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LIPID PEROXIDATIVE DAMAGE AND ALTERATIONS IN ANTIOXIDANT STATUS IN RAT ERYTHROCYTES ON REPEATED EXPOSURE OF λ-CYHALOTHRIN AND FLUORIDE ALONE AND IN COMBINATION

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ABSTRACT

Accidental exposure or injudicious application of pesticides and toxic metals cause adverse health consequence in non-target species including human and animals. The present study was aimed to investigate the lipid peroxidative damage and alterations in antioxidant status in rat erythrocytes on repeated exposure of λ-cyhalothrin (LCT) and fluoride (F) alone and in combination. Forty two wistar were divided into seven groups with six rats in each. Group I without treatment served as control. Group II and III were provided drinking water containing F at the rate of 1 and 10 ppm, whereas rats of Group IV and V were administered LCT at the dose rate of 2 and 10 mg/kg through oral gavage respectively. The animals of group VI and VII received both toxicants daily for 28 days. Repeated exposure of either LCT or F produced significant ($p<0.05$) reduction in activities of superoxide dismutase, catalase, glutathione peroxidase in erythrocyte membrane and blood glutathione level in rats as compared to control. Concurrent exposure of both the toxicants produces more pronounced effects as indicated by increased lipid peroxidative damage and decreased antioxidant status in wistar rats as compared to control and alone exposed toxicants. Observations suggests that use of LCT in areas having high fluoride content in ground water should be restricted to minimize subtle effect in non-target species.

KEYWORDS: λ-cyhalothrin; Fluoride; Antioxidant parameters; Free radicals.

INTRODUCTION

λ-cyhalothrin [α-cyano-3- phenoxybenzyl - 3- (2-chloro-3, 3, 3-trifluoro - 1- propenyl)-2, 2-dimethylcyclopropanecarboxylate] a new synthetic type II pyrethroid used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control [1]. Pyrethroids are reported to generate free radicals through hydrolytic ester cleavage and oxidative pathways by the CYP-450 enzymes [2-3]. Alteration in antioxidant status and lipid peroxidative damage has been reported with pyrethroids such as cypermethrin, deltamethrin, fipronil and fenvalerate [4-7]. These pyrethroids on metabolism produce excess intermediate free radicals which are primarily responsible for the stress induced damage to visceral organs in experimental animals [8-11]. Erythrocyte membrane is most vulnerable to free radical induced damage due to excess aerobic reaction in mitochondria and high level of hemoglobin.

Exposure to toxic metals and metalloids remains a widespread problem. In many parts of the world, toxic effects of fluoride (F) causing skeletal and dental fluorosis are a major environmental concern in human and animal health [8]. Various experimental studies suggested that antioxidant status and lipid peroxidative damage induced by repeated exposure of pyrethroids viz. cypermethrin, deltamethrin, etc were increased in presence of high level of F in drinking water [8-11]. In view of the tremendous increase in the use of λ-cyhalothrin (LCT) and ever increasing F level in drinking water, concurrent exposure of human and animals to these chemicals is a reality. Very few studies have attempted to assess the degree of hazard posed by concurrent exposure to two or more chemicals used in low doses [8, 10]. Therefore, the present study was aimed to investigate the lipid peroxidative damage and alterations in antioxidant status in rat erythrocytes on repeated exposure of LCT and F alone and in combination.

MATERIALS AND METHODS

Experimental Animals

The effects of LCT and F alone and in combination were observed on healthy wistar rats of either sex weighing 100 to 200 g procured from Indian Institute of Integrative Medicine, Jammu, India. The animals were provided standard pelleted ration and clean drinking water *ad-libitum.* All the animals were maintained under standard managemental conditions. A daily cycle of 12 h of light and 12 h of darkness was provided to animals. Prior to start of experiment, the rats were acclimatized in the laboratory conditions for a period of more than

3 weeks. All the experimental animals were kept under constant observation during entire period of study.

Experimental Design

After acclimatization forty two wistar rats were randomly divided into seven groups with six rats in each. Animals in the Group I served as control and received only normal tap water for drinking purposes. The animals of Group II and III were provided drinking water containing F (as NaF) 1 and 10 ppm respectively, whereas rats in Group IV and V were administered LCT at the dose rate of 2 and 10 mg/kg b.wt. through oral gavage. The animals of group VI were provided water containing both F (1ppm) and LCT (2mg/kg, b.wt.) through oral gavage whereas animals of group VII received both F (10ppm) in drinking water and LCT (10mg/kg, b.wt.) daily for 28 days. In order to minimize the possible instability, both toxicants were prepared freshly in tap water. All the rats were weighed weekly to make necessary corrections in the LCT dosage as per b.wt. The experimental protocol was approved by the Institutional Animal Ethical Committee.

