IN VITRO TOXICITY OF A DIOXIN (2, 3, 7, 8 TCDD) ON FEW MEMBRANE BOUND ION DEPENDENT ATPASES AND LYSOSOMAL ENZYMES IN MICE KIDNEY

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ABSTRACT
A dioxin, the 2,3,7,8-TCDD is a highly toxic POP, produced during the improper burning of paper, pulp, municipal and industrial wastes, and tend to accumulate in the adipose tissues of animals including human when exposed through different environmental sources. However, its toxicity reports on the animal organ systems in general and kidney lysosomal enzymes in particular are rare. The present communication therefore, tested the hypothesis that relatively low and environmentally available doses of TCDD affects the activities of few membrane-bound ion-dependent ATPases (Total, Na⁺-K⁺ and Ca²⁺ ATPases) and lysosomal marker enzymes which triggers the cellular apoptosis process (acid phosphatase, α-glucuronidase and α-galactosidase) of mice kidney under in vitro conditions. The results suggested a significant direct dose dependent effects of TCDD on the selected ATPases and lysosomal enzymes extracted from mice kidney tissue. The results also indicating that even a very low dose of TCDD affects the activities of membrane bound ion dependent ATPases and lysosomal enzymes which may leads to severe disturbances in the trans-membrane movements of various ions and may initiate the cellular apoptosis process in the living system.

INTRODUCTION
Dioxins are a concern because of its human health effects by altering different physiological and biochemical activities into the cell. TCDD is the most toxic dioxin that has very low degradability and high bioavailability in animal adipose tissues (Jigyasi and Kundu, 2013a). Very limited reports are available on the toxic effects of dioxins to animal systems. TCDD enters into the living organisms including humans by various routes, such as through different food sources, inhalation and by accidental and occupational exposure (Mastroiacovo et al., 1988). It has been reported that TCDD toxicity depends on its metabolites and stability of different metabolites in the body of a living organism (Fingerhut et al., 1991). These metabolites are able to severely affect the physiology in the body of the living organisms (Pirkle et al., 1989). Exposure of TCDD was found to stimulate the proliferation abnormally resulting occlusion of the lumen and hydronephrosis in the epithelial cell line and the lumen of ureter (Abbott and Birnbaum, 1989). It was also reported that the lipophilic TCDD enters into the cell by dissolving itself into the plasma membrane and affects the normal physiological processes of an animal (Jigyasi and Kundu, 2013b). TCDD, even at very low concentration, has lethal effects on the gross physiology of living organisms. The reason for its potential lethal effects might be associated with its structural similarity with different receptors which are present in the cytosol as well as nuclear membrane (Reggiani, 1989). Kociba et al. (1978) reported that the TCDD has ability to mimic the different receptor and thereby interfere into different physiological activity. TCDD was reported to cause the loss of body weight in rats after chronic oral exposure to as low as 0.1 µg/kg/day and 0.286 g/kg/day dose (Van Miller et al., 1977). Couture et al. (1990) reported that the developing mice when exposed to TCDD, showed a dose dependent increase in organ damage. Acute oral exposure of TCDD caused pale kidney in minks, enlarged convoluted tubules and Bowman’s space together with hyperplasia in rats and monkeys (Hochstein et al., 1988; Christian et al., 1986a; McConnell et al., 1978). It has also been reported that chronic oral exposure of TCDD increased renal inflammatory changes which might be secondary effects to the kidney (NTP 1982; Pegg et al., 1976). Therefore, the aim of the present study was to evaluate the dose dependent effects of very low doses of 2, 3, 7, 8-TCDD on few ATPases and lysosomal marker enzymes of mice kidney under in vitro conditions. The study tested a hypothesis that the relatively low, environmentally available doses of TCDD directly affects the activities of some selected membrane-bound ion-dependent ATPases and lysosomal enzymes under in vitro condition in mice kidney.

