Article

Toxicology and Industrial Health

Toxicology and Industrial Health
2018, Vol. 34(4) 228–236

DOI: 10.1177/0748233717754174
journals.sagepub.com/home/tih
SAGE

Melatonin preserves ovarian tissues of rats exposed to chronic TCDD: An electron microscopic approach to effects of TCDD on ovarian cells

Semir Gül, Mehmet Gül and Birgül Yigitcan

Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a toxic agent and has disruptive effects on reproductive tissues in females. TCDD disrupts the hormonal regulation of the body and decreases the production of melatonin. In this study, we investigated the protective effects of melatonin supplements against the toxic effects of TCDD on ovaries of female rats. TCDD caused a significant decrease in the average number of corpora lutea and follicles per tissue section (2.1 $\pm$ 0.7; 2.3 $\pm$ 0.8, respectively), whereas these numbers were maintained in the melatonin supplemented group (5.0 $\pm$ 0.8; 5.1 $\pm$ 0.8, respectively) and were similar to the control group (5.3 $\pm$ 1.0; 5.9 $\pm$ 0.9, respectively). Electron microscopic analysis showed that the disruption of ultrastructure components such as cell membrane and organelles due to TCDD exposure was inhibited by melatonin supplements. This study suggested that melatonin has a protective and a possible ameliorative effect over histopathological damage of rat ovaries exposed to TCDD.

Keywords

2,3,7,8-Tetrachlorodibenzo-p-dioxin, ovary, melatonin, rat, dioxins

Received 23 August 2017; Revised 25 September 2017; Accepted 11 December 2017

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic member of the halogenated aromatic hydrocarbons, is a prevalent and persistent environmental contaminant formed as a synthesis side product of herbicides production and overuse of herbicides, waste incineration and combustion processes, paper bleaching, and the manufacture of plastics (Jun et al., 2011; Poland and Knutson, 1982; Yoshizawa et al., 2007). This toxic agent is able to pass from environment to humans through the food chain by absorption by the gastrointestinal system (Hutz, 1999). TCDD has a variety of adverse biological effects, including carcinogenesis, immune and hematopoietic dysfunction, neuronal cell damage (Aylward et al., 2005; Baldridge et al., 2015; Cole et al., 2003), developmental defects, impairment ovulation, and fertility reduction (Kakeyama and Tohyama, 2003). TCDD is classified as an endocrine-disturbing compound by altering endocrine hormone synthesis (Kakeyama and Tohyama, 2003; Linden et al., 1991).

Experimental data indicated that melatonin is one of these endocrine hormones targeted by TCDD, and its production decreased dramatically and considerably in rats upon TCDD exposure (Pohjanvirta et al., 1996). Melatonin, chemically *N*-acetyl-5-methoxy tryptamine, is primarily produced by the pineal gland in humans and acts as an endocrine hormone by passing into the blood circulation (Hardeland et al., 2006). It exhibits numerous physiological and metabolic functions such as an antioxidant, circadian

Department of Histology and Embryology, Faculty of Medicine, İnönü University, Malatya, Turkey

Corresponding author:
Semir Gül, Department of Histology and Embryology, Faculty of Medicine, İnönü University, Malatya 44280, Turkey.
Email: semir.gul@inonu.edu.tr

rhythm and immune system regulation, and homeostasis (Arendt and Skene, 2003). Additionally, melatonin has a wide range of medical uses as a therapeutic agent for many diseases, including cancer, immune diseases, cardiovascular diseases, depression, and reproductive disorders. When the disturbing effects of TCDD on endocrine system and suppression of melatonin synthesis by TCDD are considered, toxic effects of TCDD may be decreased or protected by supporting the endocrine system via supplementing melatonin to the organisms. The goal of this study was to investigate the protective and possible ameliorative effects of melatonin on ovarian tissue of the rats against toxic and disruptive effects of chronic TCDD exposure.

Material and methods

Chemicals

TCDD (>99% purity) was purchased from Wellington Laboratories, Inc. (345 Southgate Dr, Guelph, ON N1G 3m5, Canada); 16 μg of TCDD was dissolved in 50-ml corn oil. Average weight of rats was assumed to be 160 g, and 0.5 ml of the final solution was administered to each animal by gastric gavage (g.g.). TCDD solution was prepared freshly for monthly usage. Stock solution of melatonin was prepared by dissolving 336 mg of melatonin in 3.15 ml of absolute ethanol. Then, 450 μl of stock solution was taken and diluted with 5% alcohol by completing to 9 ml with saline solution. According to the average weight of rats, 0.3 ml from final solution was injected intra peritonally (i.p.) to each animal. Fresh stock solution was prepared weekly.