Sample Collection and Analysis

After 28 days of daily treatment blood samples were collected from retro-orbital fossa using capillary tube in aliquots containing heparin as anticoagulant (10 IU/ml of blood). Prior to centrifugation, 200µl whole blood was used for the estimation of blood glutathione (GSH) [12]. One per cent hemolysate was prepared and used for the estimation of catalase (CAT) superoxide-dismutase (SOD) and glutathione-peroxidase (GPx) [13-15] whereas, 33 per cent of hemolysate was used for estimation of malondialdehyde (MDA) levels [16]. The results were subjected to analysis of variance (ANOVA) in completely randomized design (CRD) with statistical significance being tested using the Duncan Multiple Range Test [17].

RESULTS

The exposure of either F or LCT alone or in-combination induced lipid peroxidation of erythrocyte membrane as indicated by significant ($p<0.05$) increase in MDA levels at low and high doses as compared to control. The per cent increase in MDA level on F treatment was 57.96 % at 1 ppm and 75.66 % at 10 ppm whereas LCT treatment increased 64.63 and 92.47 % at the dose of 2mg/kg and 10 mg/kg respectively. The per cent increase in MDA levels were 84.07 % and 126.54 % in concurrent exposed toxicants with low and high dose groups respectively (figure 1).

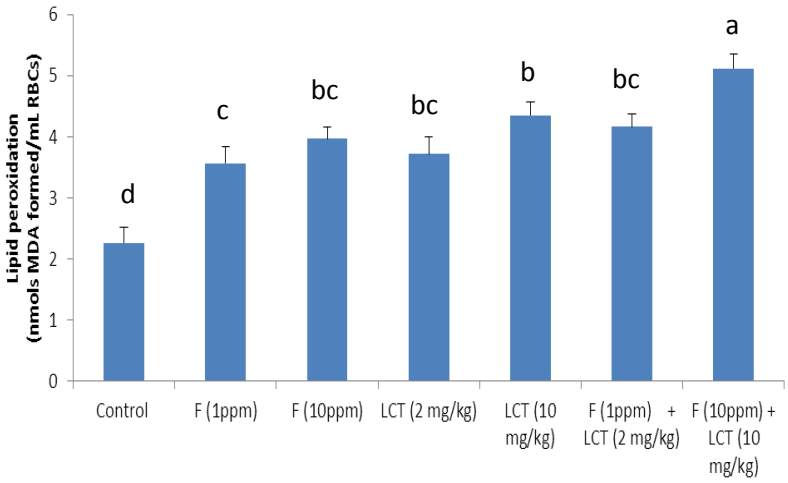

Figure 1: Effect of repeated exposure of different doses of fluoride (F) and λ-cyhalothrin (LCT) alone and in combination on lipid peroxidation (MDA level) in erythrocyte membrane of wistar rats.

Table 1 depicted the alteration in the activities of GPx, CAT, SOD and blood GSH level have been determined to assess the antioxidant status of rat erythrocytes on repeated exposure of LCT and F alone and in combination. Non-significant decrease of GSH level was observed in animals administered with F alone at 1ppm as compared to control animals. However, a significant ($p<0.05$) decline in GSH level was found in animals administered with F alone at high dose or LCT alone either at low and high doses groups. Significant ($p<0.05$) decline in GSH level was also observed in groups exposure to both F and LCT at low as well as high doses for 28 days. Significant ($p<0.05$) decrease in SOD activity was observed in all groups exposed to toxicants except the group exposed to lower dose of F alone, which shows non-significant decrease of erythrocyte SOD activity. Maximum reduction in activity was observed in concurrent exposed group i.e. in group VII. Similarly, the activity of GPx was significantly ($p<0.05$) decreased in rats treated with either repeated exposure of LCT or F alone at high doses. Such decrease was also observed when F was administered in combination with LCT at low and high doses as compared to control animals. Whereas, a non-significant decline in activity of GPx was observed in rats exposed orally with F or LCT alone at low dose. CAT activities were significantly lower in toxicant exposed at higher doses i.e. in group III and IV whereas at lower doses of toxicants activity reduced non-significantly. Concurrent exposure of F and LCT reduced significantly ($p<0.05$) CAT activity as compared to control and reduced non-significantly as compared to toxicant exposed groups.

Table 1. Effect Of Repeated Oral Administration of F And Lct Alone And In Combination At Different Dose Levels on Various Antioxidant Parameters In Blood of Rats.

