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RESEARCH ARTICLE

Impact of Chronic Dose of Dioxin (TCDD) on Haematological and Biochemical Parameters in Mice, *Musmusculus* L.

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Abstract

Persistent organic pollutants (POPs) are compounds that have been detected as contaminants in almost every component of the global ecosystem including the air, water, sediments, fish, wildlife human adipose tissue, milk, and serum. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) belongs to the group of persistent organic pollutants (POPs), and is reported to be an extremely potent toxin. The current study was designed to investigate the effects on physical, haematological and biochemical parameters due to oral administration of chronic low dose (0.00005 µg/Kg body wt.) of TCDD at the intervals of 4, 8, 12 and 16 weeks in Swiss albino mice. Results indicated marked physical changes as well as statistically very significant ($p<0.01$) decreased levels of RBC, HCT, Hb, MCV, MCH, MCHC, and WBC after all the experimental duration in TCDD treated mice. The levels of ALT, ALP, total protein, albumin, urea, and creatinine increased in time dependent manner and were statistically very significant ($p<0.01$). Hence, epidemiological studies expecting similar effects from low-dose exposure to TCDD may produce a series of haematological and biochemical changes in the body.

Introduction

Environmental contamination of air, water, soil and food threatens the continuous existence of many plants and animal communities and may ultimately hinder the survival of humans. Among these environment contaminants, 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread, persistent, and highly toxic environmental pollutant (POPs) (Niittynen et al., 2003). They are currently one of the hot spots in the field of environmental toxicology because of their extensive disposition and coexistence in the environment, persistence, bioaccumulation, migration, and high toxicity (Walker et al., 2005). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent environmental contaminant that elicits a wide range of toxic and biochemical responses including hepatotoxicity, enzyme induction, immunotoxicity and lethality (Nebert et al., 2000; Senft et al., 2002).

The haematological parameters like haemoglobin concentration, haematocrit value, blood cell counts etc. can be used to find physiological response of contaminated environment (Dethloff et al., 2001). Analysis of blood parameters is relevant to risk evaluation as the predictive value for toxicity (Olson et al., 2000). Therefore, when a clinical diagnosis of animal physiology is applied to determine the chronic effects of pollutants, the blood parameters are often measured.

Biochemical parameters are the most assessable body contents for checking the toxicity of any chemicals. Any alteration in biochemical parameters can result in serious outcomes in the form of various diseases in both the animal and its consumers (Singh et al., 2010). The activities of some enzymes like alanine amino transferase (ALT) and alkaline phosphatase (ALP) etc also indicate the impacts of pollutants.

In the present study, an attempt was made to investigate the chronic toxicity effects of TCDD on biochemical and haematological parameters of Swiss albino mice. The changes in haematological parameters

(haemoglobinconcentration, cell counts and haematocrit values etc.), biochemical changes (total protein, albumin, urea and creatinine content) and enzymes (ALT, and ALP) activities were monitored after chronic exposure of this pollutant.

Materials and Methods

Experimental animal

Healthy and sexually mature Swiss albino mice weighing 30-35gm obtained from a random-bred colony in the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, were used for the experiment. They were maintained in a well-ventilated animal house at a temperature of (24 ±1°C), humidity- (55 ± 5%), and lighting- (12-h light/dark cycle). Animals were housed in large polypropylene cages with free access to food (Amrut laboratory, Bangalore) and water *ad libitum* during the course of the experiment. All animal experiments were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA)**.**

Test Chemicals

TCDD (CAS 1746-01-6; molecular weight, 321.9; purity, 99% as analyzed by GC-MS) was purchased from Accu. Standard. It was dissolved in corn oil (Sigma Chemical Co., St. Louis, MO) to obtain the experimental chronic dose of 0.00005µg/Kg body weight.

Experimental Design

The experimental groups of mice were divided in three groups of six animals each and repetitions were made three times. After acclimatization, animals were treated as follows: Group-I (Normal) received food and water *ad libitum*. Group-II (Control) group received corn oil in addition to food and water *ad libitum*, Group-III (Test group) received a chronic dose of TCDD (0.00005 µg/Kg body weight) in corn oil solution in addition to food and water *ad libitum*.

Haematological study

Blood was drawn from normal, control and TCDD treated groups of mice at intervals of 4, 8, 12 and 16 weeks by orbital sinus puncture for the investigation of hematological parameters, using EDTA (ethylene diamine tetra acetate) as an anticoagulant. Haematological parameters such as Red Blood Cell (RBC) count, the total White Blood Cell (WBC) count the Haemoglobin (Hb) concentration, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were assessed using Cell Counter (Medonic M- Series).

Biochemical study

Another portion of blood was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 min and the clear sera aspirated off for evaluation of the following biochemical parameters: alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, albumin, urea and creatinine by using fully Automated Biochemistry Analyzer (Model No-SELECTRA-"E", VITALAB BY MERCK).

Statistical analysis

One-way analysis of variance (ANOVA) was employed to compute statistical differences between sample means. The results are presented as means ± SEM. $P<0.05$ was considered statistically significant.

