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Some Clinico and Histopathological Changes in Female Goats Experimentally Exposed to Dioxin

[1]A.S.M. Fouzy, [2]H.M. Desouky, [2]Y.A. Ghazi and [2]A.M. Hammam
[1]Food Toxicology and Contaminants, National Research Centre, Giza, Egypt
[2]Department of Animal Reproduction and A.I.-National Research Centre, Giza, Egypt

Abstract: Female Baladi goats were used for investigating the toxicological effects of dioxin. Each animal in the treated group was given an oral dose of 4 mL of stock standard solution of dioxin (labelled and native congeners) diluted in 5 mL distilled water (1/3 of LD_{50}) for three times with 2 days interval and slaughtered 16 days post treatment. Blood and tissue samples were taken and subjected for haemogram, biochemical and pathological studies as well as for determination of dioxin residues. Results revealed that exposure of female goats to dioxin induced anemia, leucocytopenia, neutropenia and eosinophilia with non significant increases in activities of serum ALT and AST as compared with untreated group. Meanwhile, activity of ALP and BUN concentration were significantly increased. Histopathological examination showed degenerative and necrotic changes associated with inflammatory reaction in liver and kidney, in addition to cystic glandular hyperplasia and adenomyosis in uterus. In ovarian tissue, marked decrease of preantral follicles together with cystic atretic follicle were noticed. The average percentage residues of pg WHO-TEQ values for dioxins (PCDDs and PCDFs) in liver, kidney, mammary gland, uterus and milk after oral dose were 0.013, 0.0012, 0.0012, 0.009 and 0.0012%, respectively. It was concluded that oral exposure to dioxin in female goats induced adverse effects on liver and kidney. Dioxins had estrogenic like effect as indicated by uterine and ovarian histopathological changes.

Key words: Goat, dioxins, biochemical analysis, histopathology, tissue residues

INTRODUCTION

Dioxins are a class of Persistent Polyhalogenated Aromatic Hydrocarbons (PHAHs) of which Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs), have been identified among the most globally distributed potent environmental pollutants (Scialli, 2001). Dioxins are unwanted by-products of many industrial processes and mainly come from industrial air emissions, waste incineration and combustion of fuels. Moreover; these pollutants are slowly degraded in environment and hence remain as persistent and toxic contaminants for long time (Prange *et al*., 2003). 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin (TCDD) is considered as one of the most potent members of PCDDs group (El-Sabeawy *et al*., 2001).

Exposure of man and various animal species to acute and chronic toxic levels of TCDD causes wide-varieties of adverse effects in different body tissue with species specific effect. The reported effects including hepatotoxicity, carcinogenicity, teratogenicity, interference with lipid metabolism, chloracne, neurobehavioral disturbance, endocrine disruption, wasting syndrome, thymic atrophy, developmental and reproductive toxicity and immunosuppression (Birnbaum, 1994; Wormley *et al*., 2004; Esser *et al*., 2005). Previous studies on female reproductive system of nonhuman primates indicated that exposure to TCDD disturbed ovarian function (Moran *et al*., 2001) and it has been implicated in development of endometriosis (Rier *et al*., 1993) and early embryonic losses (Li *et al*., 2006).

Therefore, the aim of this study is to identify the toxicological effect of dioxins on genital and vital organs of female goats with special reference to some clinico and histopathological changes, in addition to determination of its residues in different organs.

MATERIALS AND METHODS

The present research was carried at the National Research Centre Experimental Farm (Abu Rawash, Giza, Egypt) during period from July 2005 till January 2006.

Experimental animals: Six mature female Baladi goats (2-3 years old and 20-25 kg live body weight) were used in the current experiment. Animals were kept under the routine mangemental system and fed on commercial concentrate mixture with rice straw and barseem *ad libitum*.

Corresponding Author: Y.A. Ghazi, Department of Animal Reproduction and A.I.-National Research Centre, Giza, Egypt

Dioxin standard: The stock standard solution of dioxin was obtained from Freiburg, Germany (Rainer, 2002). It contained 17 native and C13-labeled 2, 3, 7, 8-substituted PCDD/F congeners with the concentrations given in Table 1.

