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Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species

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Abstract

Pyrethroid pesticides are used preferably over organochlorines and organophosphates due to their high effectiveness, low toxicity to non-target organisms and easy biodegradibility. However, it is possible that during the pyrethroid metabolism, there is generation of reactive oxygen species (ROS) and pyrethroids may produce oxidative stress in intoxicated rats. The present study was therefore, undertaken to determine pyrethroid-induced lipid peroxidation (LPO) and to show whether pyrethroid intoxication alters the antioxidant system in erythrocytes. A single dose of cypermethrin and/or fenvalerate (0.001% LD_{50}) was administered orally to rats and the animals were sacrificed at 0, 1, 3, 7 and 14 days of treatment. The results showed that lipid peroxidation (LPO) in erythrocytes increased within 3 days of pyrethroid treatment. The increased oxidative stress resulted in an increase in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). The increase in reduced glutathione (GSH) content in erythrocytes may probably be an initial adaptive response to increased oxidative stress in pyrethroid intoxicated rats. Erythrocytes and serum acetylcholinesterase (AChE) activity was measured in pyrethroid-induced oxidative stress as it may mimic inhibition in target tissues such as muscle and brain. The inhibition in erythrocytes and serum AChE activity was partially relieved over a period of time indicating recovery from pyrethroid intoxication. The increase in erythrocyte LPO correlated with the inhibition in erythrocyte AChE activity and so erythrocyte AChE can be a marker enzyme in pyrethroid toxicity. The results show oxidative stress and alteration in antioxidant enzymes in erythrocytes of pyrethroid intoxicated rats.

Keywords: Lipid peroxidation; Superoxide dismutase; Catalase; Glutathione; Acetylcholinesterase

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1. Introduction

Synthetic pyrethroids, have emerged as a new class of agricultural pesticides and have found wide use over organochlorine and organophosphate pesticides. Pyrethroid pesticides (cypermethrin and fenvalerate) show high toxicity to a wide range of insects, including resistant strains (Elliot et al., 1978) low toxicity to mammals and birds (Parker et al., 1984) and rapid biodegradibility (Leahey, 1979). However, the liberal use of pyrethroids increases the risk of intoxication to non-target organisms such as birds, animals and organisms present in soil and water. Though pyrethroids have been shown to be rapidly metabolised in mammals, their role in producing oxidative stress has not been examined. The involvement of reactive oxygen species (ROS) have been implicated in the toxicology of organochlorine (Bagchi et al., 1992, 1993) and organophosphate (Yang et al., 1996; Yang and Dettbarn, 1996) pesticides, however, there are no reports which indicate the role of ROS in pyrethroid toxicity. Recent studies by Kar and his coworkers showed thyroid dysfunction and increase in liver and kidney lipid peroxidation (LPO) due to generation of free radicals in fenvalerate treated mice (Maiti et al., 1995). The authors suggested that

