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EFFECT OF CONCURRENT EXPOSURE OF LOWER CONCENTRATIONS OF LEAD AND ENDOSULFAN ON HEPATIC DRUG METABOLIZING ENZYMES IN MALE RATS

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Abstract: The effect of concurrent repeated exposure of lower concentrations of lead and endosulfan were evaluated on hepatic drug metabolizing enzymes in male rats. Alterations were evaluated through changes in the phase I enzymes (viz. cytochrome P450 and cytochrome b5, aminopyrine *N* – demethylase and aniline p- hydroxylase) activity and phase II enzymes (viz. microsomal and cytosolic glutathione-*S*-transferase UDP-glucuronosyl transferase) activity in liver. Lead, when given alone at the dose of 100 ppm in drinking water and endosufan, when given alone at the dose of 10 ppm in feed, and given in combination did not change the activities of cytochrome b5, aminopyrine *N* – demethylase and aniline p-hydroxylase. When given in lower dose combination, they significantly inhibited levels of cytochrome 450, microsomal, cytosolic glutathione-S transferase and microsomal protein as compared to control and their single compounds when given alone.

This study suggested that activities of xenobiotic drug metabolizing enzymes by repeated exposure to lead and endosulfan at the concentrations used in the study were not modified to produce significant toxicity in case of combined exposure.

Keywords: Lead, Endosulfan, Hepatic Drug Metabolizing Enzymes.

Introduction

Lead is a major human health hazard due to its wide distribution in the environment and in biological systems (Zhen et al., 2013). Orally administered lead acetate has been demonstrated to cause cancer in animals and NOAEL (No observed adverse effect level) for lead was reported to be 100 ppm (Azar et al., 1973). Endosulfan is a member of the cyclodiene group of organochlorine pesticides used worldwide in agriculture. It is used around the world for applications on vegetables, fruits, and non-food crops such as cotton and tobacco. This colourless solid has emerged as a highly controversial agrichemical due to its acute toxicity (Wade et al., 2002). A dose of 10 ppm of endosulfan has been tested to be non toxic dose (Banerjee and Hussain, 1987). Since multiple-chemical exposure is believed to

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represent a realistic picture of the human and animal chemical toxic burden, one chemical may modify the effect of the other by altering its kinetics and/or dynamics in a co-exposure situation. In view of the increased use of endosulfan for agroproduction and high levels of lead in the ground water and environment, coexistence of lead and endosulfan seems to be a reality and simultaneous exposure of human and animals to these chemicals could be potentially hazardous.

Cytochrome P450 (CYP) isoenzymes, the phase I drug -metabolizing enzymes, in tandem with the phase II drug-metabolizing enzymes catalyse the elimination of many xenobiotics including drugs. Therefore, agents that can modulate the levels of CYPs may have important implications not only for drug treatments and also for potential toxicity. Lead, in the form of lead acetate, is known to decrease hepatic microsomal enzymes and UDP glucuronyltransferase (Pillai et al., 2002) where as administration of endosulfan is known to increase liver microsomal cytochrome P 450 enzymes (Casabar, 2006). Human and animals may be exposed to lead and endosulfan concomitantly. The interaction resulting from the concurrent exposure of lead and endosulfan cannot be predicted to be less hazardous. Hence the present study was aimed to evaluate whether repeated co-exposure to lead at lower concentration level through drinking water and to dietary endosulfan at lower concentration could modify the effect produced by each compound on hepatic drug-metabolizing systems in male wistar rats.

Materials and Methods

Colony-bred adult male albino Wistar rats (70-90g; 4-5 weeks age) were procured from Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar. As per the Institute Animal Ethical Committee guidelines they were maintained under standard managemental conditions. Four groups of six rats were taken for the study. Rats of group I served as untreated control where as Group II received drinking water containing lead as lead acetate @100 ppm (Pb100). Group III was exposed to feed containing technical grade endosulfan @10 ppm (E10). Group IV was exposed to Pb (100) +E (10). All the treatments were given daily for 28 days. At the end of the exposure period, animals were sacrificed by cervical dislocation and the liver was removed for analyzing drug metabolizing enzymes. Rats were sacrificed on the 29^{th} day after recording the final body weight. Feed was withdrawn 12 h before sacrifice. Liver was weighed and preparation of hepatic microsomes and cytosolic fractions were carried out as per the method of Suresh Babu et al., 2006. The CYP450 was determined by Omura and Sato, 1964. Cytochrome b5 was estimated as per

Omura and Takesue, 1970. Aminopyrine N-demethylase (ANDM) activity was determined by the method of Mazel (1971) and aniline P-Hydroxylase (APH) activity was measured by the method of Mazel (1971). Glutathione S-transferase (GST) activity was measured by the method of Habig et al. (1974). Uridine diphosphate glucuronosyltransferase (UGT) activity was assessed by the method of Dutten and Storey (1962). Total protein content in the microsomal and cytosolic fractions of the liver was estimated by the method of Lowry et al. (1951), using bovine serum albumin as standard. Results have been expressed as mean ± SEM. The data were analyzed by ANOVA with Duncan's multiple comparisons (Snedecor and Cochran, 1989).

