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Effect of concurrent exposure of toxic concentrations of lead and endosulfan on oxidative stress indices in rats

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ABSTRACT

The effect of concurrent exposure of toxic concentrations of lead and endosulfan were evaluated on oxidative stress parameters in male wistar rats. Group I served as untreated control whereas Group II received drinking water containing lead as lead acetate @1000 ppm (Pb1000). Group III was exposed to feed containing technical grade endosulfan @ 100 ppm (E100). Group IV was exposed to Pb (1000) +E (100). All the treatments were given daily for 28 days. Combination of lead and endosulfan modified the indices of oxidative stress in the parameters such as lipid peroxidation, reduced glutathione, superoxide dismutase and catalase in rats as compared to their individual compounds. The results suggest that the combination of these individual compounds may have the potential to modify oxidative stress produced by single compounds in male rats.

Keywords: Lead, Endosulfan, Oxidative stress, Rats, Lipid peroxidation.

INTRODUCTION

Lead is a major human health hazard due to its wide distribution in the environment and in biological systems [1]. Several studies have demonstrated that lead exposure tend to increase oxidative stress parameters [2]. NOAEL (No observed adverse effect level) for lead was reported to be 100 ppm [3]. Organochlorines are the most commonly found pesticides in the environment [4]. Endosulfan is a member of the cyclodiene group of organochlorine pesticides used worldwide in agriculture. Endosulfan has been shown to cause oxidative stress [5]. A dose of 10 ppm of endosulfan has been tested to be NOAEL [6]. Higher doses (10x or more of NOAEL dose) of lead and endosulfan have been shown to produce oxidative stress in various studies. It has been reported that lead acetate induced oxidative stress and depleted hepatic GSH in rats treated with 2000 ppm for 4 weeks [7]. It was also reported that lead acetate caused significant decreases, both in liver and brain GSH levels and caused significant induction of lipid peroxidation in rats treated with 2000 ppm for weeks [8]. It was reported that there were significant decreases in the levels of GSH in liver, kidney and brain tissues when treated with endosulfan at lower (11 mg/kg body wt) and higher (22 mg/kg body wt) doses in rats [9]. It was also reported decrease in reduced GSH and increase in lipid peroxidation in kidney of rats that received endosulfan @ 10 mg/kg body wt [10].

Since multiple-chemical exposure is believed to 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 [11]. Assessments that take into account of combined actions of pollutants reflect better the existent impact of environmental exposures than the assessments that evaluate toxicity of single chemicals [12]. In view of the increased use of endosulfan for agroproduction and accumulation 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. Hence the present study was aimed to evaluate whether repeated co-exposure to lead through drinking water and to dietary endosulfan through feed at higher concentration levels (10x of NOAEL doses) could modify the effect produced by each compound on oxidative stress parameters in liver, kidney, brain and in erythrocytes 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 @1000 ppm (Pb100). Group III was exposed to feed containing technical grade endosulfan @ 100 ppm (E10). Group IV was exposed to Pb (1000) +E (100). All the treatments were given daily for 28 days. Rats were sacrificed on the 29th day after

recording the final body weight. About 200 mg of liver, kidney and brain samples were weighed and taken in 2 ml of ice-cold saline. 200 mg of sample was weighed separately and taken in 2 ml of 0.02 M EDTA for GSH estimation. The homogenates were centrifuged for 10 min at 875 g. The supernatant was used for estimation of following biochemical parameters.

The extent of lipid peroxidation (LPO) was evaluated in terms of MDA (malondialdehyde) production, determined by thiobarbituric acid (TBA) method [13]. Reduced glutathione was determined by estimating free-SH groups, using DTNB method [14]. Superoxide dismutase was estimated by the method of Madesh and Balasubramanian [15]. Catalase was estimated by the method of Bergmeyer [16]. Protein contents were determined by the method of Lowry [17]. Results were analyzed by ANOVA with Duncan's multiple comparisons [18].