Results and Discussion

In the present study, an attempt was made to examine the chronic toxic effect of TCDD on the haematological and biochemical parameters in Swiss albino mice. The toxic impact of the TCDD was obtained from the percentage mortality of the test animals indicated 100% survival upto 4 weeks. It showed 86% survival upto 8 weeks, 71% survival upto 12 weeks and 64 % survival upto 16 weeks. The animals exhibited marked changes such as loss in body weight and became lethargic when compared with that of control. Viluksela et al., (1995) reported TCDD-induced lethality that involves feed refusal, body weight loss, and exhaustion of energy stores, collectively referred to as a "wasting syndrome".

Haematological parameters of Swiss albino mice orally treated with 0.00005 µg/Kg body weight of TCDD are recorded in Table l. A statistically significant decrease ($P<0.01$) in the RBC count as compared to the control mice after all the treatment periods were observed. Haemoglobin concentrations and haematocrit (HCT) values decreased and were statistically very significant ($P<0.01$) after 8, 12 and 16 weeks as compared to the control mice. Toxicants

might cause an adverse effect on the haematopoietic system which reduces the supply of RBC either due to less production and/or increased rate of removal from circulation (Al-Ghanim, 2012). Funseth and IIback, (1992) also found significant decrease in RBCs count in the TCDD treated rats. Fall in the level of haemoglobin may be the consequence of toxic effects of TCDD on the synthesis of this molecule. It has been reported in another study that depletion of RBC number as well as Hb percent may probably be due to defective haematopoiesis (Kim et al., 2002).

Blood cell indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values decreases and were statistically significant ($P<0.05$) after 4 and 8 weeks whereas the decreases were statistically very significant ($P<0.01$) after 12 and 16 weeks in TCDD treated mice (group-III) as compared to control (group-II) and normal mice (group-I). The decreased MCH, MCHC and MCV levels may be a sign of hypochromic microcytic anemia. Fluctuations in these indices correspond with values of RBC count, haemoglobin concentration and haematocrit (HCT) (Ahmad, 2011).

The total leukocyte count (WBC) increased and was statistically significant ($P<0.05$), in the TCDD treated group (group-III) after all the treatment periods which might be due to malfunctioning of the haematopoietic system caused by exposure to TCDD. It has been reported that TCDD adversely affected the multilineagehaematopoiesis in the haematopoietic stem cells of the bone marrow, mediated through aryl hydrocarbon receptors (AhR) in the target tissue (Sakai et al., 2003; Muranteand Gasiewicz, 2000). Yamamoto et al., (2006) also reported that TBDD (tetrabromodibenzo-*p*-dioxin)-dosed surviving rats exhibited growth retardation, decreased bone marrow hematopoiesis, decreases in red blood cell counts, haemoglobin concentrations, and haematocrit values.

Assessment of biochemical parameters is an important tool in assessing the health status of the individuals and is valuable in predicting clinical and prognostic outcomes (Krishna and Ramachandran, 2009). In the present study the biochemical parameters such as alanine transaminase (ALT) and alkaline phosphatase (ALP) increased and were statistically significant ($P<0.01$) in TCDD treated group as compared to control group (group-II) and normal group (group-I) after all the treatment periods (Table II). ALT is a very liver specific enzyme and one of the most reliable indicators of hepatotoxic damage (Ozer et al., 2008). The elevation in serum ALP may be an evidence of obstructive damage in the hepatobiliary system. Therefore, higher activities of these enzymes registered in this investigation may be due to damage caused by TCDD to liver. Some studies also found that 2,3,7,8-tetrachlorodibenzo-para-dioxin (2,3,7,8-TCDD) exposures were significantly associated with elevated alanine aminotransferase (ALT) and alkaline phosphatase (ALP) (Triebig et al., 1998; Michalek et al., 2001). Wang et al.(2011) also reported that combined exposure of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs) produced remarkable increases in the activities of ALT and ALP as compared to the control group, indicating the occurrence of hepatic injuries.

The results showed that low dose of 0.00005 µg/Kg body wt. of oral TCDD treatment induced statistically significant ($p< 0.05$) increase of serum total protein and albumin as compared to control values at all experimental durations (Table-2). Omitoyin (2007) reported that hyperproteinaemia may occur due to repercussion of water loss in plasma, elevated de novo synthesis or relative changes in blood protein mobilization. They also mentioned that such observed hyperproteinaemia may be indicative of efficient immune response and body physiological reaction to pollutants.

In the present study, the marked elevation in the serum urea and creatinine levels after 4 and 8 weeks were statistically significant ($P<0.05$) and statistically very significant ($P<0.01$) after 12 and 16 weeks following exposure to the chronic doses of TCDD which may correlate with the kidney damages. The elevated level of blood urea and creatnine in TCDD treated group of mice are in agreement with the results obtained by Lu et al. (2009). Elevated serum urea is also correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea because of increased synthesis of enzyme involved in urea production (Murray et al., 1999). TCDD induced increase in urea level observed in the present study may be due to the effect of pollutant on the liver function. Creatinine, a byproduct of muscle metabolism is more specific to the kidney, since kidney damage is the only significant factor that increases serum creatinine level (Nwanjo et al., 2007). Therefore, the significant increases in urea and creatinine levels noted in this study are classical signs of adverse effects of TCDD administration on the renal system.

