Research Article

Dose and Duration Dependent 2,3,7,8 TCDD (Dioxin) induced Enzymatic and Histopathological changes in the Liver Cells of Mice

Jyoti Jigyasi*, Aarti Chotaliya, Rahul Kundu

Department of Biosciences, Saurashtra University, Gujarat, India

***Corresponding Author**

Jyoti Jigyasi, Department of Biosciences, Saurashtra University, Rajkot-360005, Gujarat, India,
E-mail: jjigyasi@gmail.com

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Abstract

2,3,7,8 TCDD, a Dioxin, is one of the most potent carcinogenic environmental persistent organic pollutants. The present investigation was aimed to assess the histopathological and glutathione enzymes alterations caused by TCDD in the liver cells of white albino mice. The hypotheses set to assess the histopathological alterations are, (a) whether sublethal doses of TCDD exposed to mice for sub-acute duration can alter the normal activity of the antioxidative enzymes in liver cells, and (b) whether dose and duration dependenrt exposure to TCDD can induce the histopathological lesions in the mice hepatocytes. Separate groups of mice were subjected to the oral doses of TCDD (4 µg/kg bw and 40 µg/kgbw/d) for 7, 14 and 21 days of exposure durations. Control group were administered corn oil (vehicle) separately. The obtained results suggested a predominant exposure duration dependent effects in the activities of the antioxidative enzymes. The results suggested that the observed alteration in activity of the antioxidative enzymes could be due to oxidative stress which might have been an indirect effect following TCDD exposure. The results also indicated that the TCDD also caused histopathological lesions in the hepatic cells. Few of the observed lesions were severe and occurred in the high doses exposed for maximum durations.

Keywords: TCDD; Antioxidative enzymes; Liver; Mice; Histopathology; Dose; Duration

1. Introduction

Dynamic development and industrialization leads to the environmental pollution, entry of noxious agents into the environment. Among of them toxin dioxin is one of the most toxic persistent organic pollutant which has low biodegradable capacity and high biomagnificial capacity [1]. However, the major sources of dioxins are fungicide, herbicide manufacturing, paper and cellulose industry, thermal reaction of chlorinated aromatic compounds [2, 3]. Common exposure pathways of dioxins include dermal contact, inhalation and consumption of contaminated food [4]. The increasing concentrations of toxic substances in biota and their accumulation along food chains reported in many coastal areas, indicates that degradation of the natural environment is still in progress [5, 6].

However, potential health effects of dioxins are related to the actual body burden concern which can be observed after chronic exposure of TCDD. The exposure of mice and rats to different doses for different exposure duration resulted ROS and lipid peroxidation and DNA damage [7]. TCDD causes oxidative stress followed to formation of free radicals and ROS which increases the risk for chronic diseases such as cancer, diabetes and liver cirrhosis. To protect from oxidative stress, in living organisms have developed an antioxidative system including Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx) and Superoxide dismutase (SOD) [8]. The response of antioxidative enzymes is increased in response to stimuli or toxic chemicals. In present study the following Hypotheses were tested: (a) whether the sublethal dose and sub-acute exposure duration of TCDD can affect to the antioxdative system of mice hepatocytes (b) TCDD induced disturbances can affect to the histology of mice liver cells.

2. Materials and Methods

9 weeks old female Swiss albino mice weighted around 25 ± 5 gm were used as an animal model for the study. Mice were kept under standard laboratory condition approved by CPCSEA, India and acclimatized one week prior to the experiments. The groups of all experimental animals were provided rodent diet and drinking water ad libitum. All experiments were conducted according to ethical norms of CPCSEA, India (CPCSEA No. 757/PO/Re/S/03//CPCSEA for Research for education purpose on small animals, 2018). A total of 27 mice was divided into three groups. (a) Control group were receiving only corn oil vehicle, (b) 4 µg/kg bw TCDD exposure group (c) 40 µg/kg bw TCDD exposure group. 2,3,7,8 TCDD was purchased from Sigma Aldrich Pvt Ltd. CAS no. (1746-01-6) and was dissolved into corn oil and given orally for 7, 14 and 21 days of exposure duration to second and third group of animals. On scheduled days of sacrification, 0.4 gm liver tissue was excised, cleaned with sucrose buffer to remove excess blood tissue debris and was taken for antioxidative enzyme assay. Tissue extraction was done by the method of Bhor et al. [9] and Glutathione Peroxidase (GPx) enzyme assay was estimated by the method of Rotruck et al. [10], Reduced Glutathione (GSH) by Ellman [11] and Glutathione S Transferase (GST) were estimated by the method of Habig et al. [12]. Protein content in extracted tissue was measured by the method of Lowry et al. [13]. (B) Liver tissue taken for histological analysis was separated, cleaned and diced into 0.5 cubic cm in volume. Tissue was then 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 [14]. Animals exposed to toxic agent TCDD and control group was also measured body weight regularly on Experimental days, to check the effect on body weight by

