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Toxicity of low dose TCDD to body weight and glucose-6-phosphatase in liver and kidney cells of mice

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ABSTRACT

Present study communicates the dose and exposure duration dependent effects of very low environmentally available doses of TCDD to the body weight and glucose-6-phosphatase in the liver and kidney cells of mice. The study was designed to test three hypotheses viz. a) TCDD effects body weight by reducing the activity of glucose-6-phosphatse, b) TCDD causes dose and duration dependent effects on glucose-6-phosphatase and, c) The liver will be more effected than the kidney since, the gluconeogenesis mainly occurs in liver. To test the formulated hypotheses groups of female Swiss albino mice were administered different doses of TCDD (0.004 mg/kg bw/d & 0.04 mg/kg bw/d) for 2, 4 and 6 days of exposure durations. The doses were selected according to the LD_{50} of TCDD to mice and minimum required exposed dose of TCDD to human being through different environmental sources. The results revealed a clear exposure duration dependent effects of TCDD to selected enzyme and body weight. The enzyme activity was more affected in liver than the kidney hampering different metabolic processes in liver cells. The results suggest that binding of TCDD to the AhR may be one of the possibilities that affect different metabolic pathway and body weight of mice.

Keywords: TCDD, Glucose-6-phosphatase, body weight Dose & duration dependent, Liver, Kidney, mice.

INTRODUCTION

Coplanar aromatic hydrocarbons such as PCBs, TCDD and furans are highly lipophilic and Persistent Organic Pollutants, tends to bioaccumulate and biomagnify through the food chain in living organism [1,2,3]. It has been reported that exposure of TCDD for different exposure duration caused developmental toxicity, immunotoxicity, organotoxicity and lethal wasting syndrome [4,5,6]. The toxicity of TCDD principally depends on the attachment and position of chlorine atom on benzene ring. TCDD produced toxicity through AhR mediated mechanism. This receptor controls multiple target genes such as cytochrome P4501A. One of the major drastic effects of TCDD mediated AhR is lethal wasting syndrome (loss of body wt.) after long term exposure of TCDD [7]. At very High doses of TCDD causes starvation like condition, which is manifested as a cessation of weight gain. TCDD decreases liver gluconeogenesis and body weight, was considered as a prominent feature of TCDD toxicity [8,9]. TCDD is known to cause long term effects in human body since these chemicals are physically, chemically and thermally stable in environment and thus, used in industries for various applications [10]. It is reported earlier that very high doses of TCDD cause starvation like effect, which is manifested as a cessation of weight gain. As body weight is regulated by hypothalamically programmed set point [11,12]. It has been documented that daily feed intake which is

absorbed by gastrointestinal tract is similar in both control and treated animals [13,14], however; loss of body weight and appetite is prominent feature of thyroid dysfunction. TCDD impairs lipid and carbohydrate metabolism severely in adipose tissue of liver [15]. Impaired glucose transport system is one of the major possibilities for wasting syndrome in rats [16,17]. TCDD also causes decrease in hepatic Vitamin A storage in treated rats [18]. One week TCDD exposure in rats caused body weight loss and effected lipid marker enzymes [19]. It has been reported that the TCDD directly affects appetite regulation areas in brain, which is the feedback mechanism in brain [20]. It has also been reported that TCDD induced fasting (feed deprivation) which in turn caused body weight loss [21], possibly by inhibiting gluconeogenesis in liver [22]. Going through the literature it was observed that studies on the toxic effects of TCDD on gross body weight and glucose-6 phosphatase in liver and kidney tissues of mice is scanty. Therefore, the present study was aimed to test three hypotheses viz. a) TCDD effects body weight by reducing the activity of glucose-6-phosphatse, b) TCDD causes dose and duration dependent effects on glucose-6-phosphatase and, c) The liver will be more effected than the kidney since, the gluconeogenesis mainly occurs in liver.

