيرفلوروالكيل على إناث الجرذان الحوامل

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تم استخدام عدد ١٦٠ من إناث الجرذان الحوامل والتي قسمت إلى مجموعتين متساويتين. تم إعادة تقسيم كل مجموعة إلى مجموعتين: الأولى ٦٠ جرذ كمجموعة معالجة و ٢٠ جرذ كمجموعة ضابطة. تم إعادة تقسيم المجموعتين المعالجتين إلى ثلاث مجموعات فرعية متساوية حيث تم معالجة الثلاث مجموعات الأولى بمادة البيرفلوروأوكتان سلفونيت PFOS بجرعات ١ و ١٠ و ٢٠ مجم لكل كيلو من وزن جسم. وتم معالجة الثلاث مجموعات الثانية بمادة البيرفلوروأوكتانويك PFOA بجرعات ١ و ٥ و ١٠ مجم لكل كيلو من الوزن. تم التسجيل اليومي لوزن الأمهات واستهلاك العليقة والماء. تم معالجة عشر جرذان حوامل من كل مجموعة فرعية من اليوم الأول للحمل وحتى اليوم السابع عشر وفي اليوم الثامن عشر تم تخديرها لاستخراج الرحم والأجنة. بينما عولجت العشر الأخرى من اليوم الأول حتى اليوم الثامن عشر ثم تركت حتى الولادة. تم تجميع دم الأمهات وفصل المصل لقياس نشاط إنزيم اللاكتيت ديهيدروجينيز والجاما جلوتايل ترانسفيراز والأسبرتيت أمينوترانسفيريز والألانين أمينوترانسفيريز والفوسفاتيز القاعدى وكذلك مستوى الكرياتينين والبولينا والصفراء الكلية والألبيومين والجلوبيولين والكالسيوم والفوسفوروالجلوكوز والدهون الثلاثية والفوسفوليبيد والكوليستيرول الكلى والأحماض الدهنية وحمض الهيدروكسى بيوتيرك وكذلك مستوى اللبتين فى الدم. تم تسجيل كل من وزن الكبد والكلى والرئتين ومخ الأمهات ثم حساب وزنها النسبي وحفظها فى الفورمالين لحين أعدادها للفحص الباثولوجي. ثم حفظ جزء من الكبد لاستعماله مباشرة في فحص المادة الوراثية بها.

أوضحت الدراسة وجود انخفاض معنوي في كل من وزن الحيوان واستهلاك العليقة عند التركيز ٢٠ مجم من PFOS و ١٠ مجم من PFOA بينما وجد ارتفاع معنوي في كمية الماء المستهلكة عند التركيزات ٢٠ مجم و ٥ مجم من PFOS و PFOA على التوالي. كما وجد ارتفاع معنوي في كل من الوزن المطلق والوزن النسبي لكبد الأمهات مصحوبا بتضخم الخلايا الكبدية وزيادة معنوية في الوزن النسبي لرئتين ومخ الأمهات المعرضة ل ٢٠ مجم من PFOS وزيادة معنوية في وزن الكلى النسبي عند التعرض لمركب PFOA. وأوضحت النتائج وجود انخفاض معنوي في مستوى الدهون الثلاثية والفوسفوليبيد والكوليستيرول الكلى والأحماض الدهنية والبروتين واللبتين في الجرذان التي تعرضت إلى ٢٠ مجم من PFOS و ١٠ مجم من PFOA مع زيادة معنوية في نشاط أنزيم الجلوتايل والأسبرتيت والفوسفاتيز القاعدى. وجد ضرر بالأحماض النووية في كبد الأمهات التي تعرضت إلى التركيزات ١٠ و ٢٠ مجم من PFOS و ١٠ مجم من PFOA. والخلاصة أن كل من المركبين لهما تأثير سام على الأمهات وجارى استكمال الدراسة لمعرفة مدى تأثير هذه المركبات على الأجنة.