From the present investigation it can be inferred that TCDD is capable of inducing significant changes in blood parameters of Swiss albino mice. The results show that the very low dose of TCDD examined in this study also causes important changes in the haematological and biochemical parameters of mice. Hence, it is of critical importance to public health that this chemical is used carefully and selectively, and that consumers and producers are informed of the possible and real damage caused by these agents.

Table 1 Changes in heamatological parameters of Swiss albino mice exposed to oral chronic dose of TCDD (0.00005 µg/Kg body wt.) for different experimental durations.

Haematological parameters	Gr-I	After 4 weeks		After 8 weeks		After 12 weeks		After 16 weeks	
		Gr-II	Gr-III	Gr-II	Gr-III	Gr-II	Gr-III	Gr-II	Gr-III
RBC (106/mm3)	9.106 ± 0.070	9.23 ± 0.121	5.31** ± 0.098	9.12 ± 0.034	5.32** ± 0.132	8.956 ± 0.042	4.93** ± 0.031	8.88 ± 0.12	5.40** ± 0.094
HGB (gm/dl)	14.43 ± 0.176	14.77 ± 0.086	14.0 ± 0.23	13.54 ± 0.421	12.5** ± 0.361	14.23 ± 0.254	12.1** ± 0.321	14.11 ± 0.112	11.4** ± 0.372
HCT (%)	38.29 ± 0.012	34.56 ± 0.027	36.2* ± 0.21	37.45 ± 0.097	32.9** ± 0.132	37.95 ± 0.114	30.26** ± 0.352	36.87 ± 0.091	28.23** ± 0.164
MCV (µm3)	40.18 ± 0.608	41.22 ± 0.252	40.82 ± 0.12	40.45 ± 0.532	39.37* ± 0.765	41.23 ± 0.316	34.23** ± 0.532	41.65 ± 0.112	33.95** ± 0.453
MCH (Pg)	14.69 ± 0.086	14.23 ± 0.059	14.7* ± 0.076	15.64 ± 0.132	14.3* ± 0.241	16.78 ± 0.683	12.9* ± 0.531	17.11 ± 0.487	13.1* ± 0.41
MCHC (gm/dl)	36.63 ± 0.522	32.6 ± 0.041	33.8* ± 0.093	33.95 ± 0.058	31.0** ± 0.061	33.85 ± 0.042	27.85** ± 0.31	32.11 ± 0.24	25.6** ± 0.63
WBC (103/mm3)	6.91 ± 0.142	14.78 ± 0.139	9.7** ± 0.71	14.32 ± 0.632	9.2** ± 0.412	15.1 ± 0.836	9.8** ± 0.712	14.74 ± 0.081	9.6** ± 0.321

All values expressed as mean ± SEM.
*Significant ($P < 0.05$); **Highly Significant ($P < 0.01$)

Table 2 Changes in biochemical parameters of Swiss albino mice exposed to oral chronic dose of TCDD (0.00005µg/Kg body wt.) for different experimental durations.

Biochemical parameters	Gr-I	After 4 weeks		After 8 weeks		After 12 weeks		After 16 weeks	
		Gr-II	Gr-III	Gr-II	Gr-III	Gr-II	Gr-III	Gr-II	Gr-III
ALT (IU/L)	28.0 ± 0.5	27.98 ± 0.36	93.66** ± 0.14	29.5 ± 0.06	112.32** ± 0.32	34.5 ± 0.52	125.0** ± 0.274	36.4 ± 0.32	121.0** ± 0.14
ALP (IU/L)	236.6 ± 0.89	242.59 ± 0.246	259.0* ± 0.104	247.6 ± 0.86	299.32** ± 0.91	244.5 ± 0.213	308.0** ± 0.314	234.7 ± 0.247	304.56** ± 0.14
Total Protein (gm/dl)	6.14 ± 0.44	5.91 ± 0.062	6.44 ± 0.046	5.98 ± 0.38	7.12* ± 0.032	6.23 ± 0.754	7.40* ± 0.963	6.12 ± 0.36	7.32* ± 0.745
Albumin (gm/dl)	3.07 ± 0.55	3.83 ± 0.08	3.53* ± 0.05	3.5 ± 0.231	3.4* ± 0.42	3.51 ± 0.042	3.90* ± 0.052	3.54 ± 0.031	4.23* ± 0.047
Urea (mg/dl)	34.4 ± 1.44	33.9 ± 0.41	34.66 ± 0.031	32.44 ± 1.45	41.0* ± 0.04	33.41 ± 1.035	48.0** ± 1.012	33.41 ± 1.035	48.0** ± 1.012
Cretinine (mg/dl)	0.5 ± 0.04	0.50 ± 0.016	0.66* ± 0.031	0.54 ± 0.10	0.67* ± 0.04	0.57 ± 0.012	0.72** ± 0.1123	0.57 ± 0.012	0.72** ± 0.1123

All values expressed as mean ± SEM. (n=6)
Not significant; *Significant (P < 0.05); **Highly Significant (P < 0.01)

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