Experimental design: Female goats were divided into two groups:

- The first group consisted of 3 female goats, each animal was given an oral dose of 4 mL of stock standard solution of dioxin diluted with 5 mL distilled water. The amounts of stock standard solution of dioxin given to the goats were 6.9 μg which represent 0.23 μg/body weight and equal (1/3 of LD_{50}) for guinea-pig (0.6 μg kg^{-1} body weight, Kociba *et al.*, 1978) for three times with interval of 2 days.
- The second group included 3 animals and kept as control. At the end of experimental period (16 days) all animals were slaughtered.

Samplings:

- Blood samples were taken on 0, 2, 4 and 16 days post-treatments. Samples were divided into 2 parts, the first part was collected in vials containing Ethylene Diamine Tetera acetic Acid (EDTA) for haemogram and the second part was centrifuged at 3000 rpm/15 min. The obtained sera were kept at -20°C till used for biochemical analysis.
- Tissue samples were taken from liver, kidney, mammary glands, ovaries and uterus for histopathological examination and determination of dioxin residues.

Table 1: PCDDs and PCDFs concentrations in the standard stock solution and pg WHO-TEQ values for the doses per 1 μL solvent (nonane)

Compounds	C^{13}-labelled (pg μL^{-1})	Native (pg μL^{-1})
2378TCDD	9.123	9.3950
2378TCDF	7.0695	6.9550
12378PeCDF	7.5200	7.8100
12378PeCDD	7.9460	8.7380
23478PeCDF	9.4400	9.2000
123478HxCDF	6.2000	6.3580
123678HxCDF	8.2210	8.7070
123478HxCDD	9.7330	8.5260
123678HxCDD	7.0700	7.4600
12378HxCDD	6.2440	6.0800
234678HxCDF	6.7740	7.0000
123789HxCDF	9.6750	8.5400
1234678HpCDF	7.9610	7.0250
1234678HpCDD	9.6950	8.4940
1234789HpCDF	7.1450	6.8640
OCDD	6.3890	7.4100
OCDF	7.2670	7.2100

•Pg WHO-TEQ (PCDDs/PCDFs) of 17 congeners Labelled with 13C and 17 native congeners at equal preparation. Total: 57.7826 Pg WHO

- Milk samples were taken on 0, 2, 4 and 16 days post-treatments for determination of dioxin residues.

Haematological evaluation: Complete blood picture was carried out (Jain, 2000).

Biochemical analysis: Activities of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), (Reitman and Frankel, 1957), Cholinesterase (Henry, 1964) and Alkaline Phosphatase (ALP) (Kind and King, 1954) were determined. Blood Urea Nitrogen (BUN) (Patton and Crouch, 1977), creatinine (Husdan, 1968) and serum albumin (Siedel, 1983) were also assayed. All analyses were colorimetrically determined using kits purchased from Bio Merieux, France.

Histopathological study: Tissue specimens were fixed in 10% neutral buffered formalin and prepared for histopathological examination (Bancroft *et al.*, 1996).

Determination of dioxin: Dioxin residues were determined in liver, kidney, mammary gland and uterus at the Institute for Hygiene and Food Safety-Federal Research Centre for Nutrition and Food Kiel, Germany.

Analytical procedures: Fat is extracted with acetone/petroleum benzene and the fat is removed by gel permeation chromatography. The following clean up of the extracts is performed with florisil and aluminium oxide. The dioxins are determined by GC/HRMS (Fürst *et al.*, 1989).

Gas-chormotagraphy-mass spectra (GC/MC): Finnigan MAT 95/HP Series 5890. Conditions: Injector 280°C; column: DB5 60 m, 0.25 μ film thickness, 0.25 mm ID; temperature programme: 1 min at 140°C in 15 min to 240°C in 3.5 min to 300°C, 15 min at 300°C; carrier gas: Helium, 4 mL min^{-1}; SIM: 305.90-471.78; scan time: 0.2 sec; SEV: 1.6 kv; ion source pressure: 3×10^{-7} pa; system pressure: 1×10^{-10} pa; transfer line temperature: 280°C. working resolution between 6000 and 8000 (10% valley).

