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Histopathological Changes in the Intestine and Testis of Mice Treated with 2,3,7,8 TCDD

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Running Title: TCDD toxicity to intestine and testisofmice

Abstract: ***2, 3, 7, 8 TCDD being a very hazardous persistent organic pollutant which having less biodegradable and high biomagnification capacity to different trophic level. The aim of the present study was to evaluate the toxicological effects of sublethal dose of TCDD exposed for subacute duration which causes alteration in cellular activity of intestine and testis of mice. Three groups of mice were treated individually, receiving corn oil vehicle, TCDD 4µg/kgbw/d and TCDD 40µg/kgbw/d respectively for 7,14 and 21 days of exposure duration. On the scheduled days selected tissues were excised, cleaned and blocks were prepared for the analysis of histopathological effects. The results suggested that increased dose of TCDD causes drastic changes in villi and cryptic cells of intestine. Increased goblet cells in intestinal cells are providing protective mechanism for the pathological changes created by TCDD. Similar degeneration was observed in testicular germinal epithelium and morphological changes in primary spermatocyte and spermatid. Increased dose of TCDD (40µg/kgbw/d) exposed for higher exposure duration (21 days) causes degenerative changes in testicular and intestinal cells.***

Keywords: Dose and Duration, Intestine, Testis, Histopathology, TCDD toxicity

1. Introduction

2,3,7,8 TCDD refers to a very well known group of dioxins congeners in persistent chlorinated chemical compounds, which have similar potency of toxicity among of all group congeners. TCDD is one of the most toxic congeners to mammals.[1,2]It has been noticed that increase of awareness of the possible effects of Persistent organic pollutants on male reproductive system and different sites of system, different physiological processes such as Spermatogenesis and androgen production.[3]TCDD induces many damages at the cellular level including lipid peroxidation, cell membrane disruption, DNA, and protein damage by accelerating ROS generation.[4,5]

TCDD acting through Aryl Hydrocarbon receptor (AhR) mediated signalling pathways and produces various toxic byproducts and biochemical effects, reproductive effects, liver damage, wasting syndrome.[6]TCDD binds with Aryl Hydrocarbon receptor (AhR) which is a ligand dependent transcription factor widely expressed in the cytosol of various cells and tissues.[7-10] Administration of TCDD at the dose of 2µg/Kg/week shows histopathological deformation of sperms.[11]Serum TCDD levels and its relationship with reproductive hormones with effect on serum testosterone, luteinizing and follicle-stimulating hormones were observed.[12-13] TCDD also interferes with cholesterol transport to the inner mitochondrial membrane which effects to steroidogenesis in granulosa cells and leydig cell.[14-15]

In earlier report TCDD administered with low dose causes destroy of many germ cells at every stage and different lesions of testes.[16]Route of administration and exposure of TCDD causing effects on foetal and neonatal development of rat male reproductive system.[17]It was also noted by earlier report that TCDD affects to the permeability of intestine and motility and gut microbiota, bile acid metabolism.[18]Although the developing male reproductive organs are very much sensitive to TCDD. It was noticed that decrease in testis weight, dissolution of germinal epithelium, degeneration of male germ cells, decreased spermatogenesis occurred in TCDD treated adult animals.[19-20]It was also observed that after TCDD intoxication for different exposure durations, various effects on male reproductive system occurred. However, going through the literature it was observed that the histopathological effects of TCDD for subacute duration is not known. Therefore, the present study was designed to test the hypotheses i.e., the low dose of TCDD exposed for subacute duration will be causing histopathological effects to intestinal cells and testicular cells.

2. Material and Methods

(a) Animals and Treatment

A total of 27 male *swiss* albino mice, aged4 weeks old weighted around 30±5 g,was used as an animal model for the present study. The experimental procedure was approved by ethical norms of CPCSEA, India (CPCSEA No.757/PO/Re/S/03//CPCSEA *for Research for education purpose on small animals*, 2018). The animals were divided into three groups (a) Control group were receiving only corn oil vehicle, (b) 4µg(0.004mg/kg bw/d) TCDD exposure group (c) 40 µg (0.04 mg/kg bw/d) TCDD exposure group. The selected groups were provided standard commercial rodent diet and drinking water *ad libitum*.