MATERIALS AND METHODS

The dioxin used in this study, 2,3,7,8 TCDD were purchased from Sigma-Aldrich Chemicals Ltd. (CAS No. 1746-01-6). All other chemicals were used for this study was analytical grade and were purchased from sigma chemical co. (St. Louis, MO, USA) for the assessment of glucose-6-phosphatase in liver and kidney tissues of mice. Inbred female Swiss albino mice around 2-3 months of age and 30-40 g of weight were used for entire study. The animals were kept in departmental animal house with hygienic facilities and prescribed conditions as per CPCSEA, India. Animals were provided commercially available rodent diet and water *ad libitum*. Different animal groups were kept in controlled humidity and temperature (25± 2° c; 44-55% RH and 10:14 h light and dark cycles) for one week before the experiment [5]. All studies were conducted according to the ethical norms approved by the CPCSEA, India (CPCSEA/CH/RF/ACK-2003, 29-07-2003). A total of 63 adult female Swiss albino mice were used for the study. The selection of the doses were based on the (a) available reports of the doses causing non-carcinogenic effects in the liver and kidney tissue of mice, especially on the enzymes following an acute to sub-acute exposure, and (b) evaluation of toxicity studies and application of factors (LOAEL) for extrapolating from animal model to human for TCDD administered through oral route [23]. The doses selected therefore, were very low concentrations of TCDD, comparable to that of a possible human exposure from different environmental sources. Different groups of mice were given oral administration of TCDD (0.004 and 0.04 mg/kg body weight /d) dissolved in corn oil (vehicle) for three different exposure durations of 2, 4 and 6 days. After completion of toxic treatment of TCDD, the liver and kidney tissues were rapidly removed and washed in ice cold Sucrose – EDTA - Imidazole buffer (SEI buffer). Known amount of tissue was homogenized using Potter- Elvehjem glass homogenizer to make a 10% (w/v) tissue concentration. The tissue preparation and enzyme extraction procedure were as per the method of Zaugg [24] with appropriate modifications. Activity of Glucose-6-phosphatase was estimated by the method of Shimeno [25] with appropriate modifications Jigyasi and Kundu [5]. Inorganic phosphate was measured by the method of Fiske and Subbarow [26]. To calculate the specific activities of the enzymes studied, protein content of each sample was estimated as per the method of Lowry et al. [27] using Folin phenol reagent and bovine serum albumin as the standard. The obtained data were subjected to various statistical analyses for their cumulative acceptability and for testing the hypotheses formulated. Comparison between control and doses were made using one-way ANOVA. A two-way nested ANOVA was done to check the significance in the variations between different doses and amongst different exposure durations. In addition to those tests, Comparison for the significance variations between control and each durations within a given dose were performed using two-tailed Student's 't' test. All statistical procedures were done as per Sokal and Rohlf [28].

RESULTS AND DISCUSSION

The effects on the body weight of the exposed mice were observed after the intoxication of TCDD for 2,4 and 6 days of exposure durations. The body weight in the exposed mice showed an initial stimulation followed by inhibitory trend after the exposure of TCDD (Fig. 1). The specific activity of glucose-6-phosphatase in liver tissue showed inhibition in all exposure durations while, more effects were observed in 0.04 mg/kgbw/d dose of TCDD after 6 days of exposure durations (Fig. 2a). The specific activity of glucose-6-Phosphatase showed inhibitory trend after the intoxication of both the doses of TCDD in kidney tissue (Fig. 2b).

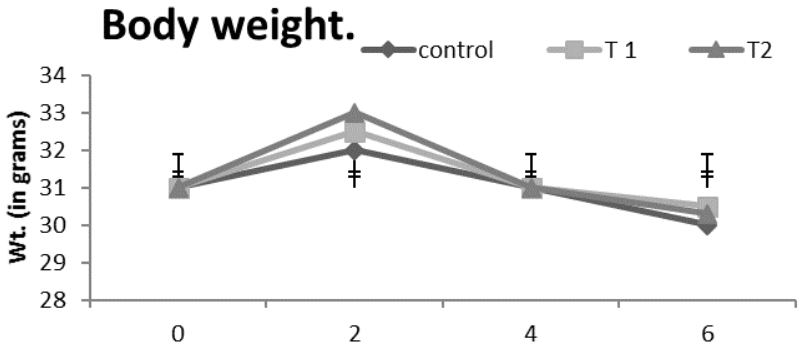

Fig.1 Graphs showing dose and duration dependent alterations in the body weight after *in vivo* TCDD intoxication
Error bars represent the standard deviation