Validation and detection limit: The method was validated by the Fraunhofer Institut für Verfahrenstechnik und Verpackung, Munich Germany. The detection limit for all compounds determined is 0.003 pg g^{-1} fat.

Statistical analysis: The obtained data were computed and statistically analysed according to Snedecor and Cochran (1980).

RESULTS

Clinical signs: The clinical symptoms of female goats drenched dioxins were ranging from general depression,

Table 2: The effect of oral administration of Dioxins (PCDDs, PCDFs) on blood picture of Egyptian female Baladi goats

Group	Hb (mg %)	PCV (%)	MCH (pg)	MCHC (g dL^{-1})	MCV (fl)	RBCs (×10^{6} mm^{-3})	WBCs (×10^{3} mm^{-3})	Differential leucocytic count (%)				
								Monocytes	Basoph.	Eosinoph.	Lymph.	Neutroph.
Untreated group	8.0±0.04	25.0±0.002	42.0±0.004	32.0±0.008	13.160±0.001^{a}	19.0±0.003^{ab}	17.50±0.002^{a}	1.0±0.20	1.0±0.00	1.0±0.20^{ab}	36.0±6.13	62.0±5.43^{a}
48 h Post-treated	8.27±0.20	22.9±0.71	48.37±3.39	88.78±25.97	8.193±15.71^{b}	17.39±0.84^{b}	8.02±0.20^{ab}	1.6±0.24	1.0±0.00	1.80±0.20^{b}	59.8±4.94	35.60±4.70^{ab}
96 h Post-treated	8.03±0.66	19.50±2.29	50.65±5.21	104.35±19.63	7.087±12.15^{c}	15.15±1.08^{c}	8.91±1.17^{c}	1.75±0.47	1.0±0.00	4.5±1.32^{a}	53.81±3.73	40.0±3.95^{c}

Means with different superscript are significantly different within columns at p<0.05

Table 3: The effect of oral administration of Dioxins (PCDDs, PCDFs) on some serum biochemical parameters of female Baladi goats

Groups parameters	Untreated	Treated		
		Post 48 h	Post 96 h	Post 16 days
ALT (U mL^{-1})	82.80±0.92	81.20±0.96	85.20±5.06	85.0±2.88
AST (U mL^{-1})	102.60±3.96	91.80±2.55	97.20±5.06	91.25±1.25
ALP (U mL^{-1})	51.60±1.89^{a}	57.80±1.93^{a}	59.60±1.28^{a}	89.0±7.85^{b}
BUN (mg %)	24.10±2.05^{a}	25.94±2.17^{a}	28.70±2.59^{a}	42.50±3.37^{b}
Creatinine (mg %)	1.24±0.09	0.98±0.12	0.99±0.18	1.22±0.06
Albumin (mg %)	3.99±0.19	4.57±0.15	4.33±0.22	4.74±0.16
Cholinestrase (U L^{-1})	2.54±0.19^{c}	3.53±0.28^{b}	3.82±0.33^{ab}	4.59±0.30^{a}

Means with different superscript are significantly different within rows at p<0.05

different degrees of inappetaness, poor body condition, pale mucous membranes and staggering gaits. All animals had normal temperatures (39°C) and having respiratory manifestations in the form of continuous nasal discharges and cough, together with different degree of pica.

Clinicopathological changes

Blood picture: Significant decrease in RBCs count (96 h) and MCV (48 and 96 h) was noticed post treatment as compared with untreated group. Meanwhile, non significant increase in Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were recorded 96 h post-treatment with dioxin (Table 2).