MATERIALS AND METHODS
Adult Swiss albino female mice around 3 months of age and weighing 30 ± 5 g were used for study. The animals were provided commercially available rodent diet with water ad libitum and kept under hygienic conditions, controlled relative
humidity, temperature (25 ± 2°C) and diurnal cycle of 14:10 h in the animal house facilities. All experiments were conducted according to CPCSEA, India. The TCDD was purchased in its purest form from Sigma (CAS No. 1746-01-6). All other chemicals used for this study were of analytical grade and procured from reputed chemical companies. Various concentration of TCDD like 200, 400, 600, 800, 1000 ng/ml were dissolved separately in DMSO using its stock solution. As far as the toxicity of DMSO is concerned, it has been reported that the final concentration of DMSO in assay buffer (< 0.5% v/v) did not significantly affect any cellular activity (Pathak and Kundu, 2013e). The kidney tissue was suspended in chilled Sucrose-EDTA-Imidazole buffer (SEI) at pH 7.1 to remove excess blood and other membranes. The enzyme extract preparation and assay for membrane bound ion dependent ATPases was carried out by the method of Zaugg (1982) with appropriate modifications (Lakshmi et al., 1991; Pathak and Kundu, 2013a). The lysosomal fraction was obtained by the method of Beaufay (1972). The activity of Acid phosphatase, beta-glucuronidase and alpha-galactosidase were estimated using this lysosomal fraction by the method of Tettamanti and Masserini (1984). Enzymes were exposed to different dosed of TCDD by pre-incubating for 10 minutes at 37ºC before the addition of the respective substrate. Controls and blanks for all the enzyme assays were run separately for all TCDD concentrations. The obtained data were subjected to different statistical analyses for the testing of the formulated hypothesis (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The result of the present investigation showed a general trend of inhibition in the enzymes extracted from the kidney cells. The specific activity of Total ATPase showed an inhibitory trend in all doses of TCDD (Fig. 1a). The specific activity of Na+-K+-ATPase showed a progressively inhibitory trend in higher dose of TCDD. However, a slight stimulation was observed in lower dose (Fig. 1b). In case of lysosomal enzymes, the specific activity of acid phosphatase showed high degree of inhibition in all the selected doses and was found to be fully inhibited in 800 ng/ml and 1000 ng/ml doses (Fig. 1c). On the contrary, the specific activity of α-galactosidase showed a stimulatory trend in 800 ng/ml dose (Fig. 1d). The activity of α-glucuronidase showed a general progressive inhibitory trend in all the doses of TCDD (Fig. 1e). In the present study, the drastic changes observed in the enzymatic activity might be indicating a general metabolic changes in the kidney cells after TCDD exposure. In a living system, the main regulatory enzymes which is responsible for the transport of ions across the plasma membrane are ATPases (Jigyasi and Kundu, 2013b, Pathak and Kundu, 2013e). It has been reported earlier that the metabolism of endosulfan may affect the configuration and active transport of the cell membrane (Verma et al., 1978; Rudrama Devi et al., 2010; Awasthi et al., 2013). The activities of total and Na+-K+-ATPases inhibited by many xenobiotics, this effect mainly contribute to functionally damage to ion channels and protein which could impair the Na-K pump resulting to irrepressible entry of water molecules followed by increased osmotic gradient which may lead to the cell rupture (Jernelov, 1978). TCDD molecules bind with intracellular receptors after entering into the cell. Previous studies of our laboratory reports that disturbances in intracellular ions can interrupt to homeostasis of cell (Pathak and Kundu, 2013c). TCDD compounds are known to induce hydrenephrosis in the developing stage of mice kidney and defined the pathology of accumulation of urine due to obstructed outflow (Abbott and Birnbaum, 1989). It was also reported that organochlorine metabolites are capable to induce the activities of enzymes related to the defense mechanism of the cell against the oxidative stress (Cheeseman and Slater, 1993; Pathak and Kundu, 2013d). The common cause of toxicity is oxidative stress and toxic metabolites which is produced by different cellular reactions through specific organs (Rao and Murthy, 1980).

In the present study, it was evident that lysosomal marker enzymes were also severely affected by TCDD molecules. The result of statistical analysis in the present investigation also proved significant variation in all functional enzymes of lysosome. The result of One way ANOVA showed highly significance in estimated membrane bound and lysosomal enzymes in kidney cells (Table 1). In Total ATPase and Na+-K'-ATPase (F5-6 = 37.98, p = 0.05; F5-6 = 1.74, p = 0.05 level respectively). POPs normally are lipophilic so it can easily enter into the cell by affecting lipid bilayer system of the cell. Uncontrollable entry of Na+ and K+ ions can lysis to the cell or can disturb to the volume of cell (Jigyasi and Kundu 2013c; Pathak and Kundu 2013b). The Results suggested highly

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Table 1: Results of single factor ANOVA of the activities of different enzymes estimated in the kidney cells of the mice exposed to different doses of TCDD under in vitro conditions

Table 2: Results of Student's 't'-test in the activities of different enzymes estimated in the kidney cells of the mice exposed to different doses of TCDD

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*Significance at p = 0.05 (F crit = 4.30; dF = 5, 6)

*Significance at p = 0.05 (t crit = 4.30; dF = 2)
significant variation in acid phosphatase, α-glucuronidase and α-galactosidase ($F_{5,6} = 48.46$, $p = 0.05$; $F_{5,6} = 14.23$, $p = 0.05$; $F_{5,6} = 8.38$, $p = 0.05$ respectively) (Table 1). Acid phosphatase enzyme is the main enzyme of lysosome which composed of different proteins that hydrolysis to different chemical reactions and break bonds between phosphate groups. The results of t-test also support to these studies and shows significant variation in specific activity of all estimated enzymes between each dose groups (Table 2).

The present study showed high inhibition in the activity of α-glucuronidase in the lysosomal sub-fraction of the kidney cell extract. It is possibly related to the disturbances caused by the catalysis of complex carbohydrates into the cell through breakdown of glycosidic bonds between glucuronic acid and some other compounds (Pathak and Kundu, 2013e). The inhibition in the activity of liver α-glucuronidase may be related to less production of glucuronyl group as a substrate for Glucuronyl Transferase (UDP-GT) (Jozwik et al., 2005). Like α-glucuronidase, α-galactosidase catalyzes the hydrolysis of galactosidae into galactose and glucose. If TCDD, in anyway, alters the activity of these enzymes then the disturbances in the metabolic pathways occur may leads to the metabolic disorders in a living organisms including humans (Jigyasi and Kundu 2013d, Jigyasi and Kundu, 2014). On the other hand, it may also release the different macromolecules and contents into the cytosol through lysosomal permeabilization which can interrupt the different cellular activities (Stoka et al., 2007) and initiate the apoptosis or damage of the cell (Zhao et al., 2003).

The overall results of the present study showed that these alterations in the activities of selected membrane-bound ion-dependent ATPases and lysosomal enzymes of mice kidney may create the cellular disturbances by affecting the transport of ions and metabolic pathways which leads to alterations in the normal cellular physiological processes (Bhuvu et al., 2014). The results of the present study answer the hypothesis that the exposure of very low and environmentally available doses of TCDD has a direct dose dependent effects on the activities of selected ATPases and lysosomal enzymes under in vitro condition.

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Figure 1: Dose dependent effects of 2,3,7,8 TCDD in the specific activity of ion dependent ATPases in mice kidney cells. A ‘**’ sign denotes the significant variations in each dose.
aphid and their safety to predatory Coccinellids. The Bioscan. 8(3): 1007-1010.