2,3,7,8 TCDD selected doses was dissolved in corn oil and given orally for 7, 14 and 21 days of exposure duration to second and third group of animals. The selection of the doses was based on the available reports of the doses causing effects on enzymatic activity in the selected tissues of mice acute to sub-acute exposure and evaluation of toxicity studies ATSDR.[11]On the scheduled days 8,15 and

22nd days, the animals were weighted and sacrificed. Testis and intestine were excised, weighted and cleaned.

(b) Histopathological Studies

Selected tissues were diced into 0.5 cubic cm in volume and fixed in buffered formaldehyde (4% final concentration) prepared in phosphate buffer saline (PBS) (pH 7.4) by completely immersing the tissues and left overnight at room temperature (8–12 hrs). The buffered formaldehyde was drained and 70% ethanol was added and kept at 4°C until paraffin blocks were made. The blocks were cut into 5 μm thickness using rotary microtome and stained with haematoxylin and eosin. [21]

3. Results and Discussion

Several types of lesions were observed in the Intestine and testis tissues exposed to two sub-lethal doses for 7, 14 and 21 days of exposure durations. After the exposure of 0.004 mg/kg b.w./d and 0.04 mg/kgbw/d dose of TCDD in intestine for 7 days, marked changes like initial stages of degenerationin villi and changes in mucosal architecture with level of lysis was multifold. In the next higher duration (14 days), in lower dose the initial stages of necrosis and development of subepithelial space with integration of villi were observed. However, in higher dose destruction of villi and severe degeneration in intestinal cells were observed. In the next higher duration (21 days), cryptic cell degeneration and increased in goblet cell number with vacuolation were noticed, with the degeneration of villi sloughing of villi into intestinal lumen were also found However, intestine is vital tissue which absorb the entire nutrient after digestion. The damage after lower dose of exposure is due to the interruption of functional aspect of Intestine or some morphological changes in intestinal cells which could lead to necrosis of villi cells (Fig. 1 A-G). One of the common marked changes in intestinal cells of mice is swelling and increased in the number of goblet cells indicating the defence mechanism against the severe pathological damages.[22]The mucous secreting from the goblet cells is providing the protection for the epithelium and facilitates the food transition.[23-24] it was also reported that ingestion of toxic substances which cause damage to gastrointestinal tract may also affect the absorption of different substances through the internal organ by endocytosis.[25-26]it was also suggested that exposure to the toxic substances isassociated with changes in intestinal epithelium, indicated disturbances in intestinal absorption.[27]Although, the similar effects were observed in earlier report that intestine of some fishes exposed to chlorinated pesticides eldrin, dieldrin, DDT showed fusion of intestinal villi and acute epithelial necrosis.[28]

A previous study suggested even a very low dose of chlorinated pesticide has the potential to induce histopathological lesions in intestine.[29]Toxic pollutant which enters to the digestive tract via the food and water causing a deterioration of structures and the function in the gut.[30-31] Through exposure of TCDD oxidative stress is believed to be a crucial mechanism of dioxin caused pathology, due to imbalance between oxidants and antioxidants.[32]

Similarly, histopathological changes in the testicular cells exposed to different sub-lethal doses of TCDD were evident compared to their controls. After the exposure of TCDD for 7 days, marked changes like, breakdown of germinal epithelium, degeneration of primary spermatocytes and decrease the number of spermatids with destruction and level of lysis was multifold. In the next higher duration (14 days) change the shape of seminiferous tubules, degeneration of spermatogonium and primary spermatocytes with necrosis of leydig cells were observed. In higher exposure duration (21 days) degeneration of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatid, swelling of sertoli cells and the wide gap between seminiferous tubules were observed. The damage after lowerdose of exposure is due to the interruption of functional aspect of testes or some morphological changes in testicular cells which could lead to necrosis of seminiferous tubules (Fig.2 A-G).

