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AROCLOR 1254 PROVOKES THE ALTERATIONS IN THE LYSOSOMAL FUNCTIONS THROUGH THE PRODUCTION OF OXIDATIVE STRESS IN MICE HEPATIC CELLS

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ABSTRACT: Lysosomal Membrane Permeabilization (LMP) is a potentially lethal event because the ectopic presence of lysosomal proteases in the cytosol causes digestion of vital proteins and the activation of additional hydrolases including caspases. In the present communication we studied the activities of some antioxidative enzymes viz., Glutathione-S-transferase (GST), Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) and Reduced Glutathione level (GSH), as the indicative of oxidative stress. The studies were also carried out on the activities of few lysosomal enzymes, viz., Acid phosphatase, α-galactosidase, β- galactosidase and β-glucuronidase, which indicates the disturbances in the LMP. The results indicated that the exposure of low doses of Aroclor 1254 for short durations significantly provoked oxidative stress by altering the natural antioxidative mechanism which disturbed the LMP and caused alterations in the lysosomal enzymes activities in the liver of mice. This study thus, reports the dose and duration dependent effects of Aroclor 1254 produced the disturbances in the LMP, by inducing a plethora of distinct stimuli including ROS, with a possible involvements of lysosomotropic compounds and endogenous cell death effectors, in the liver of white Swiss albino mice.
Key words: Aroclor 1254, oxidative stress, LMP, lysosomal enzymes, liver

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INTRODUCTION

PCBs are very stable, strongly lipophilic and persistent in the ecosystem. These compounds are generally bioaccumulated through the food chain due to their affinity for lipids and are almost resistance to metabolism(Pathak *et al*., 2013) [1].Rates of PCB metabolism vary greatly with the degree of chlorination of the biphenyl rings and the position of the chlorines on these rings (ATSDR, 2016) [2]. PCBs, especially the highly chlorinated congeners, tend to accumulate in lipid rich tissues due to their lipophilic nature therefore greater relative amounts of PCBs are usually found in adipose tissue, breast milk, liver and skin (ATSDR, 2000; Matthews and Dedrick, 1984) [3,4]. Liver is the primary site of metabolism of PCB, which occurs via hydroxylation and conjugation with glucuronic acid and sulfates (ATSDR, 2016) [2].The metabolism of PCBs is complex and has an impact on toxicity and there by on the assessment of PCB risk. A large number of reactive metabolites are formed in the processes of biotransformation, which leads to generate oxidative stress (Grimm *et al.*, 2015) [5]. SOD, GST, GR and GSH, play an important role in the biological systems to act against oxidative stress (Akyol *et al.*, 2002) [6].The antioxidant enzymes such as GR, SOD and GST, take part in maintaining GSH homeostasis in tissues which prevents the oxidative stress (Abdel-Moneim *et al.*, 2010) [7].Oxidative stress is responsible to create havoc in the normal physiological cellular processes. The most harmful effect is; it can cause direct, intra-lysosomal damage or cause secondary lysosomal damage through the increased production of damaged macromolecules or organelles.

Lysosomes contain many different types of hydrolytic enzymes including proteases, lipases, nucleases, glycosidases, phospholipases, phosphatases and sulfatases that usually exert their maximal enzymatic activity at low pH. The acidic milieu of lysosomes (pH 5) is maintained by a vacuolar ATPase that pumps protons from the cytosol into the lysosomal lumen (Luzio *et al.,* 2007) [8].The lysosomal membrane is protected from the acidic hydrolases by lysosome specific expression of membrane proteins such as Lamp-1 (Lysosomal-associated membrane protein-1) and Lamp-2 (Lysosomal-associated membrane protein-2), which are heavily glysosylated and hence resist digestion (Eskelinen, 2006) [9].Several degradation pathways converge at the level of lysosomes. This applies to the endocytotic degradation of plasma membrane receptors and proteins from the extracellular matrix, as well as the phagocytotic degradation of bacteria and apoptotic cells (Mizushima, 2007) [10].Several studies reported that the increase in the reactive oxygen species disturbs the LMP which cause cell death (Boya and Kroemer,2008; Guicciardi *et al.,* 2004; Kroemer and Jaattela, 2005; Tardy *et al.,*2006; Terman *et al.,* 2006) [11-15].The distinctive sign of LMP is the translocation of soluble lysosomal components (including enzymes) from the lysosomal lumen to the cytosol (Boya and Kroemer,2008) [11]. Under conditions of cell stress, however, lysosome function and integrity may become compromised and can trigger regulated cell death (Pivtoraiko *et al.,* 2009) [16].In this study, we checked the hypothesis that the administration of low doses of Aroclor 1254 (a, PCB) exerts its toxicity by the induction of intracellular oxidative stress which creates the disturbances in the lysosomal enzymes activities by affecting the LMP which may ultimately lead to cellular injury.