Moreover, significant decrease in total WBCs count associated with neutropenia was recorded 48 and 96 h post-treated with dioxins as compared with untreated group. On the other hand, eosinophilia was obvious in dioxin exposed female goats as shown in samples taken after 96 h post treatment compared with untreated group.

Serum biochemical values: Table 3 indicated the changes in studied biochemical parameters in female goats received dioxin. Animals in the treated group showed significantly increased (p<0.05) activity of ALP and cholinesterase and concentration of BUN with the peak values on day 16 post-treatment. Meanwhile, activities of serum ALT and AST and albumin concentration showed non significant changes as compared with the untreated group.

Histopathological findings: Liver displayed diffuse granular and vacuolar degeneration (Fig. 1). The hepatic

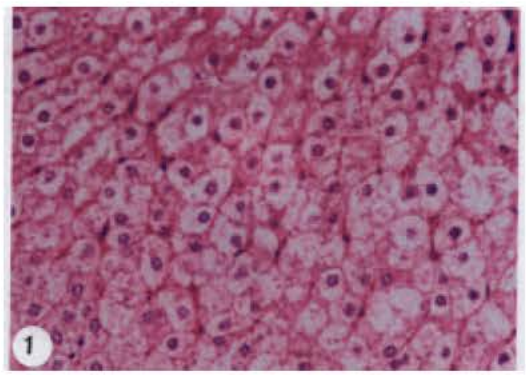

Fig. 1: Liver, showing diffuse vacuolar degeneration of hepatic cells (H & E, X200)

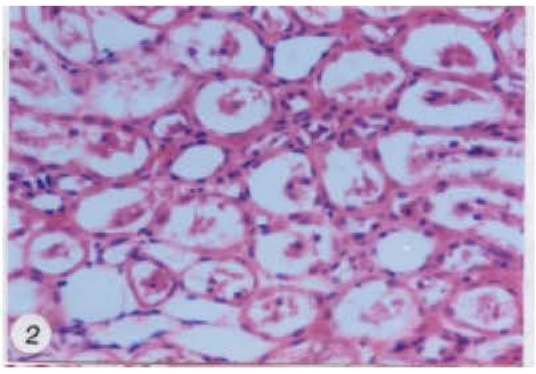

Fig. 2: Kidney, showing necrotic changes and desquamation of the epithelium lining of renal tubules (H & E, X200)

cells appeared markedly swollen, with finely granulated and vacuolated cytoplasm and thickening of the cell membrane. Activation of Kuppfer cells was noticed.

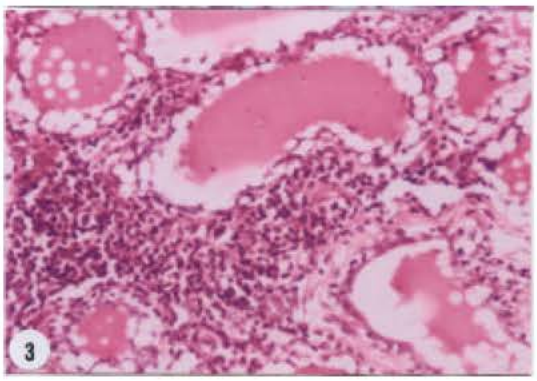

Fig. 3: Mammary gland, showing massive aggregations of lymphocytic cells (H & E, X200)

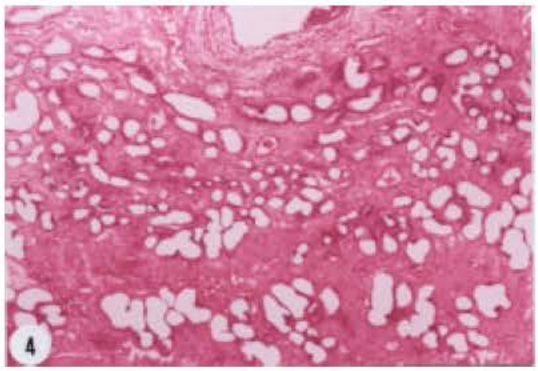

Fig. 4: Uterus, showing cystic glandular hyperplasia (H & E, X40)

Interstitial foci of round cell aggregation, mostly of lymphocytes were seen. In addition to these lesions, mild hyperplasia of epithelial cell lining of bile duct as well as infiltration of portal area with mononuclear cells were also noticed.