The major reproductive toxicity induced by TCDD was an impediment to the development of the reproductive organ; the testis and epididymis were considered to function in a normal manner based on histology.[33]Histopathological changes in the seminiferous tubules of testes ranging from vascular congestion to focally diffuse interstitial edema, focal area of tubular degeneration and coagulative necrosis of spermatozoa were also observed after the TCDD exposure to the experimental rats. Furthermore, interstitial tissue in the treated rats showed a relative increase in edema of the interstitium and congested blood vessels suggesting inflammation. In addition, there were apoptotic signs in leydig cells followed to seminiferous tubules were relatively widely separated from each other. In agreement with the present results, TCDD was found to produce atrophy, morphological changes, impaired spermatogenesis, in the testes of experimental animals.[34]2,3,7,8 TCDD administered to rats, histopathologic examination revealed a decrease in the diameter of seminiferous tubules and the number of testicular sperm and testicular lesions.[35-36]

TCDD being a most toxic dioxin having xenobiotic mechanism. Apart of xenobiotic mechanism, AhR plays important role in developmental pathway, such as haemopoiesis, cell differentiation, cell proliferation and carcinogenesis. AhR mechanism can also activate to the upregulation of inflammatory cytokinesis, formation of ROS and also related to induction of some antioxidative enzymes which proceeds to the oxidative stress and tissue damage.[37]

4. Conclusion

The overall findings of the present study suggestedthat TCDD was causing severe and drastic changes in a dose and duration dependent manner in the intestinal cells and testicular cells. Higher dose of 40 µg/kg bw/d caused alteration in villi and cryptic cells. Similarly, reduction in spermatogenesis followed by degeneration of spermatocyte were observed in testicular cells exposed to higher dose of TCDD. The observed increment of the goblet cells was possibly due to the protective mechanism for the pathological changes caused by TCDD in intestinal villi. The overall morphological alterations in villi and cryptic cells could lead to the changes in metabolic activity.

The treatment of TCDD caused morphological changes which could reveal the early sign of apoptosis. Seminiferous tubules, with only Sertoli cells existed with few germ cells found, were affected by necrosis and damaged. Thus, it can be concluded that the toxicity of TCDD in the testicular tissue might have caused abnormal spermatogenesis followed by reproductive disorder and infertility. However, the exact mechanism of damage in intestinal cells and testicular cells created by toxic was not clear at this point. It might be due to the lipophilic dioxin interrupting the channels and causes various damages into the cells.

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Conflict of Interest: The authors have no conflict of Interest.