Activity/Groups	Control	F (1 ppm)	F (10 ppm)	LCT (2 mg/kg)	LCT (10 mg/kg)	F (1ppm) + LCT(2mg/kg)	F (10ppm) + LCT (10mg/kg)
GSH (nmol/ml)	69.01^{e} ± 1.13	66.37^{e} ± 2.20	61.14^{d} ± 0.58	58.05^{cd} ± 1.13	52.05^{b} ± 0.84	55.12^{b} ± 1.14	47.28^{a} ± 1.26
SOD (Units/mg Hb)	78.13^{a} ± 7.24	70.41^{ab} ± 5.83	61.28^{bc} ± 6.01	48.70^{cd} ± 4.28	43.15^{de} ± 3.89	31.87^{ef} ± 1.47	27.35^{f} ± 1.89
GPx (U/mg Hb)	8.82^{a} ± 0.23	8.65^{ab} ± 0.25	7.96^{d} ± 0.24	8.58^{abc} ± 0.21	7.76^{cd} ± 0.18	8.10^{bcd} ± 0.19	6.64^{e} ± 0.18
CAT (µmol H_2O_2 utilized/min/mg Hb)	106.27^{a} ± 6.70	82.19^{ab} ± 10.12	77.53^{b} ± 7.75	81.52^{ab} ± 9.85	69.81^{b} ± 9.93	68.79^{b} ± 10.27	52.75^{b} ± 5.97

Values are mean ± SEM (n=6)
Means with at least one common superscript do not differ significantly ($p<0.05$).

DISCUSSION

Free radicals or reactive oxygen species (ROS) are the byproduct of oxidative reactions, which are necessary for maintaining cellular activities. Exposure of LCT or F increases the oxidative reactions leading to excess formation of ROS or reactive intermediate compounds during metabolism. Though, mammalian cells are endowed with extensive antioxidant defense mechanisms which counteract the damaging effects of toxic ROS/free radicals [18-21]. But excessive production of these radicals during increasing dose of toxicants produces deleterious effects on different organs by attacking on -SH group of enzymatic and structural protein or polyunsaturated fatty acid of lipid membrane leading to peroxidation of lipids [7, 22]. Superoxide radicals are produced in mitochondria and endoplasmic reticulum as a consequence of auto-oxidation of electron transport chain components. The superoxide radicals are also produced during monovalent reduction of oxygen molecules in living tissues. SOD is the first line of defense for scavenging superoxide and peroxide radicals to H_2O_2 and H_2O. Decreased SOD activity in present study is suggestive of excess free radical generation or interference of reactive metabolite to SOD activities, which impairs natural defense mechanism. Accumulation of superoxide in medium is toxic to biological systems by denaturing cellular proteins and by breaking DNA strands and thereby disrupting cellular functions [22-23]. Decreased activity of SOD has also been reported from exposure of different pyrethroids and F [7, 8, 24-25]. CAT catalyzes the hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2). The enzyme is found in all aerobic eukaryotes and is important in

the removal of H_2O_2 generated in peroxisomes involved in ß-oxidation of fatty acids, the glyoxylate cycle (photo-respiration) and purine catabolism. Stress conditions in which there is an excessive free radicals generation results in the depletion in CAT activity leading to accumulation of peroxide radicals [22, 26]. The decrease in the CAT activity has also been reported in rats co-exposed with F along with deltamethrin [8]. Similar decrease in CAT activity has been reported following exposure of rats to cypermethrin [7], deltamethrin [8] and bifenthrin [27]. Reduced glutathione, a major endogenous antioxidant is counter balances by providing –SH group to free radicals or RSO thus decreasing the free radicals mediated damage by inactivating or eliminating them [22]. Significant reduction in the GSH level by toxicants exposure is either due to increase utilization or decrease production of GSH [28]. As the activity of GPx is dependent upon the level of GSH, depleted GSH, concomitant with increased utilization of GPx to detoxify the toxicant induced free radicals and H_2O_2 production, could result in the significant decrease of GPx in toxicants exposed groups especially the concurrent exposed groups. Similar decrease in GPx activities has also been reported in rats exposed to deltamethrin, cypermethrin and F [7-8, 25, 27]. Significant decreased in activities of SOD, CAT, GPx and GSH level reducing the natural antioxidant defense of mammalian body. Accumulation of free radicals and ROS in cellular milieu due to reduced antioxidant system leading to free radical induced damage to cellular and sub-cellular components. Increased MDA level results from one of the most frequent reactions caused by free radical attack on biological structures as reflected in disturbance of the oxidant/antioxidant balance in the biological system [5, 29-32]. In present study increasing dose of both toxicants increases MDA level indicate more lipid peroxidative activities which may be due to excessive production of free radicals/ROS during metabolism of toxicants. Similarly increased MDA level has also been reported from concurrent exposure of F and pesticide in experimental studies [7, 8, 25, 27].

CONCLUSION

Balance between antioxidant and oxidant status is required for proper functioning of antioxidant system in mammals. In the present study decreased activities of SOD, CAT and GPx as well as the GSH level suggested that exposure of either F or LCT altered the antioxidant defense leading to increased radicals induced damage in erythrocyte membrane as indicated by increased MDA level. Further, concurrent exposure of F and LCT potentiated lipid peroxidative damage and alteration in antioxidant status in rat erythrocytes.

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Conflict of interest: Nil

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