Experimental protocol

Studies were performed on female Wistar albino rats. Animals were housed in cages under standard conditions at constant temperature (22°C) in a 12-h light/dark cycle. All experiments were approved by the Ethics Committee of Inonu University Experimental Animals Production and Investigation Centre (approval no. 2014/A-11, 31 January 2014). Seventy-two rats of 10 weeks age were used in this experiment and randomly divided into six groups: control group ($n = 10$), oil group ($n = 10$), TCDD group ($n = 15$), alcohol group ($n = 10$), melatonin (Mel) group ($n = 12$), and TCDD+Mel group ($n = 15$). Control group received nothing. Oil group received corn oil weekly for 16 weeks (0.5 ml/kg/week × 16, g.g.). Alcohol group received ethanol daily (0.3 ml/kg/day × 115 i.p.). Mel group received melatonin (10 mg/kg/day × 115 i.p.). TCDD group received TCDD dissolved in corn oil (1 μg/kg/week × 16 TCDD g.g.). TCDD+Mel group received TCDD weekly and melatonin daily for 16 weeks (1 μg/kg/week TCDD, g.g. + 10 mg/kg/day mel, i.p.). Animals were weighed each week from the beginning to end of the experiments. At the end of the experiments, rats were decapitated and their ovaries were removed. One ovary of each rat was used for light microscopic examination and the other one was used for electron microscopic examination.

Light microscopy

For light microscopic examination, ovarian tissues were fixed by 10% formaldehyde for 2 days. After routine histological tissue processing, tissues were embedded to paraffin blocks. Ovarian tissue sections of 0.5-μm thickness were stained with Hematoxylin and eosine (HE). Stained sections examined under light microscope and images were taken via image analysis system (Nikon Optik Hot Light Microscopy, DS L3 Image Analysis system, DS F2 Nikon Camera (Nikon, Tokyo, Japan)). Histopathological changes, number of corpus luteum, and number of follicles after primordial phase were evaluated.

Electron microscopy

The tissues taken for electron microscopic examination were fixed by 3% glutaraldehyde for 1 day. After fixation, electron microscopic tissue processing procedure was applied and tissues were embedded to resin. Then, 80-nm thick tissue sections were cut by (Leica Ultracut R (Leica Microsysteme vertrieb GmbH, Wetzlar, Germany)) and stained with uranyl acetate and lead citrate. Stained sections were examined under transmission electron microscopy (Zeiss Libra 120 TEM (Carl Zeiss NTS GmbH, Oberkochen, Germany)) in respect of ultrastructural compartments of the ovarian cells.

Statistical analysis

Kruskal–Wallis test was used for comparison of data between groups. Conover test was applied for binary comparison, resulted in a p-value lower than 0.05 indicating the significant difference. IBM SPSS STATISTICS 22.0 program was used for statistical analysis.

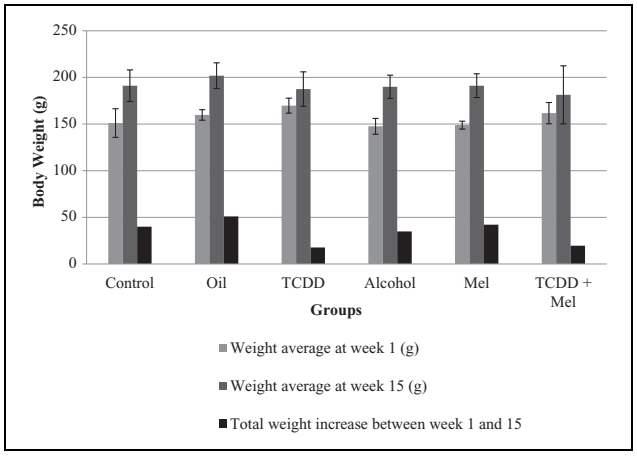

Figure 1. Comparison of body weight increase among the groups. Increase in weight of rats in control (40.0 g), oil (51.0 g), mel (42.2 g), and alcohol (34.9 g) groups were close to each other and significantly higher than the TCDD (17.7 g) and TCDD+Mel (19.6 g) groups ($p < 0.05$). TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

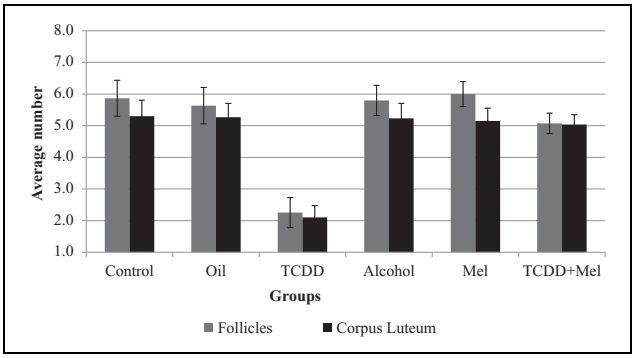

Figure 2. Histogram showing the comparison of number of follicles and corpora lutea among the groups. TCDD caused a significant decrease in the average number of follicles and corpus luteum per tissue section (2.3 $\pm$ 0.8; 2.1 $\pm$ 0.7, respectively), whereas these numbers preserved in TCDD+Mel group (5.1 $\pm$ 0.8; 5.0 $\pm$ 0.8, respectively) and were lower than control group (5.9 $\pm$ 0.9; 5.3 $\pm$ 1.0, respectively). TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Results

Effect of TCDD on body weight

Based on the weight measurements (data not shown), there was a predictable weight increase in control, oil, Mel, and alcohol groups from the 1st to 15th weeks. However, increased body weights in the TCDD and TCDD+Mel groups stabilized approximately at the 10th week and started to decrease until 15th week. Total weight increase between the 1st and 15th weeks in the groups of control (40.0 g), oil (51.0 g), mel (42.2 g), and alcohol (34.9 g) was significantly greater than the TCDD (17.7 g) and TCDD+Mel (19.6 g) groups (Figure 1).