MATERIALS AND METHODS

Experimental Animal Model and Ethics:

Inbred male mice were utilized in present investigation. They were maintained as per CPCSEA, Govt. of India, guidelines in the departmental animal house facilities, in highly hygienic condition in the mice cages. Healthy animals with the same body weight of about 30-40g were considered for the experimental purposes after acclimatization for a period of 2-3 days prior to the experiment. The animals were grouped before the experiments and kept under standard conditions. The experiments were conducted according to the ethical norms approved by the CPCSEA (No.757/PO/Re/S/03//CPCSEA for Research for Education purpose on small animals, dated 24-04-2017). Animals were provided with rodent diet (Keval Sales Corporation, India) and water ad libitum.

Chemicals and reagents:

Polychlorinated Biphenyls (Aroclor 1254) (CAS No. 11097-69-1)) and all the other chemicals used in this study were procured from Sigma Chemical Company Inc. All the chemicals used in this study were of analytical grade.

Experimental Design:

Animals of the same weight group were selected and used for experimental studies. For each experiment four groups of animals were used. Each group contained three animals. Each experiment was repeated at least three times. Since, the present study was aimed to assess the sub-lethal doses and exposure duration dependent effects of selected Aroclor 1254 on few organs in mice, experiment were conducted with two sub lethal doses (0.1 and 1 mg kg^{-1} d^{-1}) and four different exposure durations 7, 14, 21 and 28 days.

Homogenate Preparation:

10% homogenate of liver tissue was prepared by the method of Bhor *et al.,* 2004 [17].The supernatant so obtained was used for all the assays.

Estimation of Reduced Glutathione Content (GSH) and Antioxidative enzyme activities assays:

Glutathione peroxidase (GPx) activity was measured by the method of Rotruck *et al.,* 1973 [18].GSH was determined Ellman, 1959 [19]. The absorbance was read at 412 nm. Glutathione peroxidase activity was expressed as µg of GSH consumed/min/mg protein and reduced glutathione as mg/100gm of tissue.

Glutathione-S-transferase (GST) activity was determined spectrophotometrically by the method of Habig*et al.,* 1974 [20]. The absorbance was followed for 5 min at 340 nm. The activity of GST is expressed as µmoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity was determined by kit method purchased from Sigma-Aldrich Ltd. and the absorbance read at 450 nm using micro plate reader.

Preparation of liver lysosomal fraction:

Liver lysosomal fraction was prepared by the method of Beaufay, 1972 [21]. The collected liver lysosomal fraction was used for all the assays.

Lysosomal enzymes activity:

The activity of Acid phosphatase, α-galactosidase, β- galactosidase and β-glucuronidase were estimated using this lysosomal fraction extract of Tettamanti and Masserini, 1984 [22]. The absorbance was read at 410 nm. All enzymes' activity were expressed as µ mol p-nitrophenol produced mg $protein^{-1}$ h^{-1} of tissue.

Protein:

Protein was determined by using Bovine Serum Albumin (BSA) as standard, at 660 nm Lowry*et al.*, 1951 [23].

Statistical analysis:

The obtained data were analyzed by a two-factor ANOVA and single-factor ANOVA. All statistical procedures were computedusing SPSS version 21.0 (SPSS, Inc., Chicago, USA) and Sokal and Rohlf, 1969 [24].The p-value of $p<0.05$ and $p<0.01$ were considered as statistically significant.

RESULTS

Effect of Aroclor 1254 on Antioxidant enzymes in mice liver:

In the present study, the GSH level was significantly declined in all the doses exposed for almost all the durations (Fig. 1a). Similarly, the GST activity was also significantly increased in all doses and exposure durations (Fig. 1b). On the other hand, the GPx activity was showing an increasing trend with the increasing exposure durations in the lower dose. However, in higher dose the activity was significantly high in all the exposure durations (Fig. 1c). SOD activity was significantly increased in all doses and all exposure durations (Fig. 1d).