Kidney showed periglomerular and interstitial lymphocytic infiltrations which were focally invaded the renal parenchyma. There were proliferation of endothelial cells lining of the tuft in some glomeruli filling the subcapsular space with adhesion between the glomerular tuft and parietal layer of Bowman's capsule. Focal areas of necrotic changes of renal epithelium were seen (Fig. 2). Some renal tubules were dilated, lined with flattened epithelium and contained eosinophilic and granular casts inside its lumens. Extravasated erythrocytes were observed among the renal tubules in addition to medullary blood vessels were severely congested.

Mammary gland revealed multiple interstitial foci of lymphocytic cell aggregations in addition to intraluminal accumulation of leukocytes mainly of neutrophils in some acini was found (Fig. 3). In non lactating gland

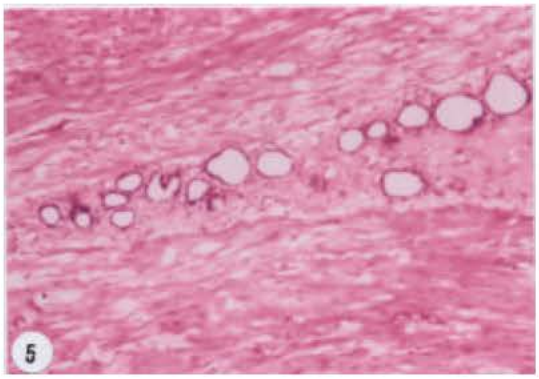

Fig. 5: Uterus, showing presence of nests of endometrial glands in between the muscles bundles of myometrium (H & E, X100)

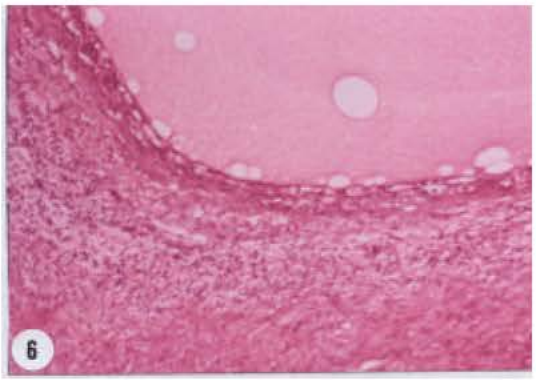

Fig. 6: Ovary, showing a part of cystic atretic follicle, note absence of granulosa cell (H & E, X100)

of one case, the lobules composed of well developed inter and intralobular ducts in addition to atrophied secretory acini.

Uterus, the epithelium lining of endometrium of examined cases was of columnar type and showed partial stratification and desquamation. The endometrial stroma was oedematous and infiltrated with mononuclear round cells.Blood vessels were highly dilated and congested. Most of uterine glands appeared increased in numbers and size, irregularly distributed and cystically dilated (cystic endometrial hyperplasia). These cysts were lined by single layer of flattened epithelium (Fig. 4). In addition to, nests of endometrial glands among the muscle bundles of myometrium (adenomyosis) was seen (Fig. 5).

Ovaries showed a marked decrease in the number of preantral follicles per section. Bilateral cystic atretic follicles embedded in ovarian stroma were found. The granulosa cell layer was completely degenerated and absent. The theca cell layer appeared thick and easily differentiated from the ovarian stroma (Fig. 6).

Table 4: Residue levels of dioxins (PCDDs, PCDFs) in the liver, kidney, mammary gland, uterus and milk of female goats 16 days after oral administration of dioxins

Organs and milk	Concentration of dioxin in organs and milk after 16 days Pg WHO-TEQ (PCDD/PCDF)/g	Dioxin residues in organ (%)*
Liver	655.9719	0.013
Kidney	81.1476	0.0011
Mammary gland	85.6546	0.0012
Uterus	32.7667	0.0009
Milk	85.08	0.0012

*Ratio of residues/oral dose

Dioxin residues in body organs: The highest percentage of dioxin residue was in the liver. Meanwhile, low percentage level of dioxin residue was observed in uterus (Table 4).