Effect of TCDD on the number of ovarian follicles and corpus luteum

Sections from each ovarian tissue were taken from different levels and were analyzed with respect to number of follicles at post-primordial phase in the corpus luteum. Average numbers of oocyte follicles in control (5.9 $\pm$ 0.9), oil (5.6 $\pm$ 1.0), alcohol (5.8 $\pm$ 1.0), mel (6.0 $\pm$ 0.8), and TCDD+Mel (5.1 $\pm$ 0.8) groups were similar but were significantly greater than the TCDD group (2.3 $\pm$ 0.8; $p < 0.05$). The numbers of corpus luteum in TCDD group (2.1 $\pm$ 0.7) were significantly less than control (5.3 $\pm$ 1.0), oil (5.3 $\pm$ 0.9), alcohol (5.2 $\pm$ 0.9), mel (5.2 $\pm$ 0.8), and TCDD+Mel (5.0 $\pm$ 0.8; $p < 0.05$; Figure 2).

Light microscopic results

HE-stained sections showed that general histological structures such as corpus luteum, ovarian follicles, cortex, medulla, and germinal epithelial of ovarian tissue were of normal histological appearance and similar to each other in the control, oil, alcohol, and mel groups (Figure 3(a), (b), (d), and (e)). In the TCDD group, there were structural and cellular disruptions compared to control groups (Figure 3(c)). Ovarian germinative epithelium was loss in some regions and it had flattened epithelial cells in remaining parts. Diffuse and high level of heterochromasy and pyknosis were observed in corpus luteum cells. In some of corpora lutea, inflammatory regions and edema were detected (Figure 4(c)). Also, vacuolar degenerations were observed in some corpus luteum cells. In addition to these histological changes, there was a decrease in the number of ovarian follicles at the post-primordial phase and corpus luteum compared to other groups (Figure 3(c)). In TCDD+Mel group, general histological structures were similar to control, oil, alcohol, and mel groups (Figure 3(f)). However, granulosa cells in ovarian follicles had occasionally heterochromatic nucleus. Also, there were dilatations at minimal level in intercellular areas of granulosa cells in the follicles. Granulosa lutein cells of corpus luteum were in normal appearance with big, euchromatic nucleus, and clear colored cytoplasm. Theca lutein cells between granulosa lutein cells were also of normal appearance (Figure 4(f)). Additionally, numbers of follicle and corpus