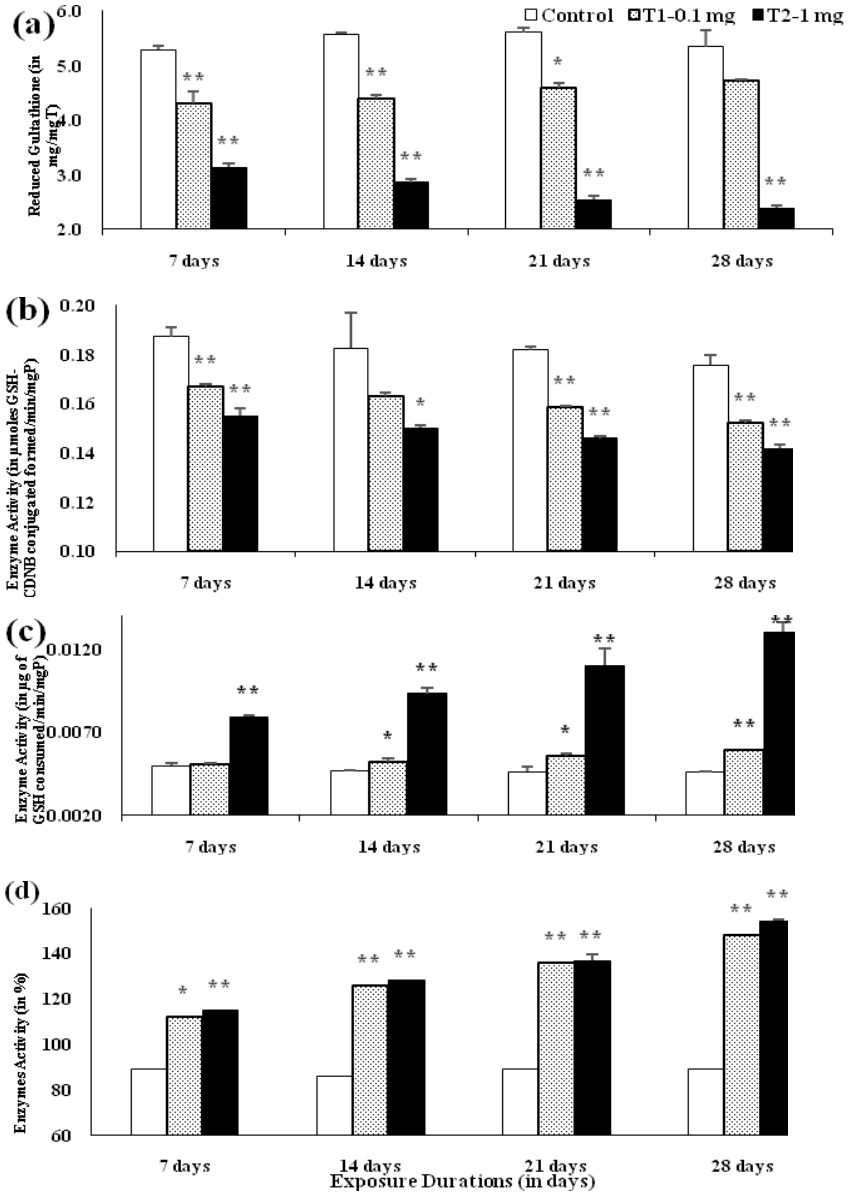

Fig. 1. Effects of oral dosage of 0.1 and 1 mgkg^{-1}d^{-1}Aroclor 1254 on various enzyme activities in the liver of mice. (a) Reduced Glutathione (GSH); (b) Glutathione-S-transferase (GST); (c) Glutathione peroxidase (GPx); (d) Superoxide dismutase (SOD). Data denoted as mean ± standard error (n=3). Differences relative to control were considered to be statistically significant at $p < 0.05$ (*) and $p < 0.01$ (**).

Effect of Aroclor 1254 on lysosomal enzymes in mice liver:

Results revealed that the dose and duration dependent effects of Aroclor 1254 on the activities of the selected lysosomal enzymes, viz. Acid Phosphatase, α-Galactosidase, β-Galactosidase and β-Glucorounidase, showed stimulations compared to the control. The observed changes in the specific activity of the acid Phosphatase was statistically significant ($p<0.05$ and $p<0.01$) in longer exposure durations of both the doses (Fig. 2a). In case of α-Galactosidase, the variations in the specific activity were statistically significant in all doses and exposure durations expect in lower dose and shorter exposure durations (Fig. 2b). Similarly, the specific activity of β-Galactosidase revealed statistically significant variations in almost all doses and durations except in 7 days exposure to 0.1 mg kg^{-1} d^{-1} of Aroclor 1254 (Fig. 2c). On the other hand, significant variations in the activity of β-Glucorounidase was observed in all the exposure durations of the higher dose (Fig. 2d).