TCDD. The obtained data were also subjected for various statistical analyses. Two Way ANOVA were implicated to check the significance between control and both toxicated group and Student t-test was implicated to check the significance between both selected doses and exposure duration. All statistical analyses were analyzed by the method of Sokal and Rohlf [15].

3. Results and Discussion

3.1 Effect on body weight

The effect on body weight shows drastic alteration after the exposure of TCDD. The body weight shows initial stimulation then inhibition in higher exposure duration. Inhibition in body weight is directly proportional to feed consumption (Figure 1). Dioxin and dioxin like PCBs causes drastic effects into the cell after binding with AhR [16]. The inhibition in body weight is possibly due to disturbances in glucose homeostasis leads to less energy production into the liver cells [17]. Previous study suggests that TCDD causes reduction in body weight is independent to lipid peroxidation process. AhR regulates glucose homeostasis, energy level with ATP production through multiple chains of reaction [18].

3.2 Effect on antioxidative enzymes

Glutathione Peroxidase showed the stimulation after low exposure duration in both selected doses whilst, declination after 21 days of exposure durations as compared to control after the exposure of both the doses (Figure 2a). Activity of Reduced glutathione showed the stimulation after the exposure of both the doses of 2,3,7,8 TCDD in all the exposure durations. But, 40 µg/kg/ b.w./d dose of 2,3,7,8 TCDD showed the inhibition in the activity after 14 days of exposure compared to control (Figure 2b). However, the activity of glutathione-S-transferase showed the stimulatory trend after the exposure of both the doses of 2,3,7,8 TCDD in all the exposure durations (Figure 2c). Results of two-factor ANOVA showed the clear cut exposure duration dependent significant alterations on the anitoxidative enzymes extracted from mice liver. The reduced glutathioneshowed the high significant alterations in the level as compared to other enzymes after the exposure of 2,3,7,8 TCDD (Table 1). The results of statistical analysis, student's t-test suggests the dose and duration dependent manner of significant changes in the activities of all the selected antioxidative enzymes after the exposure of 2,3,7,8 TCDD. The maximum significant alterations in the activities of all the antioxidative enzymes after the exposure to higher dose, i.e., 40 µg/kg b.w./d dose of 2,3,7,8 TCDD (Table 2).

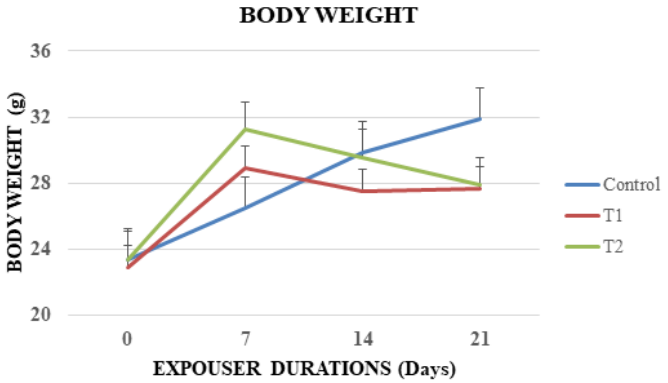

Figure 1: Variation in body weight in Control and Toxicated group of mice in different doses (4 and 40 µg/kg b.w. /d) of TCDD exposed for different durations.