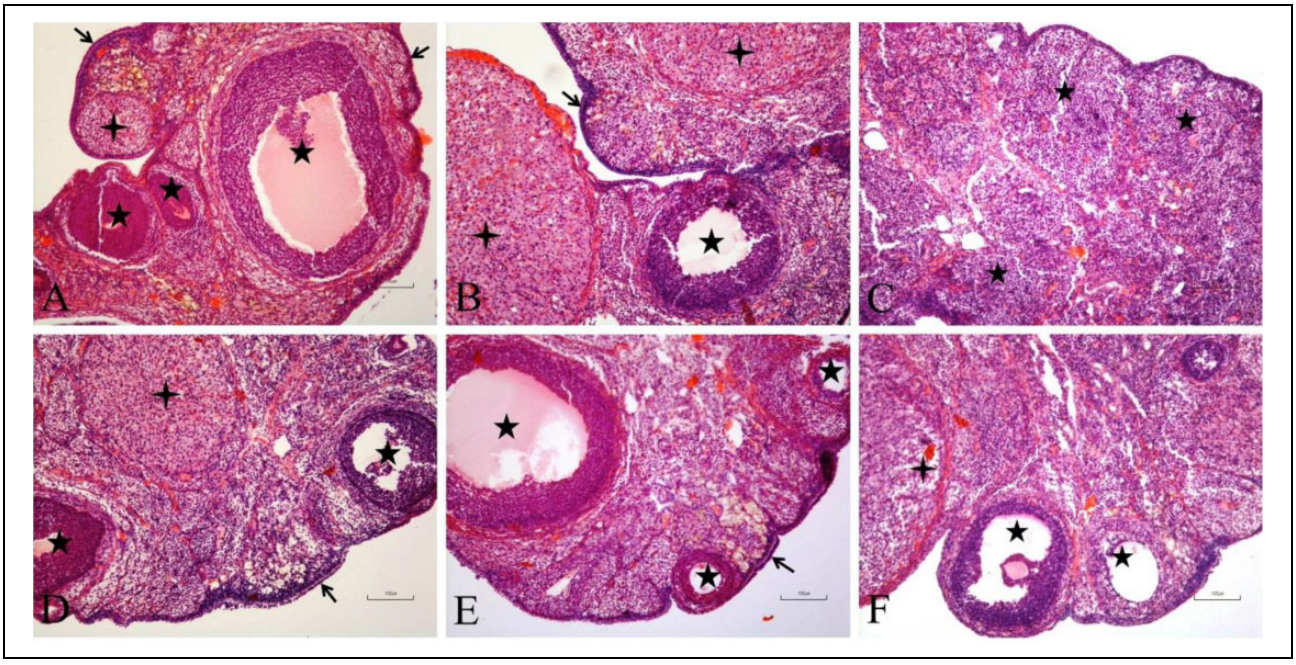

Figure 3. Ovary sections with follicles (five-pointed stars) and corpora lutea (four-pointed stars). Arrows showing the germinal epithel. Notice the abundant number of follicles and corpora lutea in control (a), oil (b), alcohol (d), mel (e), TCDD+Mel (f) groups and degenerated and undifferentiated follicles in TCDD group (c). Hematoxylin eosine, 4x objective (HE-4x). TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

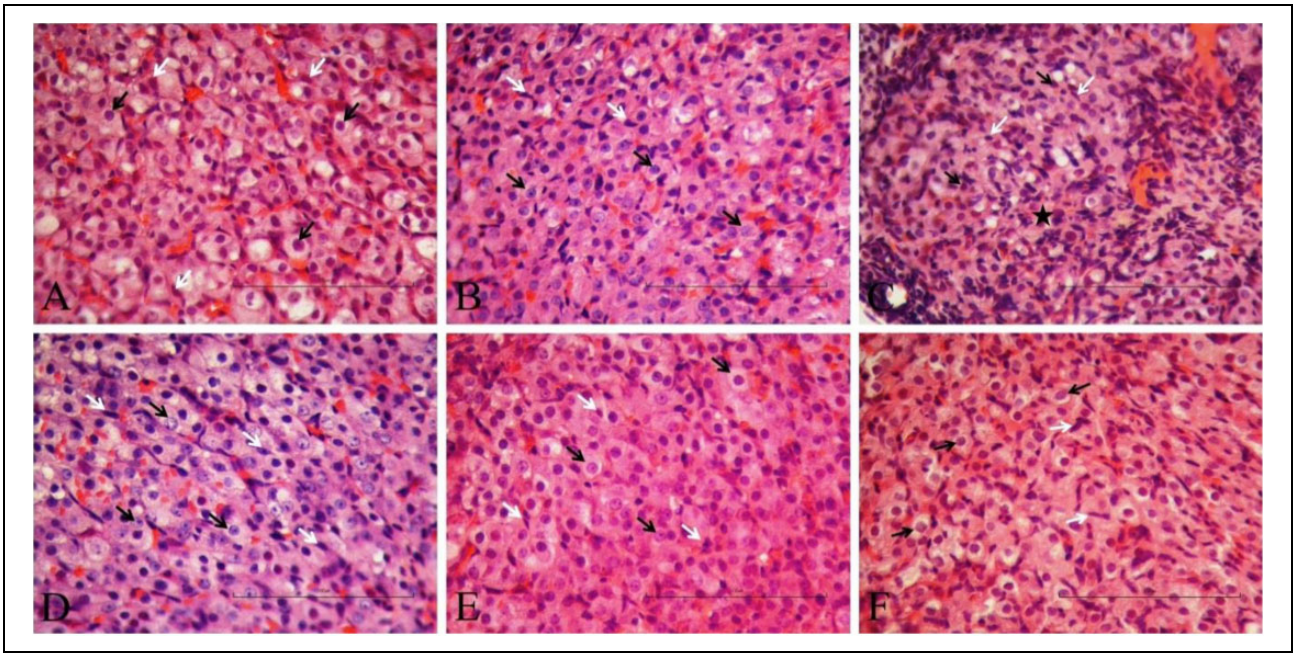

Figure 4. Ovary sections showing corpus luteum cells. Granulosa lutein cells (black arrows) and theca lutein cells (white arrows) in (a) control, (b) oil, (d) alcohol, (e) mel, and (f) TCDD+Mel groups. (c) Degenerated, condensed, and disordered granulosa lutein cells (black arrows), shrunken and condensed theca lutein cells (white arrows), and cellular infiltration (star) in TCDD group. Hematoxylin eosine, 40x objective (HE-40x). TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