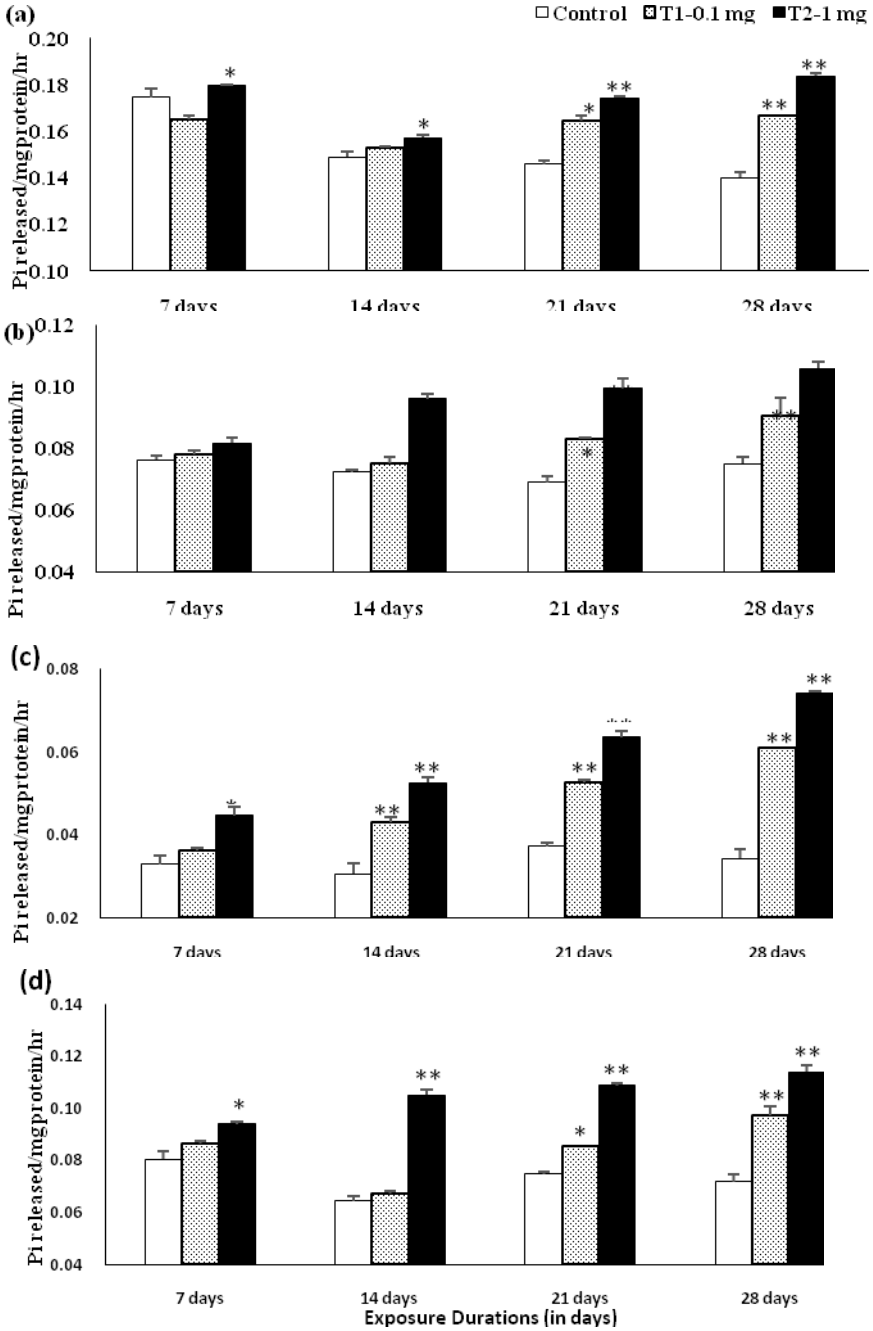

Fig. 2. Effects of oral dosage of 0.1 and 1 mg kg^{-1} d^{-1} Aroclor 1254 on various enzyme activities in the liver of mice. (a) Acid Phosphatase; (b) α-Galactosidase; (c) β-Galactosidase; (d). β-Glucorounidase. Data denoted as mean ± standard error (n=3). Differences relative to control were considered to be statistically significant at $p < 0.05$ (*) and $p < 0.01$ (**).