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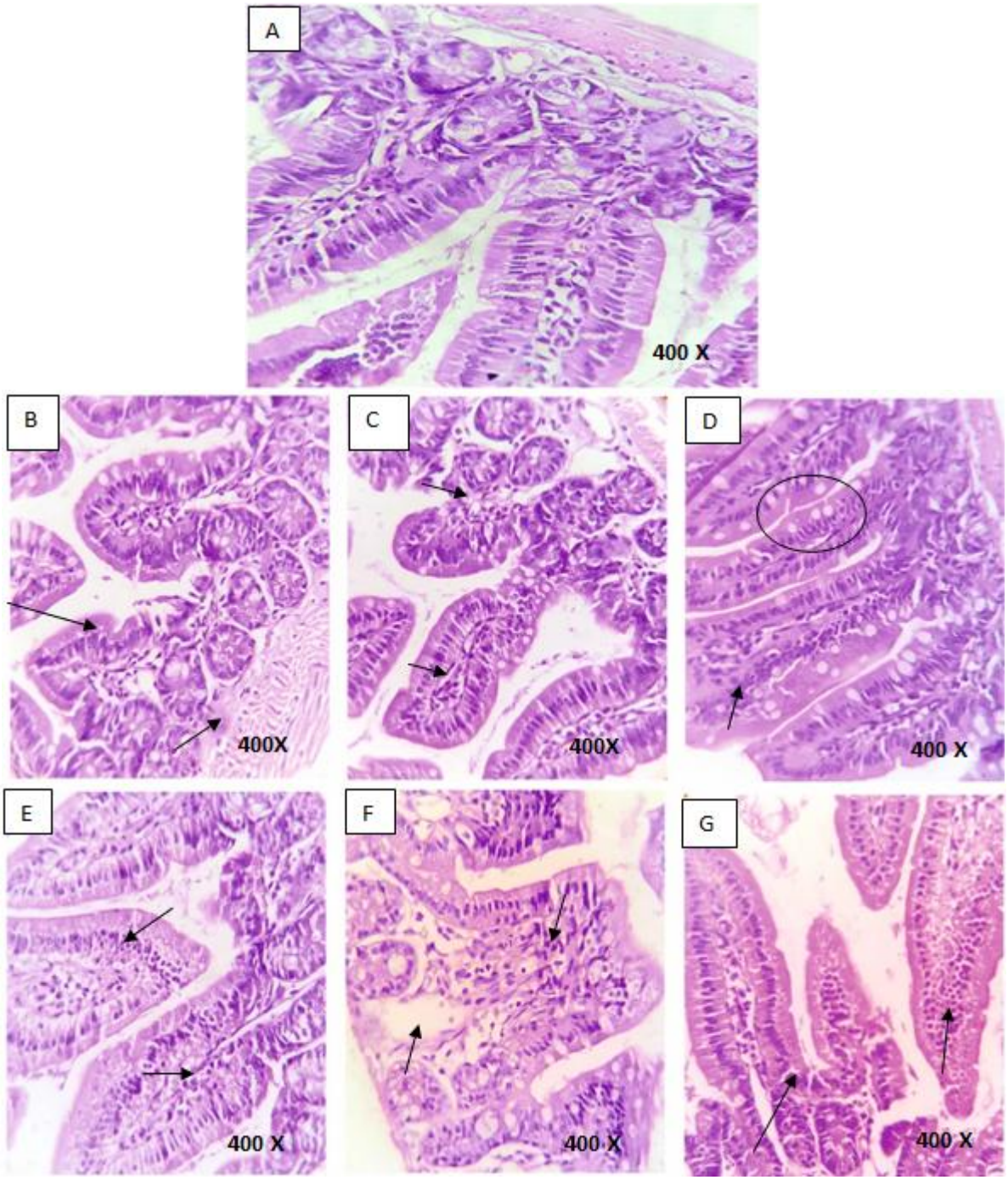

Figure 1: (A) Photograph of control showing normal pattern of Villi, Cryptic cells, Enterocytes and lumen **Histology of the Intestine tissue (H & E Staining) magnification (400X) after0.004 mg/kg bw dose TCDD intoxication** (B) degeneration in villi and changes in muscular architecture, (C) necrosis and development of subepithelial space with integration of villi (D) cryptic cell degeneration and increased in goblet cell number with vacuolation**Histology of the Intestine tissue (H & E Staining) magnification (400X) after 0.04 mg/kg bw dose TCDD intoxication**(E) destruction of villi and severe

degeneration in intestinal cells (F) more development of subepithelial space and integration of villi with degeneration of villi and sloughing of villi into intestinal lumen (G) necrosis of villi, distortion of mucosal architecture, with severe degeneration in cryptic cells and intestinal villi.