RESULTS AND DISCUSSION

After 28 days, there were no significant changes in the body weights of rats given the higher concentrations of lead, endosulfan and lead plus endosulfan in all groups taken for the study. The results of oxidative stress parameters are depicted in Tables 1,2,3,4. Groups treated with lead and endosulfan has shown significantly increased MDA levels, decreased levels of reduced glutathione, decreased superoxide dismutase increased catalase activity and decreased levels of protein content as compared to control group ($P < 0.05$). There was an elevation in the levels of lipid peroxidation and reduction in reduced GSH in liver (61.89 % and 21.69 %), kidney (70.87 % and 28.23 %), brain (209.06 % and 37.33 %) and erythrocytes (145.45% and 21.56 %) of rats receiving higher concentration of endosulfan (100 ppm) as compared to control. Significant induction of MDA levels also observed in liver and kidney of Imidacloprid and Dichlorvos treated group [19]. In another study, MDA concentrations increased significantly in liver, kidney and brain and GSH levels decreased in liver and brain significantly in Fischer 344 rats that received 1100 ppm lead in drinking water for 6 weeks [20]. It was also observed an increase in MDA levels in erythrocytes and decrease in GSH levels in blood and brain of rats that received 2000 ppm of lead [21].

There were significant decreases in the levels of GSH in liver, kidney and brain tissues when treated with endosulfan at higher doses (33 mg/kg body wt) in rats [9]. The results were also consistent with another study which reported that there was significant increase in the levels of TBARS with single (30 mg/kg) and repeated doses of endosulfan (10 or 15 mg/kg/day) for 5 days in liver and brain of rats [22]. As compared to control, there was reduction in activities of SOD and elevation in the levels of catalase in liver (27.95% and 37.89%), kidney (27.40% and 28.39%), brain (20.22% and 80.83%) and erythrocytes (33.72% and 54.03%) in animals given higher concentration of endosulfan (100 ppm). Another study reported decreased SOD in erythrocytes by 21% and liver SOD by 12% in rats exposed to 1.9, 3.8, 7.6, 15.2 and 22.8 mg/250g body wt doses of endosulfan [23].

There was elevation in the levels of MDA and reduction in the levels of reduced GSH in liver (114% and 28%), kidney (113% and 20%), brain (344% and 16%) and erythrocytes (165% and 17%) of rats receiving higher concentration of lead (1000 ppm) as compared to control. Several studies have pointed to ROS generation, namely hydrogen peroxide and superoxide anion in lead toxicity [24, 25]. Lead is reported to alter antioxidant activities by inhibiting functional SH groups in several enzymes such as ALAD, SOD and catalase [26].

In the present study, as compared to control, there was elevation in the levels of catalase and reduction in the activities of SOD in liver (40.27% and 43.75%), kidney (31.04% and 45.37%), brain (52.91% and 41.38%) and erythrocytes (47.58% and 32.30%) in animals exposed to higher concentration of lead (1000 ppm). This may be attributable to the elevation of production of catalase in response to increase in MDA levels and could play a significant role in protecting cells. These results were also consistent with the study which also reported increase in catalase levels in rats received higher concentration (2000 ppm) of lead as lead acetate [7]. Reduction in the activity of SOD may be attributed to the utilization of SOD in response to increased production of ROS. The findings corroborated earlier reports of reduced activities of SOD in erythrocytes, liver, kidney and brain of lead-exposed rats [27, 28, 29].

In liver, combination of Pb (1000) +E (100) showed non significant antagonistic effect on MDA levels as compared to their single administration. In kidney, combination of Pb (1000) and E (100) also showed significant antagonistic effect on MDA levels as compared to Pb (1000) and E (100) when given alone. There was weak antagonistic effect on SOD activities in liver, kidney, brain and erythrocytes in animals treated with lead plus endosulfan in higher doses as compared to their single administration. Another study suggested that co-exposure of endosulfan and arsenic induced comparatively less oxidative stress than the expected additive effects induced by individual chemicals in chickens [30]. Higher dose combination showed synergistic effect in catalase activity in liver, kidney and brain as compared to their single administration. Most of the studies indicate that most of the metal mixtures frequently produce synergistic effects [31].

The results of the study indicate that oxidative stress related parameters such as lipid peroxidation, reduced glutathione and superoxide dismutase activity showed weak antagonistic effect and catalase activity showed synergistic effect in the higher dose combination as compared to their individual compounds This may be due to the activity of antioxidants to minimize further damage developed by individual dose ranges. It is suggested that combination of higher doses (10x of NOAEL) of lead and endosulfan may modify the activity of the individual compounds on oxidative parameters in male rats.