DISCUSSION

The important outcome of the present study was that the exposure of very low doses of Aroclor 1254 for short time period also induced oxidative stress that significantly provoked the lysosomal enzymes activities indicating disturbances in the LMP which might have lead to cellular injury or cell death.Several studies on PCBs reported that these cause many have deleterious effects on different organ systems in rodents (Berberian *et al.,* 1995; Glauert *et al*., 2005; Hemming *et al.,* 1993; Tharappel *et al.,* 2002) [25-28].It is also known that oxidative stress leads to many physiological disorders like autism, liver disorders, cellular injury, cancer and heart disease through endothelial damage (Chauhan *et al.*, 2004; James *et al.*, 2004; Nair, 2006; Tas *et al.*, 2005; Valko *et al.*, 2006; Yoshida and Ogawa, 2000; Ramadass *et al*., 2003) [29-35]. It has been reported that PCBs show a higher affinity for liver than other adipose tissues when compared to those PCB congeners which have chlorine atoms in ortho positions(Yoshimura *et al*., 1985; van Birgelen *et al.,* 1994) [36-37].The increased affinity of Aroclor to liver is possibly associated with the induction of hepatic binding proteins. These proteins are excellent indicators to follow the toxic effect of a huge variety of compounds and excessive amount of PCBs. Aroclor 1254 is known to induce hepatic tissue damage which is mediated by reactive oxygen species and acetaldehyde (Zima *et al.,* 2001) [38] In mammals, highly chlorinated Aroclor 1254 congeners are reported to have slow metabolism and tend to increase their potential to disrupt the signal pathway (Whitlock, 1999) [39]. These are capable to generate transient reactive oxygen species and free radicals which in turn increase oxidative stress (Giacco and Brownlee, 2010) [40].In the current study, the GSH and activity of GST were significantly decreased as compared to control which may be due to enhanced oxidation and consumption in the detoxification of highly reactive peroxides and its use in the glutathione peroxidase reaction (Fig. 1 a & b) (Ugochukwu *et al.,* 2004) [41]. While, the activities of antioxidative enzymes present in liver, GPx and SOD were significantly increased after the administration of low doses of Aroclor 1254 for short exposure duration as compared to control (Fig. 1 c & d). This increase in the GPx and SOD indicates the possible elevation in the amount of superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2).This elevation in the amount ofreactive oxygen species and free radicals are able to increases oxidative stress (Giacco and Browke, 2010) [40]. This oxidative stress can affect the lysosomes and cause alterations in the lysosomal enzyme functions directly and the lysosomal properties. In the present study, lysosomal enzymes activities viz. acid phosphatase; α and β galactosidase and α and β glucoronidase showed significant increase after the exposure of Aroclor 1254 indicating leakage of lysosomal content due to increased membrane permeability and damage which may lead to possible cellular injury in the liver (Fig. 2) (Adewusi and Afolayan, 2010) [42]. These alterations may be due to the oxidative stress induced by Aroclor 1254 administration, which can increase the fragility of lysosomal membranes and modulate intra lysosomal pH (Ishibashi, 2006) [43]. This in turn may alter lysosomal enzymes activity as these enzymes typically require acidic pH (Patschan and Goligorsky, 2008; Hideshima *et al.*, 2005) [44-45].

Damage of the lysosomal membrane often results in cytosolic leakage of potent hydrolases which could cause intracellular havoc (Roberta *et al.,* 2006) [46]. Direct damage of the lysosomal membrane by reactive oxygen species during oxidative stress has been extensively reported (Kiffin *et al.,* 2004) [47]. Alterations in the lysosomal enzymes activities could be attributed to the variability in LMP which affects the outward leakage of these enzymes. Lysosomal enzymes are known to be involved in cell death and tissue damage (Fushimi *et al.,* 1974) [48]. So, antioxidant and lysosomal enzymes are important biomarkers for physiological disturbances after the living being is exposed to the lipid soluble xenobiotics like PCB. Basically, antioxidants indirectly present the leakage of lysosomal enzymes in to cytosol and ameliorate the altered lysosomal enzyme activities. In conclusion, the overall results of our study proved that Aroclor 1254 induces oxidative stress by altering the activities of antioxidative system in liver which leads to leakage of lysosomal enzymes.This alterations of lysosomal enzymes activities are capable to create cellular damage. Lysosomes have been classically considered one of the main targets of the reactive oxygen species. In fact, the destabilization of the LMP during oxidizing conditions promotes the leakage of the enzymes contained in these organelles and contributes to cellular damage.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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