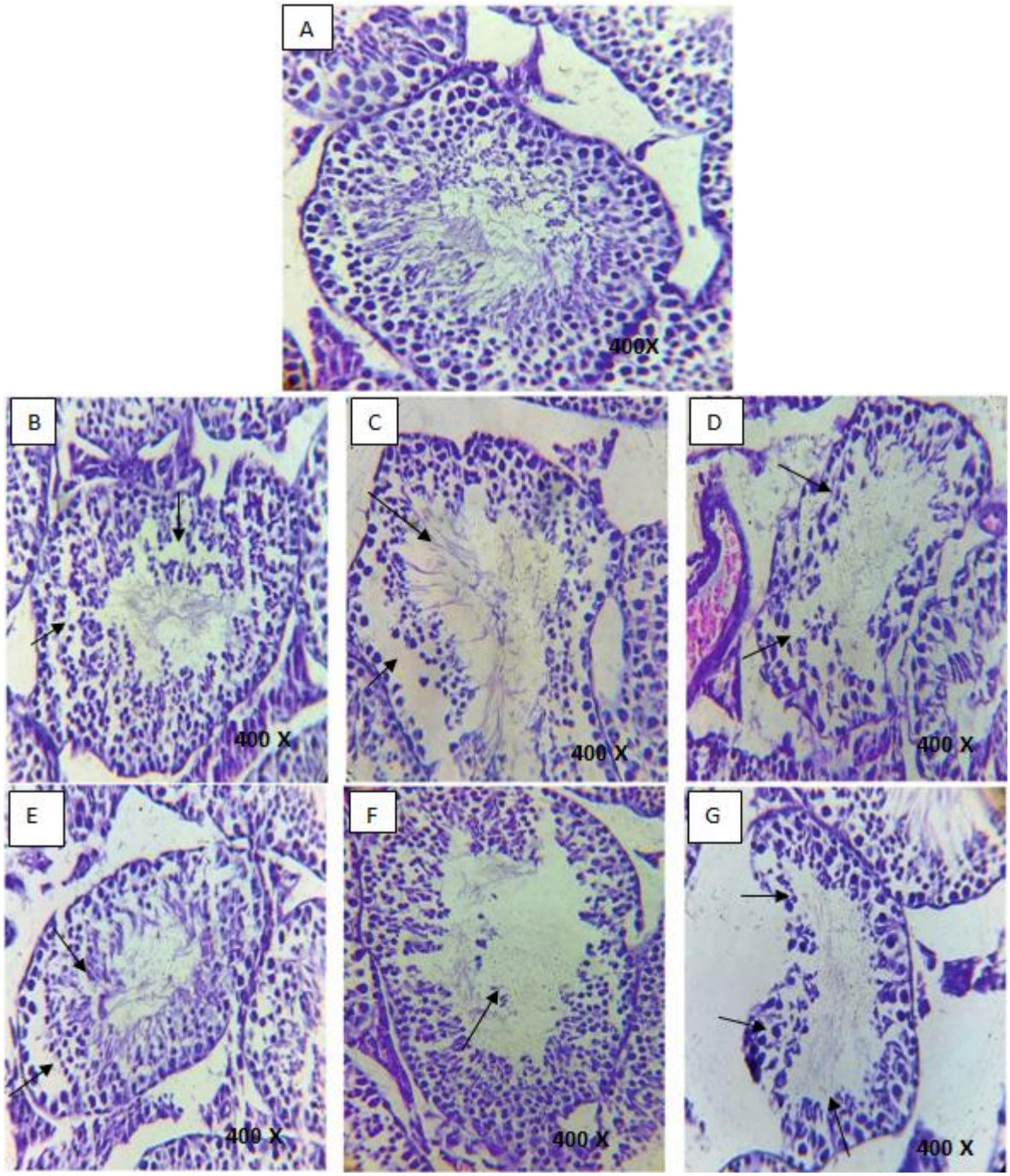

Figure 2: (A) Photograph of control showing normal pattern of seminiferous tubules with spermatogenesis **Histology of the Testicular cells (H & E Staining) magnification (400X) after 0.004 mg/kg bw dose TCDD intoxication**(B) damage in germinal epithelium and decreases the number of spermatid (C) Primary spermatocytes are degenerated, necrosis in leydig cells, and change the shape of seminiferous tubules (D) Enlargement of intracellular spaces, irregular lumen¸wide gap between seminiferous tubules and degenerate spermatids**Histology of the Testicular cells (H & E Staining) magnification (400X) after 0.04 mg/kg bw dose TCDD intoxication**(E) Severe degeneration of primary spermatocytes and leydig cells (F) necrosis and vacuolations in seminiferous tubules and irregular lumen (G) destruction of germinal epithelium,degeneration of spermatogonia, primary spermatocytes and spermatid, wide gap between neighbouring cells and deshaped seminiferous tubules.

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