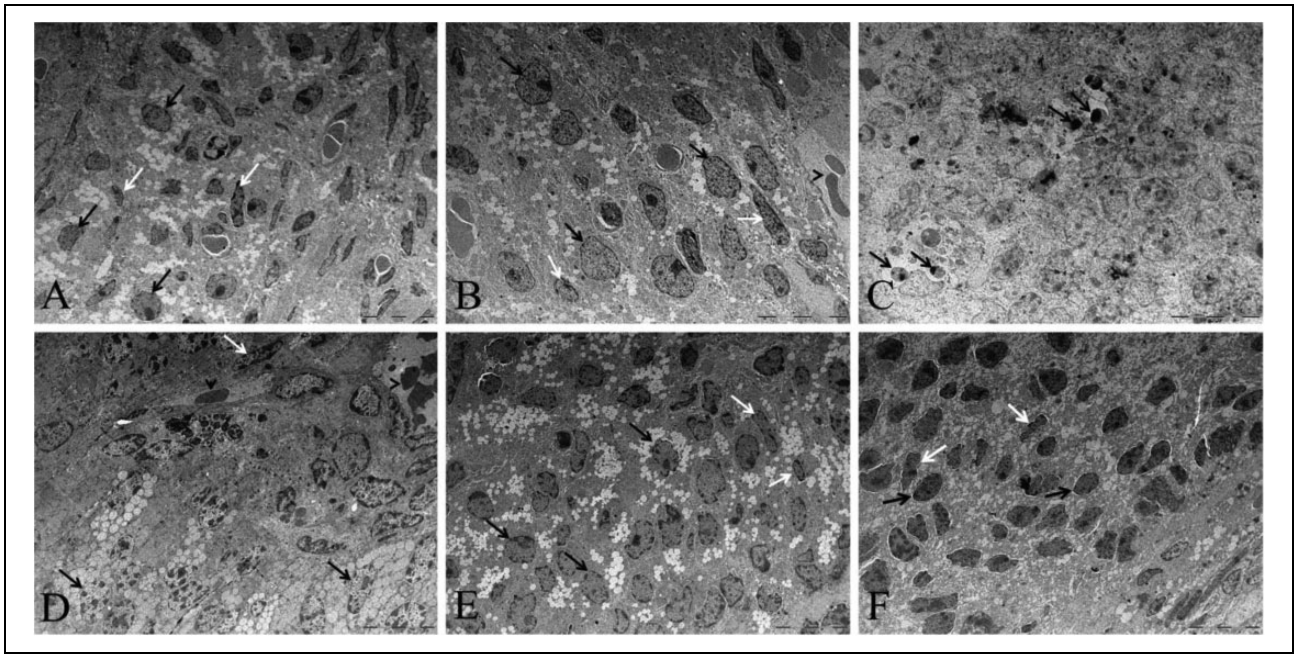

Figure 5. Electron microscopic appearance of granulosa lutein and theca lutein cells. Euchromatic granulosa lutein cells (black arrows) and oval-shaped theca lutein cells (white arrows) in (a) control, (b) oil, (d) alcohol, (e) mel, and (f) TCDD+Mel groups. (b) and (d) Erythrocytes in capillers (arrow heads). (c) Note that there is organizational malformation throughout the tissue in TCDD group. Black arrow represents the apoptotic bodies in granulosa cells. Scale bar: 100 μm. TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

luteum were conserved compared to the TCDD group (Figure 3(f)).

Electron microscopic evaluation

Ultrastructures of ovarian cells were of normal appearance in control, oil, alcohol, and mel groups with regard to intracellular content and organization (Figures 5 and 6). In the TCDD group, stromal cells had pyknotic nuclei and undulation at the nuclear periphery. There were degenerated granulosa cells, large and irregular ordered hydrophilic vacuoles between stromal cells, numerous apoptotic bodies, and autophagosome structures in the cytoplasm of granulosa cells (Figure 6(c)). Furthermore, corpus luteum cells had disassociated nuclear periphery and dilatations at perinuclear region (Figures 5(c) and 6(c)). Theca cells of the corpus luteum had large hydrophilic vacuoles. Additionally, severe pyknotic nucleis, nuclear border irregularity, peripheral chromatin condensation, and disassociation at myofibril organization were observed in the stromal smooth muscle cells in the ovary. In the TCDD+Mel group, stromal, smooth muscle, and vascular endothelial cells were of normal ultrastructure appearance. In this group, granulosa cells were generally polygonal shaped and had euchromatic nuclei. Granulosa lutein cells of corpus luteum were seen as having cytoplasmic vacuoles with low electron density. Their nuclei were screened as oval shaped and euchromatic. However, there were a few pyknotic nuclei in corpus luteum cells, dilatation at perinuclear regions and undulations at nuclear outlines at a minimal level. Similar to control, oil, alcohol, and mel groups, theca lutein cells of corpus luteum were flat shaped with more heterochromatic nuclei compared with the granulosa lutein cells (Figures 5(f) and 6(f)).

Discussion

TCDD is a reproductive toxicant and endocrine disrupter that is known to block ovulation (Birnbaum and Fenton, 2003). This study demonstrated that melatonin supplementation significantly reduced the severity of ovarian malformations caused by TCDD exposure in rat. The most evident finding was the preservation of the number and structure of ovarian follicles at post-primordial phase and corpus luteum by melatonin supplement upon TCDD exposure to rats. Additionally, melatonin supplementation played a protective role over intracellular integrity of the ovarian tissue which disturbed by TCDD.