Results and Discussion

After 28 days, there were no significant changes in the body weights of rats given lower concentrations of lead, endosulfan and lead plus endosulfan in all groups taken for the study. The weights of the liver were not altered appreciably in the rats exposed to lower doses of lead and endosulfan alone or in combination. Groups exposed to lower doses of lead (Pb - 100) and endosulfan (E-10) alone did not produce significant changes in the levels of microsomal CYP and cytochrome b5 (Table 1). The lower dose combination showed significant decrease in the levels of microsomal CYP 450 (13.15%). Groups treated with lower dose combination showed cytochrome b5 contents comparable to control group. Groups exposed to lower doses of lead and endosulfan alone and their combination did not produce significant changes in the levels of ANDM, APH and UGT activity in rats (Table 1&2). Groups exposed to lower doses of lead and endosulfan alone did not produce significant changes in the levels of hepatic microsomal and cytosolic Glutathione -*S*-transferase (GST) activity as compared to control (Table 2). Other groups treated showed significant changes in the levels of enzymes as compared to control. Group treated with lower dose combination showed decreased levels of microsomal GST (31.29%) and cytosolic GST levels (31.29% and 67.57%). However, there was increase in the activities of cytosolic GST as compared to control. Similar effects were observed in the study of Groten *et al.* (1997) which showed adverse effects not only in the MOAEL (Minimum Observable Adverse Effect level) but also in some NOAEL groups. The changes in the levels of drug metabolizing enzymes by these chemicals may be attributed to the regulation of transcription factors in the cells which are controlled by ROS that has been produced by compound. The result in the present study could also be due to the higher total dose due to metal and pesticide in the mixture. The data does not provide a basis for predicting the response. This type of result is

useful, however, it demonstrates that subthreshold doses of the individual chemicals can, when administered in combination, result in a response, and suggests that assessment of exposure to each chemical separately may underestimate the effect of combined exposure (ATSDR, 2001).

In conclusion, it is suggested that the activities of xenobiotic drug metabolizing enzymes by repeated exposure to lead and endosulfan at the concentrations used in the study were not modified significantly to produce toxicity in case of combined exposure.

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Table 1: Effect of 28- day treatment with lead, endosulfan and their combination on hepatic microsomal cytochrome P450, cytochrome *b*5 contents (nmole /mg microsomal protein) aminopyrine N-demethylase (ANDM) (nmole formaldehyde formed/min /mg microsomal protein) and on aniline hydroxylase (APH) (nmole p-aminopyrine formed /min /mg microsomal protein) in rats

Groups	Cytochrome P450	Cytochrome b5	ANDM	APH
Control	0.715±0.051^{d}	0.058±0.003^{b}	7.554±0.33^{b}	0.649±0.037^{b}
Pb-100	0.721±0.031^{d}	0.059 ±0.005^{b}	7.685±0.61^{b}	0.628±0.026^{b}
E-10	0.726±0.031^{d}	0.061±0.007^{b}	7.877±0.21^{b}	0.639±0.042^{b}
Pb-100+E-10	0.621±0.053^{bc}	0.063±0.003^{bc}	7.644±0.32^{b}	0.620±0.022^{b}

Table 2: Effect of 28- day treatment with lead, endosulfan and their combination on microsomal, cytosolic glutathione-s-transferase (µM CDNB-GSH conjugate formed/ min/ mg protein) and UDP-glucuronosyltransferase (nmole/ min /mg microsomal protein) in rats

Groups	Microsomal GST	Cytosolic GST	UDP-glucuronosyl transferase
Control	0.163±0.004^{c}	08.48±0.18^{b}	3.492±0.81^{c}
Pb-100	0.160±0.003^{c}	07.29±0.36^{b}	3.241±0.23^{c}
E-10	0.167±0.006^{c}	07.12±0.41^{b}	3.338±0.51^{c}
Pb-100+E-10	0.112±0.004^{b}	14.21±1.32^{c}	3.015±0.12^{c}

Table 3: Effect of 28- day treatment with lead, endosulfan and their combination on microsomal and cytosolic protein (mg/g tissue) in rats

Groups	Microsomal protein	Cytosolic protein
Control	26.96±2.15^{c}	84.00± 2.11^{c}
Pb-100	28.41±3.11^{c}	88.00± 1.54^{c}
E-10	27.34±2.15^{c}	86.17± 3.11^{c}
Pb-100+E-10	20.18±4.20^{b}	74.40± 5.12^{b}

Pb-100 indicate lead 100 ppm and E-10 indicate endosulfan 10 ppm. Values (mean ± S.E.M., n=6) in the same column bearing no superscript common vary significantly ($P \leq 0.05$) in Duncan multiple comparison post hoc test.