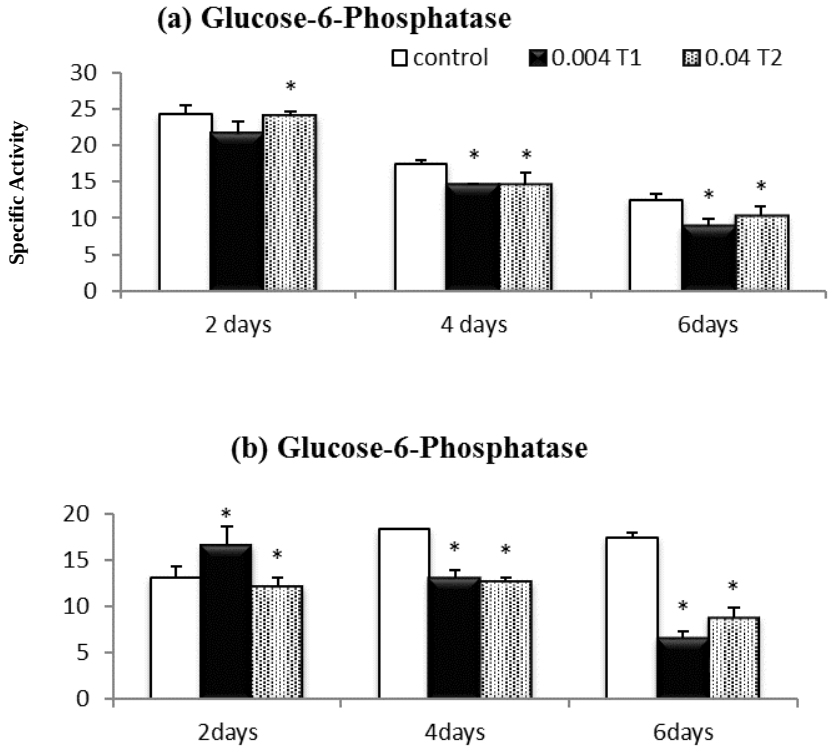

Fig. 2. Graphs showing the dose and duration dependent alterations in the specific activity of Glucose-6-phosphatases after TCDD intoxication(a) in liver (b) kidney
The error bars represents the standard deviation and '' sign represents the significant variations at P = 0.05 level in the activity of Glucose-6-phosphatases in tissues concern*

TABLE 1. Results of Two-factor ANOVA between control and each toxicated groups

	Body Weight	Liver	Kidney
Amongst doses	1.45	0.08	1.02
Within duration	93.50**	24.95**	48.04**

**Significance at p = 0.05(F crit (dF = 3,8) =3.44)*
***Significance at p = 0.05 (F crit (dF = 8,35) = 2.35)*

Dioxin or dioxin like PCBs has adverse effects after binding to the AhR [29]. These effects are possibly due to the irregular metabolism and energy production in the affected cells [30]. The inhibition of the Glucose – 6 phosphatase enzyme activity due to the TCDD intoxication possibly caused disturbances in physiological activities such as

glucose homeostasis, gluconeogenesis and energy transport pathways etc. [31,32]. In mammals, liver and kidney tissues possesses high amount of glucose utilization for their normal cellular activities. Therefore, the dioxin induced inhibition in the activity of glucose-6-phosthatase might have induced the precursor of metabolic pathogenesis. The results of two way nested ANOVA showed a clear exposure duration dependent effects of TCDD in liver and kidney tissue as well as body weight of mice after TCDD exposure (Table 1). This was possibly due to the lower concentration of TCDD which were not capable of producing any direct dose dependent effects in the exposed cells [33,34]. Results of the one-way ANOVA also showed significant variation in all exposure duration of TCDD intoxication (Table 2). The results of student's 't' test showed significant alteration between control and individual treated groups within each dose group (Table-3). Maximum significant alterations were observed in liver tissue since more metabolic activities are normally occurred in liver than the kidney tissue [35]. The statistical analyses showed significant alterations in the body weight of mice as well as the activity of glucose-6-phosphatase in liver and kidney cells of mice. In mammalian liver, TCDD is known to be decrease the expression and activity of glucose-6-phosphatase enzyme that mainly control gluconeogenic flux [9,36], however, the exact line of action is not clear at this stage.

The present investigation reports an exposure duration dependent effects of TCDD on body weight and the activity of glucose-6-phosphatase. As the majority of the studies pertaining the effects of dioxin exposure on various metabolic activities used mostly rodents as preferred animal models, the broad strain specificity demonstrated the sensitivity to wasting effects of dioxins [37,38,39]. The observed effects of TCDD are more sensitive for metabolic pathogenesis [38].The earlier reports also suggested that hypophagia induced weight loss is one of responses that contributes the death of TCDD treated rats [40]. These are the direct effects of TCDD on appetite regulating areas in brain which affect to body weight of organism and key enzymes of gluconeogenesis. It has been suggested that increased hypoglycemia developing as a consequences of feed intake and decreased ability to form glucose via the gluconeogenic pathway [41]. Affected glucose homeostasis and glucose level of plasma, liver glycogen is due to the acute exposure of TCDD [42].

TABLE 2. Results of single-factor ANOVA between individual exposure durations within each toxicated group

	Body weight	Liver	Kidney
Control	41.26	12.65	14.5
0.004 mg	65.26*	27.45*	24.8*
0.04 mg	34.26*	23.25*	64.08*

**Significance at p = 0.05(F crit. = 4.06)*

TABLE 3. Results of Student's 't'-test between control$_1$ and individual exposure durations within each toxicated dose

	Body weight		Liver		kidney	
	0.004 mg	**0.04 mg**	**0.004mg**	**0.04 mg**	**0.004 mg**	**0.04 mg**
2 d	5.21*	1.23	1.96	5.76*	2.87*	11.54*
4 d	7.29*	6.21*	21.79*	17.79*	12.36*	9.95*
6 d	5.25*	2.35	4.92*	9.34*	15.73*	7.83*

**Significance at P = 0.05 (F crit = 2.77)*

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