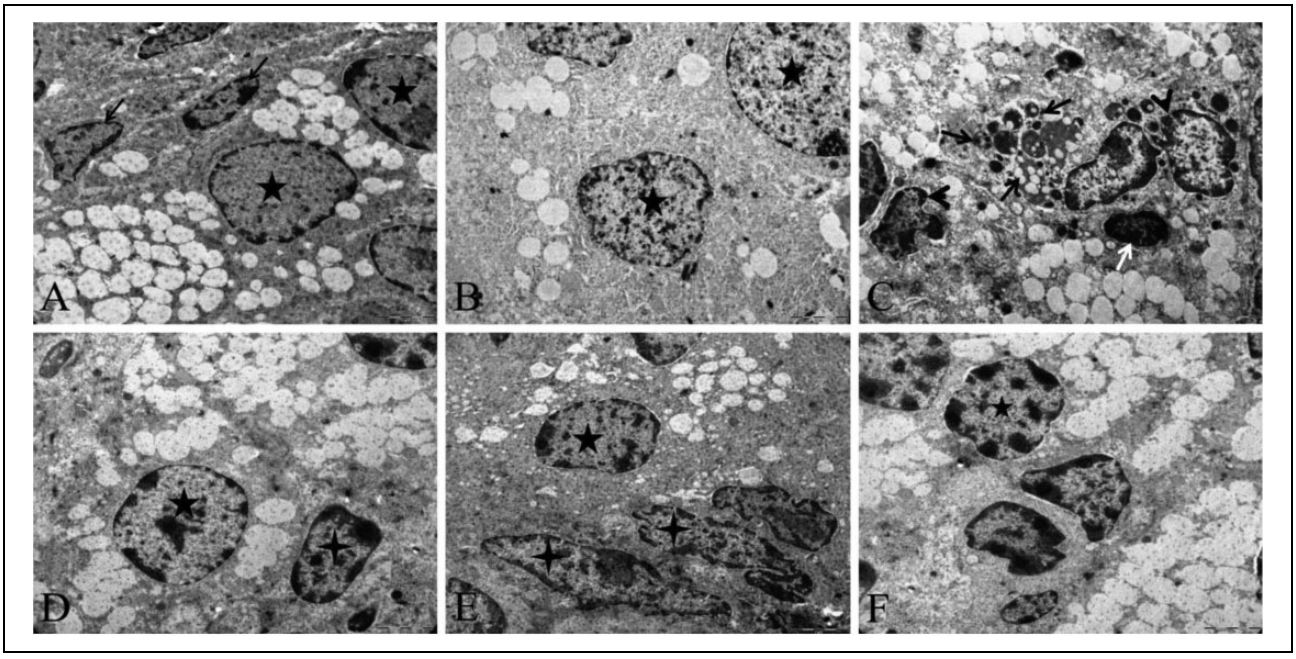

Figure 6. Ultrastructural appearance of ovarian cells. Granulosa lutein (five-pointed stars) and theca lutein cells (four-pointed stars) were seen in normal ultrastructure in (a) control, (b) oil, (d) alcohol, (e) mel, and (f) TCDD+Mel groups. (c) Large autophagosomes (black arrows) in the cytoplasm of corpus luteum cells, irregularity at nuclear periphery (arrow head), and pyknotic nucleis (white arrow) were detected in TCDD group. Scale bar: 2 μm. TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Previous studies showed that chronic or acute exposure to TCDD leads to lose of body weight in adult rats or decrease in body weight gain in young rats (Wolf et al., 1999; Xuelin et al., 1995). Furthermore, it was demonstrated that weight loss or decrease in weight gain is correlated with the dose of TCDD (Cummings et al., 1999). Likewise, in the present study, we observed that TCDD decreases weight gain in young rats as the result of weekly exposure for 16 weeks to TCDD at the dose of 1 μg/kg (Figure 1). All the control and vehicle groups had a normal increase while the TCDD group showed stabilization at the 10th week and showed a tendency to decrease until 16th week. Total weight difference between the end of the study and first day of the study in control and vehicle groups was significantly greater than the TCDD and TCDD+Mel groups. There was a slight increase in TCDD+Mel group compared to TCDD group. The reason for the weight loss was proposed that it may be related to appetite disorder or decrease in absorption of food from the intestinal system as a result of toxic effect of TCDD on nerve system and metabolism of the body (Mitrou et al., 2001; Tuomisto et al., 1999).