DISCUSSION

TCDD has received much attention as a developmental and reproductive toxicant with endocrine disruption capability (Peterson *et al*., 1993; Pohjanvirta and Tuomisto, 1994) The lipophilicity and resistance of TCDD to metabolization allow this compound to accumulate within target tissues where most of its toxicity is due to binding to and activating the Arylhydrocarbon Receptors (AhR), which trigger a number of biologic responses (Wilson and Safe, 1998; Laiosa *et al*., 2002).

In the present study, dioxins exposed female goats showed mild signs of adverse healthy condition; Schiller *et al*. (1985) reported similar findings in rats. Such clinical signs could be due to appetite suppressive effect of TCDD which related to its feedback mechanism originating in the periphery and not to a direct effect on appetite-regulating areas of the brain (Stahl and Rozman, 1990). Moreover, in this study, dioxins exposure was accompanied with macrocytic anemia, renal and hepatic disorders. Additionally; nervous manifestation in form of staggering gaits was accompanied with significant increase in the activity level of cholinestrase. The occurrence of anemia in this study was in line with the results reported by Funseth and IIback (1992) who found significantly decreased in RBCs count in the TCDD treated rats. In this respect, Viluksela *et al*. (1998) mentioned that the highest mortality rates recorded in rats treated with mixture of four chlorinated dibenzo-p-dioxins was related to wasting, hemorrhage and anemia, in addition to prolongation of the prothrombin times with decreased platelet counts in some rats receiving high doses. In this study, female goats exposed to dioxin revealed significant decrease in total WBCs count associated with neutropenia. It is well known that dioxin had immune suppressive effect on bone marrow lymphocyte stem cells by mechanism mediated directly or indirectly through estrogenic action (Frazier *et al*., 1994). Murante and Gasiewicz (2000) added that proliferation and/or differentiation processes of hemopoietic stem cells are affected by TCDD and these effects contribute to a reduced capacity of bone marrow to generate pro-T lymphocytes. Moreover, it was found that TCDD-treated hematopoietic stem cells almost lost long-term reconstitution activity (Sakai *et al*., 2003).

In the current investigation, BUN concentration was significantly increased at 16 days post-treatment. This result was coincided with the histopathological picture of kidney. This finding was supported by Payne and Payne (1987) who added that urea nitrogen concentration reflects the balance between its opposing rates of entry and excretion from blood, so that kidney failure prevents its excretion and lead to uraemia. Moreover increase of the copper levels in the kidney tissue after acute intoxication with-dioxin lead to nephritis and kidney dysfunction (Elsenhans *et al*., 1991). On the other hand, non significant histopathological changes in mice kidney following single dose of TCDD exposure were recorded by Esser *et al*. (2005).

The activities of serum ALT and AST of female goats received dioxin showed non significant changes as compared with untreated group. This finding was confirmed by histopathological examination of liver that showed vacuolar degeneration of hepatic cells. However, Patterson *et al*. (2003) found that treatment of mice with TCDD alone at 100 μg kg^{-1} increased serum enzyme activity of ALT and AST, at 14 days, indicating that peak liver damage occurred at that time. Recently, Chang *et al*. (2005) demonstrated that all the hepatocytes exhibiting pathological changes were AhR-positive and they provided direct evidence on the interaction and causal relationship between AhR expression and hepatocellular toxicity.