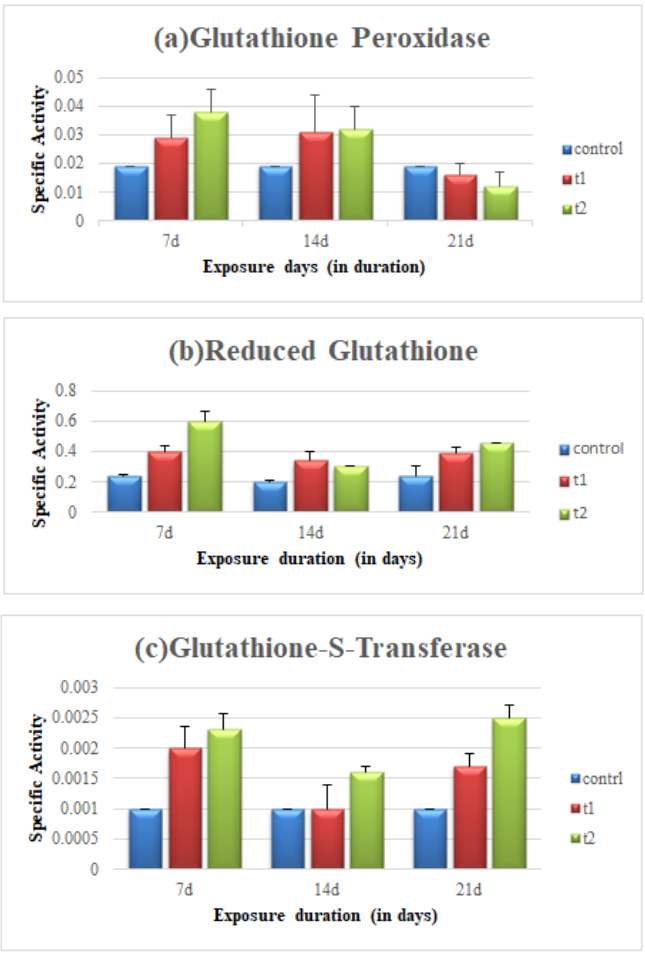

Figure 2: *In vivo* dose and duration dependent effects of 2,3,7,8 TCDD on (a) glutathione Peroxidase; (b) reduced Glutathione and (c) Glutathione-S-transferase, antioxidative enzymes extracted in mice Liver.

	Glutathione Peroxidase	**Reduced Glutathione**	**Glutathione-S-transferase**
Amongst doses	1.62	0.0009	0.32
Within durations	9.68**	86.85**	5.38**

*Significant at P = 0.05 (*F crit (df= 1, 8) = 5.31),* ** Significant at P = 0.05 (*F crit (df= 8, 17) =3.43)*

Table 1: Result of Two-Factor ANOVA between control and toxicated groups in mice hepatocytes.

	Glutathione Peroxidase		**Reduced Glutathione**		**Glutathione-S-transferase**	
	4µg	40µg	4µg	40µg	4µg	40µg
7 days	0.16	0.27	35.0*	0.27	8.12*	10.9*
14 days	0.58	0.99	0.54	0.17	0.04	0.63
21 days	0.71	1.63	0.71	4.65*	2.31	6.92*

*Significant at P = 0.05 (*F crit.* = 2.91)

Table 2: Result of Student's t-test between control and individual exposure duration within each dose in hepatocytes of mice.

One of the mechanisms by which xenobiotics produce effects is through producing the reactive oxygen species (ROS)/ free radicals like the sulfhydryl groups. Sulfhydryl groups serve as a source of electrons for reduction and also mediate the methylation process due to their ability to interact with the cellular sulfhydryl groups in proteins. When availability of free thiol group is low, enhanced expression of toxicity could lead to oxidative stress. Thus, the reduction in total -SH group in our study indicates toxicity status of the tissue by these toxicants. Glutathione performs a pivotal role in maintaining the metabolic and transport functions of cells. Its conjugation helps in detoxification by binding electrophiles that could otherwise bind to proteins or nucleic acids, resulting in cellular damage and genetic mutation [19]. The increased glutathione levels in present study could be due to its involvement in the mechanisms of detoxification of various xenobiotics which is supporting the results of our study [20], inhibition of lipid peroxidation by scavenging free radicals as well as reducing dehydroascorbic acid to reduced form [21, 22].