Ovary is one of the most dynamic tissues in the body due to being subject to structural and functional changes controlled by hormonal regulation monthly. There are very important structures like follicles and corpus luteum in the ovary under the control of endocrine and nervous systems that makes it quite sensitive to toxic agents. Our results are consistent with past studies in the rat in which TCDD caused inhibition of follicular development, ovulation, and hormonal secretion (Heimler et al., 1998; Prooije et al., 1994; Shiverick and Muther, 1982) in ovary. Xuelin et al. (1995) showed that 1 μg/kg/day TCDD exposure leads to disruption of menstrual cycles and inhibition of development of follicles. In an another study carried out by Roby (2000), it was shown that TCDD exposure in immature rats inhibited ovulation in some of the rats and reduced ovulation in some of the rats. Heimler et al. (1998) also showed that TCDD exposure resulted in the decrease in number of large volume follicles. In our study, we observed that the number of follicles significantly decreased in the TCDD group while this number was conserved in the control groups and melatonin supplemented group. Similarly, the number of corpus luteum was significantly decreased in TCDD group and conserved in TCDD+Mel group. Furthermore, the density of the follicles at primordial phase didn't change in TCDD group. So we think that TCDD inhibits or affects the

follicles especially during the differentiation from primordial phase stock follicles. This put forth the disruption of hormonal regulation required for follicle development by TCDD, so we propose that inhibition of follicle and corpus luteum development may be related to the inhibition of hormonal regulation of ovarian follicles. We also observed that TCDD caused to disorganization of granulosa cells, pyknotic nucleus, and vacuolization in corpus luteum cells and focal inflammation in corpus luteum paranchima. However, these structural changes were at minimal level and they were similar to control groups in TCDD+Mel group. This protective effect of melatonin may be related to its regulator effect on endocrine system, cytoprotective, antioxidant, and free radical scavenger properties.

Structural and functional changes due to TCDD in the ovary tissue were seen more strikingly in cell and tissue components at the ultrastructural level. In the transmission electron microscopy, findings of the control groups were consistent with the literature (Roby, 2000; Wolf et al., 1999; Xuelin et al., 1995). Heimler et al. (1998) reported in their *in vitro* studies that TCDD at different doses and durations caused cell damage at ultrastructural levels in human luteinized granulosa cells in cell culture. These findings are in the form of apoptosis increase, DNA fragmentation, and peripheral chromatin condensation in granulosa cells in proportion to the duration of TCDD applied (Heimler et al., 1998). In our study, electron microscopic examination of the ovary sections of the TCDD group revealed numerous apoptotic bodies within the autophagosomes in the granulosa cells of atretic follicles and in some other granulosa cell cytoplasm of normal follicles. They also reported that TCDD administration in cell culture medium disintegrated the cell membrane in human luteinized granulosa cells and distributed the nuclear and cytoplasmic material out of the cell. The findings of this type of cellular degeneration appear compatible with cell necrosis. In our study, necrotic degeneration was also found in many areas as well as apoptosis in granulosa cells. In these areas, the granulosa cell cytoplasm disintegrated, organelles integrity and association disrupted, and the presence of erythrocytes was detected between the granulosa cells. In this case, the increase in apoptotic changes in granulosa cells may have been chosen as a less traumatic cell death route than cell necrosis, in order to minimize the destructive effect of TCDD toxicity of the cells. As a result, TCDD administration has shown a strong toxic effect on ovarian granulosa cells, which has both increased apoptosis and triggered intense necrosis. In our study, while the apoptotic bodies and necrosis were not found, lysosomal structures were identified in the granulosa and corpus luteum cells of TCDD+Mel group.

Pharmacological studies have shown that melatonin is absorbed in rats at high rates by oral, intravenous, and intraperitoneal routes (Yeleswaram et al., 1997). However, it has been shown in many studies that melatonin is more absorbed by intraperitoneal, transdermal, intranasal, and sublingual ways compared to oral intake (Zetner et al., 2016). Melatonin, used by humans, is present in tablets between 0.1 mg and 50 mg in health food stores and taken orally. So, we administered higher dose of melatonin (10 mg/kg/day) per kg in our study than humans used. However, the amount of TCDD (1 μg/kg/week) used in the experiment is also too high compared to normal environmental exposure (119 pg/day, Kang et al., 2007). Hence, except for acute exposures, administration of melatonin at the dose of 10 mg/kg or above is useful against normal environmental TCDD exposure. In summary, we recommend that the best dose of melatonin for human upon TCDD exposure should be determined according to the age and body weight of the individual and serum level of TCDD in the patient by taking into consideration of lower oral absorption compared to mentioned ways.

Conclusions

It is stated in this study that TCDD has a disruptive effect on ovarian tissue by disturbing the proper organization of the ovarian cells and may reduce fertility by decreasing the mature ovarian follicle numbers. Furthemore, it was shown that melatonin plays a protective and possible ameliorative role to overcome the toxic effect of TCDD. Because of this crucial function of melatonin, when it is confirmed by more research both *in vivo*, *in vitro*, and clinically, it may be considered as an important therapeutic agent in order to overcome the toxic effects of TCDD.

Acknowledgments

The authors would like to thank all members of Histology and Embryology Department of Medical Sciences, Inonu University Experimental Animals Production and Investigation Centre, and Özkan Fidan for their support during the study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Inonu University Scientific Research Projects Committee (BAP).

References

Arendt J and Skene DJ (2003) Melatonin as a chronobiotic. *Sleep Medicine Reviews* 9: 25–39.

Aylward LL, Brunet RC, Carrier G, et al. (2005) Concentration-dependent TCDD elimination kinetics in humans: toxicokinetic modeling for moderately to highly exposed adults from Seveso, Italy, and Vienna, Austria, and impact on dose estimates for the NIOSH cohort. *Journal of Exposure Analysis and Environmental Epidemiology* 15: 51–65.