The most striking feature of histopathological findings of uterus of female goats treated with dioxin was occurrence of cystic glandular endometrial hyperplasia and adenomyosis. It is well known that uterine epithelium plays a critical role in uterine function and in reproduction and fertility in general. From the present histopathological picture of uterus, it is evident that dioxin has estrogenic like effect in goats. It is noteworthy, that dioxin and dioxin like compounds play an important role in the pathogenesis of endometriosis in rats (Vernon and Wilson, 1985), mice (Cummings and Metcalf, 1995), human and monkeys (Scialli, 2001; Rier and Foster, 2002) whereas the condition is estrogen dependence. It has been found that TCDD could mimic the effects of estrogens (Ohtake *et al*., 2003; Vajda and Norris, 2005).

In contrast, antiestrogenic effect of TCDD in mouse uterus was well described by Buchanan *et al.* (2000) and its negative effect on ovarian function and fertility (Li *et al.*, 1995). The observed toxic and biochemical responses following TCDD treatment are dependent on several factors including age, sex, species of animal and the target organ or cell type (Peterson *et al.*, 1993; El-Sabeawy *et al.*, 2001). Endometriosis might be explained on basis that dioxins induces inappropriate estrogen production in the endometrium. The (AhR) is believed to mediate most of the biological and toxicological effects of dioxins. AhR responsive genes function in reproductive process within the uterine endometrium (Rier and Foster, 2002). Previous reports indicated marked increases in concentrations of triglyceride and cholesterol in the TCDD-exposed rats, (Schiller *et al.*, 1985; Brewster *et al.*, 1988; Stanton *et al.*, 2002)through mobilization of adipose tissue lipid resulting increased plasma free fatty acid that esterified into triacyglycerides by liver (Swift *et al.*, 1981) also another study suggested that TCDD induces increase *de novo* fatty acids synthesis in the liver (Gorski *et al.*, 1988). On the other hand, the histopathological findings of ovarian tissue were cystic atretic follicles and a marked decrease in the number of growing follicles. Similar results were observed by Moran *et al.* (2001). It was reported that single exposure to TCDD can lead to long-term adverse effects on ovarian function in primates whereas TCDD could act directly on follicular development and granulosa cell division and did not affect ovarian steroidogenesis (Son *et al.*, 1999; Moran *et al.*, 2001). In this respect, Heimler *et al.* (1998) added that apoptosis does not appear to be the underlying mechanism of dioxin in reduction of growing follicles number. Benedict *et al.* (2000) stated that (AhR) regulates the toxicity of TCDD that the (AhR) may play a role in the formation of primordial follicles and the regulation of antral follicle numbers. On the other hand, Franczak *et al.* (2006) stated that the number and size of ovarian follicles were not altered by TCDD that induced endocrine disruption rather than depletion of follicular reserves as a primary mechanism of the premature transition to reproductive senescence following activation of the AhR pathway by TCDD in female rats. Moreover, Dioxins have been found in human ovarian follicular fluid (Tsutsumi *et al.*, 1998). These alterations indicated the possibility that TCDD might directly alter ovarian functions, including steroidogenesis and ovulation. Li *et al.* (1995) stated that TCDD alters reproductive function via effects on the hypothalamic-pituitary axis as well as by direct effects on the ovary.

Regarding to dioxin residues in organs, our investigation showed that the highest percentage of dioxin residue was in the liver, it is well established that dioxins had high lipophilicity and low metabolization rate. On the other hand, low percentage of dioxin residues was observed in uterus. In this respect, Grova *et al.* (2002) noticed that excretion route of the largest part of radioactive ingested TCDD remained in the organs (71.2%) and also these results were confirmed by observations of Fouzy and Rouff (2006). Dioxins residue level in milk was the same in mammary glands (0.0012%) as ratio between oral dose and residues, these findings agree with Grova *et al.* (2002) as well as Fouzy and Rouff (2006) who reported that a small amount of 2, 3, 7, 8-TCDD transfers into milk of lactating goats after oral ingestion.

Finally, it could be concluded that oral exposure to dioxin in female goats induced adverse effects on liver and kidney. Moreover, dioxin had estrogenic like effect as indicated by uterine and ovarian histopathological changes. Such effect may lead to endocrine disruption and subsequently influence on the reproductive performance of animals.

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