The stimulation in the activities GPX, GST and reduced glutathione suggested that large amounts of peroxide were generated and these enzymes tried to neutralize the amount of reactive oxygen species production. The activation of antioxidant systems in response to exposure to pollutants has been reported in various fish tissues [23, 24]. Liver is the major target organ for ingested oxidants that increases glutathione peroxidase activity. This probably reflects an adaptation to the oxidative conditions to which the fish have been exposed [25]. Significant increase in glutathione peroxidase activity was observed predominantly in the liver indicates the protective role of the enzyme against lipid peroxidation.

4. Histopathology

In present investigation, significant histopathological changes in the liver tissue exposed to different sub-lethal concentration of 2,3,7,8 TCDD were evident compared to their controls. Several types of lesions were observed in the liver tissue exposed to two sub-lethal doses for short to long durations. After the exposure of lower dose of TCDD for 7 days showed, significant changes like intravascular blood corpuscles accumulation (IGA) and binucleate cells with cytoplasmic alteration were observed. The initial stages of portal fibrosis were observed in the cells, however, appeared to maintain the normal activities up to certain extent as well as defined nucleus were seen. In many cells, fragmentation of chromosomal materials was observed. In the next higher duration (14 days), more fragmentation of nuclear material, interstitial gap between cells and cell destruction were observed. Portal fibrosis were also observed in 14 days of exposure duration. In the next higher duration (21 days), massive cellular destruction, lysis of nucleus and cell destruction were observed in most of the hepatocyte cells. In same duration cell accumulation with demarcation of nucleus were observed (Figure 3).

Hepatocyte cells located in the periphery of the tissue were found to be less affective. On the other hand, in the higher dose (40 µg/kg b.w./d), similar types of destruction were observed but the level of cellular destruction was multifold. In the lowest exposure duration (7 days), interstitial gap between cells, Lipid globules, and cytoplasmic alteration was found to be initiated. In the next higher exposure duration (14 days), the more nuclear fragmentation associated with intra- and inter- cellular lipid accumulation was observed. Cell accumulation with portal fibrosis and sinusoidal degeneration were also observed (Figure 4). In the next higher exposure duration (21 days), maximum massive cell destruction, cytoplasmic fragmentation and interstitial erythrocyte accumulation were more frequently observed. The most affected hepatic cells lost much of its cytoplasm and cellular organelles which were

replaced by lipid (Figure 2). The inner region of the liver tissue was found to be most adversely affected than the peripheral region. The complete cellular destruction of majority of the hepatocytes and few tumors were noticed but their malignancy has not been checked.

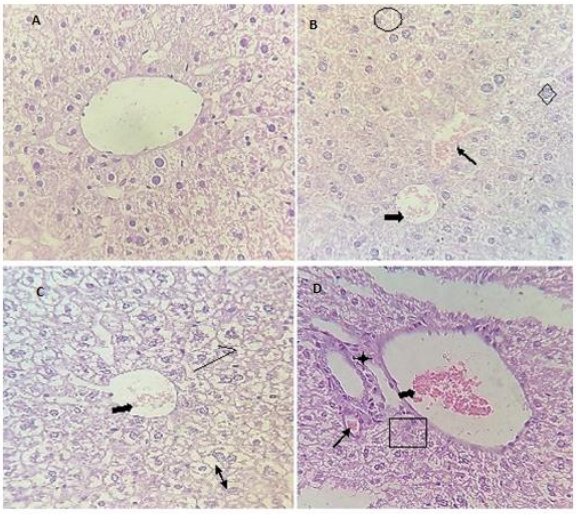

Figure 3: Histology of the liver at magnification 400× (H&E Staining). (A) Photograph of control showing normal pattern of Central vein (CV) and Portal vein branch (PVB); (B) Photograph of toxic effect after 7 days exposure, Circle indicates Parenchymal degeneration, Arrow indicates intravascular blood corpuscles accumulation (IGA), Diamond indicates Nuclear changes, Thick arrow indicates Portal Fibrosis; (C) Photograph of toxic effect after 14 days of exposure, Thick arrow indicates Portal Fibrosis (PF), Single line indicates Lipid Globules (LG), Double Arrow Head Interstitial gap between two adjacent cell (IG); (D) Photograph of toxic effect after 21 days exposure, Thick arrow indicates Portal Fibrosis(PF), Four pointed star indicates Cluster of necrotic hepatocytes, Square indicates Massive Cellular Destruction(MCD), Arrow indicates Inflamed cells.