Baldridge MG, Marks GT, Rawlins RG, et al. (2015) Very low-dose (femtomolar) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) disrupts steroidogenic enzyme mRNAs and steroid secretion by human luteinizing granulosa cells. *Reproductive Toxicology* 52: 57–61.

Birnbaum LS and Fenton SE (2003) Cancer and developmental exposure to endocrine disruptors. *Environmental Health Perspectives* 111: 389–394.

Chang GK, Seung HL and Eui KK (2007) Endocrine disruptors. *Journal of Korean Medical Association* 50(4): 359–368.

Cole P, Trichopoulos D, Pastides H, et al. (2003) Dioxin and cancer: a critical review. *Regulatory Toxicology and Pharmacology* 38: 378–388.

Cummings AM, Hedge JM and Birnbaum LS (1999) Effect of prenatal exposure to TCDD on the promotion of endometriotic lesion growth by TCDD in adult female rats and mice. *Toxicological Sciences* 52(1): 45–49.

Hardeland R, Pandi-Perumal SR and Cardinali P (2006) Melatonin. *The International Journal of Biochemistry & Cell Biology* 38(3): 313–316.

Heimler I, Trewin AL, Chaffin CL, et al. (1998) Modulation of ovarian follicle maturation and effects on apoptotic cell death in Holtzman rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in utero and lactationally. *Reproductive Toxicology* 12(1): 69–73.

Hutz RJ (1999) Reproductive endocrine disruption by environmental xenobiotics that modulate the estrogen-signaling pathway, particularly by tetrachlorodibenzo-p-dioxin (TCDD). *Journal of Reproduction and Development* 45: 1–12.

Kakeyama M and Tohyama C (2003) Developmental neurotoxicity of dioxin and its related compounds. *Industrial Health* 41: 215–230.

Kang CG, Lee SH and Kim EK (2007) Endocrine Disruptors. *Journal of the Korean Medical Association* 50(4): 359–368.

Li X, Johnson DC and Rozman KK (1995) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on estrous cyclicity and ovulation in female Sprague-Dawley rats. *Toxicology Letters* 78: 219–222.

Linden J, Pohjanvirta R, Rahko T, et al. (1991) TCDD decreases rapidly and persistently serum melatonin concentration without morphologically affecting the pineal gland in TCDD-resistant Han/Wistar rats. *Pharmacology and Toxicology* 69: 427–432.

McLachlan JA and Korach KS(1995) Estrogens in the environment, III: Global health implications. In: Barney SP and Schroeder JC (eds) *Symposium on estrogens in the environment*, Vol. 4. Washington, DC: Environmental Health Perspectives, 1–178.

Mitrou PI, Dimitriadis G and Raptis SA (2001) Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. *European Journal of Internal Medicine* 12: 406–411.

Pohjanvirta R, Jarmo L, Vakkur O, et al. (1996) Mechanism by which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) reduces circulating melatonin levels in the rat. *Toxicology* 107: 85–97.

Poland A and Knutson JC (1982) 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annual Review of Pharmacology and Toxicology* 22: 517–554.

Prooije A, Waalkens-Berendsen D and Wijnands M (1994) Oral two-generation reproduction study with PCBs in rats. *TNO-Report* 92: 503.

Roby KF (2000) Alterations in follicle development, steroidogenesis, and gonadotropin receptor binding in a model of ovulatory blockade. *Endocrinology* 142: 2328–2335.

Shiverick K and Muther T (1982) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on serum concentrations and the uterotrophic action of exogenous estrone in rats. *Toxicology and Applied Pharmacology* 65: 170–176.

Tuomisto JT, Pohjanvirta R, Unkila M, et al. (1999) TCDD-induced anorexia and wasting syndrome in rats: effects of diet-induced obesity and nutrition. *Pharmacology Biochemistry and Behavior* 62(4): 735–742.

Wolf CJ, Ostby JS and Gray LE (1999) Gestational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) severely alters reproductive function of female hamster offspring. *Toxicological Sciences* 51: 259–264.

Wang J, Liu X, Li T, et al. (2011) Increased hepatic Igf2 gene expression involves C/EBPβ in TCDD-induced teratogenesis in rats. *Reproductive Toxicology* 32: 313–321.

Yeleswaram K, McLaughlin LG, Knipe JO, et al. (1997) Pharmacokinetics and oral bioavailability of exogenous melatonin in preclinical animal models and clinical implications. *Journal of Pineal Research* 22(1): 45–51.

Yoshizawa K, Heatherly A, Malarkey DE, et al. (2007) A critical comparison of murine pathology and epidemiological data of TCDD, PCB126, and PeCDF. *Toxicologic Pathology* 35: 865–879.

Zetner D, Andersen LPH and Rosenberg J (2016) Pharmacokinetics of alternative administration routes of melatonin: a systematic review. *Drug Research* 66: 169–173.