Table 1: Effect of 28- day treatment with lead, endosulfan and their combination on LPO (nmoles of MDA formed /g of wet tissue/ml erythrocytes) in organs and erythrocytes of rats (Mean ± S.E.M; n=6; $P \leq 0.05$)

Groups	Liver	Kidney	Brain	RBC
Control	13.28 ± 1.33^{a}	15.21 ± 0.64^{a}	07.06 ± 1.46^{a}	1.32 ± 0.21^{a}
Pb-1000	28.49 ± 0.89^{c}	32.40 ± 2.93^{d}	31.38 ± 0.82^{c}	3.51 ± 0.25^{b}
E-100	21.50 ± 3.84^{b}	25.99 ± 1.18^{c}	21.82 ± 0.89^{b}	3.24 ± 0.21^{b}
Pb-1000+E-100	20.60 ± 1.21^{b}	17.97 ± 1.10^{b}	21.88 ± 1.11^{b}	3.25 ± 0.25^{b}

Different superscripts in a column differ significantly ($P < 0.05$)

Table 2: Effect of 28- day treatment with lead, endosulfan and their combination on reduced glutathione levels (mM of GSH /g of wet tissue/ml erythrocytes) in organs and erythrocytes of rats(Mean ± S.E.M; n=6; P≤0.05)

Groups	Liver	Kidney	Brain	RBC
Control	0.106±0.001^{b}	0.085±0.004^{c}	0.075±0.006^{c}	0.51±0.04^{c}
Pb-1000	0.076±0.005^{a}	0.068±0.004^{b}	0.063±0.006^{b}	0.42±0.01^{a}
E-100	0.083±0.004^{a}	0.061±0.005^{a}	0.047±0.005^{a}	0.40±0.02^{a}
Pb-1000+E-100	0.080±0.007^{a}	0.065±0.004^{a}	0.053±0.007^{a}	0.46±0.04^{b}

Different superscripts in a column differ significantly (P < 0.05)

Table 3: Effect of 28- day treatment with lead, endosulfan and their combination on superoxide dismutase (U/mg of protein) in organs and erythrocytes of rats (Mean ± S.E.M; n=6; P≤0.05)

Groups	Liver	Kidney	Brain	RBC
Control	5.76±0.23^{c}	5.51±0.51^{c}	5.34±0.45^{c}	4.21±0.29^{c}
Pb-1000	3.24±0.41^{a}	3.01±0.23^{a}	3.13±0.41^{a}	2.85±0.21^{a}
E-100	4.15±0.23^{b}	4.00±0.25^{b}	4.26±0.41^{b}	2.79±0.27^{a}
Pb-1000+E-100	4.94±0.25^{bc}	4.66±0.31^{b}	4.73±0.32^{bc}	3.46±0.34^{b}

Different superscripts in a column differ significantly (P < 0.05)

Table 4: Effect of 28- day treatment with lead, endosulfan and their combination on catalase levels (mmol/min/ mg of protein) in organs and erythrocytes of rats (Mean ± S.E.M; n=6; P≤0.05)

Groups	Liver	Kidney	Brain	RBC
Control	211.12±15.1^{a}	186.33±14.5^{a}	113.20±12.3^{a}	124±15.0^{a}
Pb-1000	296.15±15.1^{b}	244.18±12.5^{b}	173.10±13.5^{b}	183±25.5^{b}
E-100	291.12±12.1^{b}	239.23±25.1^{b}	204.70±21.4^{c}	191±20.5^{b}
Pb-1000+E-100	346.30±14.1^{c}	293.08±12.6^{c}	264.00±11.5^{d}	188±17.6^{b}

Different superscripts in a column differ significantly (P < 0.05)

Table 5: Effect of 28- day treatment with lead, endosulfan and their combination on protein levels (mg/g of tissue) in organs of rats (Mean ± S.E.M; n=6; P≤0.05)

Groups	Liver	Kidney	Brain
Control	143.20±3.19^{b}	89.12±3.10^{b}	63.25±1.25^{b}
Pb-1000	081.45±3.17^{a}	66.20±2.55^{a}	45.12±3.10^{a}
E-100	084.00±5.10^{a}	69.15±3.10^{a}	43.44±3.41^{a}
Pb-1000+E-100	084.12±3.25^{a}	64.71±4.12^{a}	40.84±2.15^{a}

Different superscripts in a column differ significantly (P < 0.05)

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