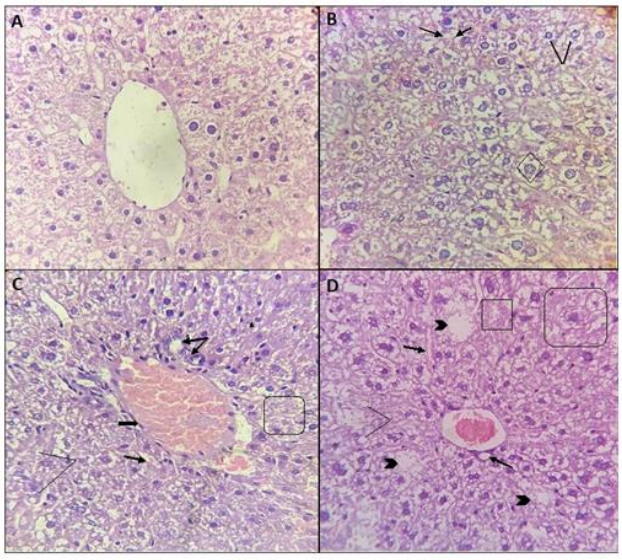

Figure 4: Histology of liver at magnification 400x (H&E) Staining. (A) Photograph of control showing normal pattern of Central Vein (CV), and Portal Vein Branch (PVB); (B) Photograph of toxic effect after 7 days exposure, Short Arrow indicates intravascular blood corpusclesaccumulation (IGA), Single line indicates Lipid accumulation

in cytoplasm, Diamond indicates Chromosomal fragmentation; (C) Photograph of toxic effect after 14 days exposure, Thick arrow indicates Portal Fibrosis (PF), Short arrow indicates interstitial gap between two adjacent cells (IG), two short arrow indicates inflamed cells, Single line indicates Lipid Globules and Square circle indicates Parenchymal degeneration; (D) Photograph of toxic effect after 21 days exposure, Square indicates Massive Cellular Destruction (MCD), Circle Square indicates Parenchymal degeneration, Arrow indicates inflamed cells, single line indicates Lipid Globules, Arrow head indicates Hepatocytes filled Lipids representing macrovesicular stetosis.

Increasing epidemiological evidences revealed that exposure of persistent organic pollutant can promote to the chronic liver fibrosis particularly in non-alcoholic fatty liver disease [26]. 2,3,7,8 TCDD upon binding with AhR transcriptionally activates to the elimination of the xenobiotic through detoxification process. However, detoxifications of toxicant also lead to cellular stress, due to multiple chain of reaction such as oxidative stress [27]. In role of detoxification AhR is found to be affect to lipid metabolism which is correlated to development of hepatic stetosis. TCDD promotes liver fibrosis followed by obesity in mice.

It has been reported that the entry of lipophilic toxicant can cause asphyxia which in turn damage hepatocyte cells [28]. The intracellular granulocyte accumulation may be due to the direct effect of the lipid soluble toxicant [29]. It has been suggested that TCDD induced hepatomegaly by depositing lipid molecules inside the cell and inducing higher metabolic requirement [30]. In the present investigation predominant exposure duration dependent effect observed for different cellular lesions was evident [31]. The fermentation of nuclear material was possibly a direct effect of the TCDD which entered in to the cell by dissolving itself into the plasma membrane [32]. Hepatic lesions similar to those observed in the present studies have also been found in different controlled laboratories studies [33]. The TCDD could have caused the hepatocyte cell destruction after the chronic exposure it may be possible that 2,3,7,8 TCDD may be transformed in to primary and secondary metabolites depending on the tissue oxygen contractions [34]. This may have caused the observed cellular damage and damage to the cellular components which resulted in to the massive scale cellular necrosis [35]. The present study increased seniority of the damaged with increasing exposure duration possibly indicative of both direct and indirect effect of TCDD in to the liver cells of mice [36].

5. Conclusion

Present study showed that TCDD damaged the mice liver in a dose and exposure duration dependent manner. A principally exposure duration dependent effects were observed in the activities of the antioxidant enzymes studied. However, both dose and exposure dependent histopathological lesions were observed in the liver cells of exposed mice. The administration of high dose (40 µg) for maximum exposure duration (21 d) caused major liver damages which might have been fatal.

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