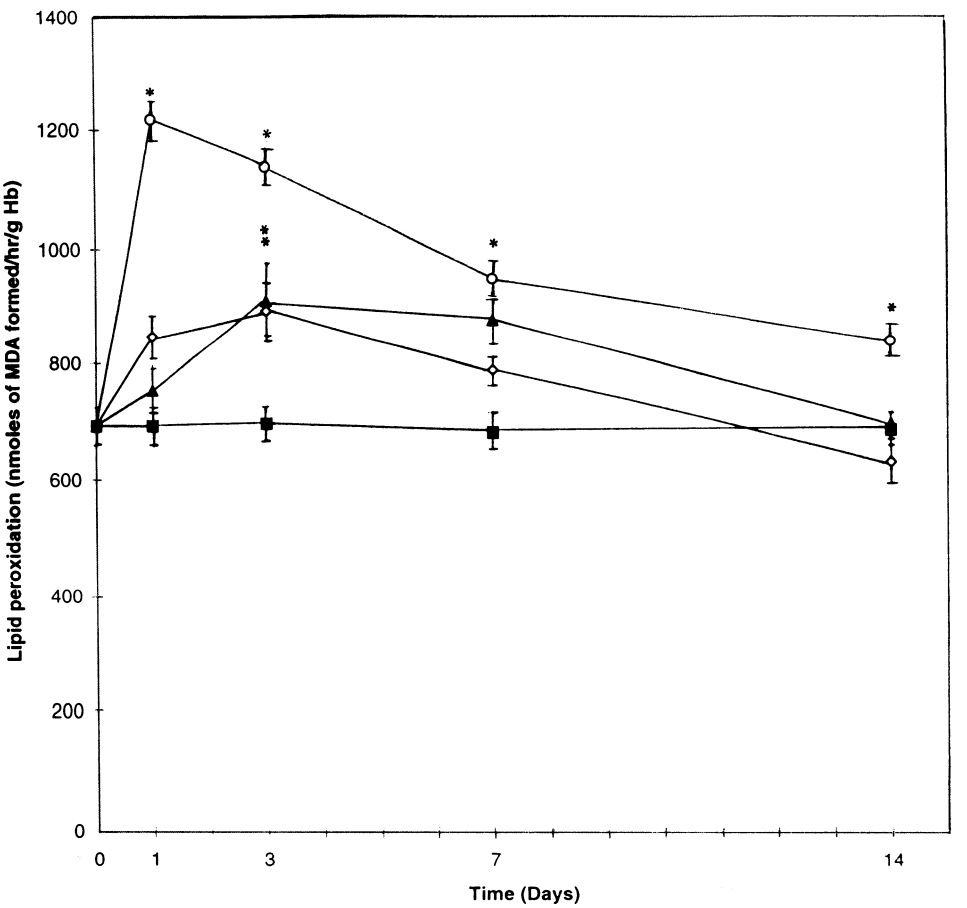

Fig. 1. Lipid peroxidation in erythrocytes. Rats were treated with a single dose of cypermethrin (◇), and fenvalerate (▲) or both the pesticides (○). The animals were followed at varied time intervals up to day 14. The animals treated with vehicle served as control (■). Values are mean ± SEM of six animals in treated and three animals in control. * $P < 0.001$ significantly different from control at each time interval by ANOVA and Student's t-test.

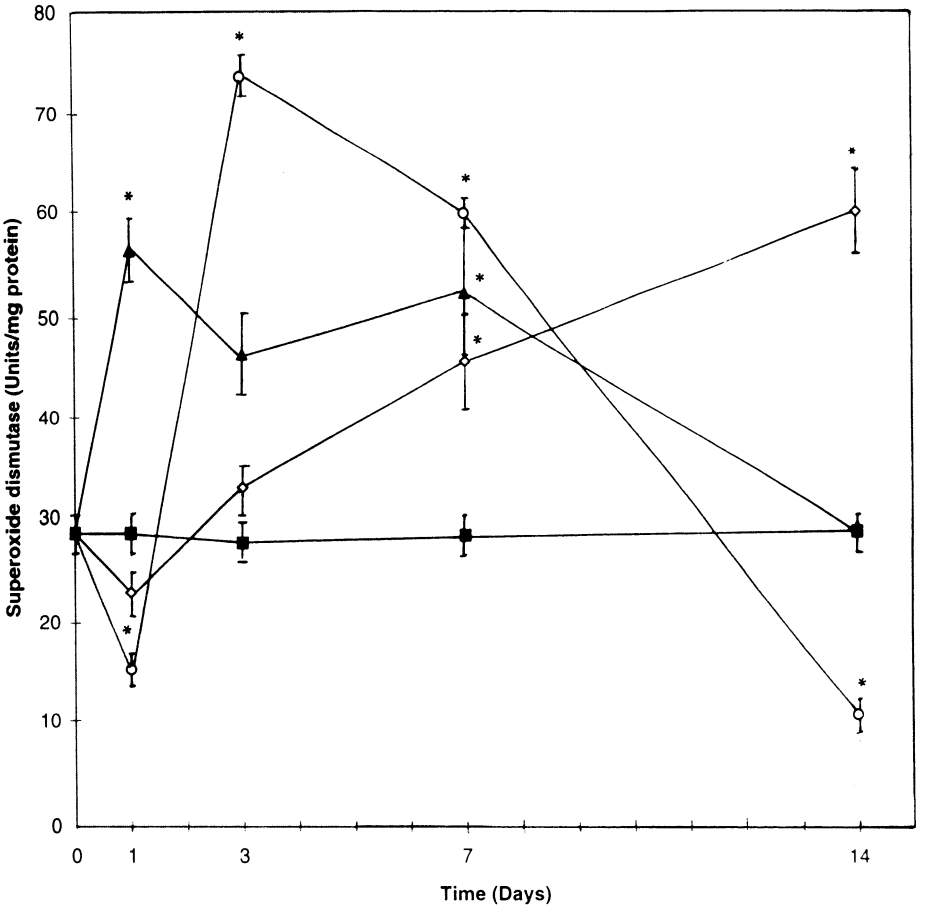

Fig. 2. Superoxide dismutase activity in erythrocytes. Other details are as shown in legend to Fig. 1.

testosterone could ameliorate fenvalerate induced toxicity as indicated by decreased hepatic LPO with increased activity of superoxide dismutase (SOD) and catalase (CAT) in intoxicated mice (Maiti and Kar, 1997). The cleavage of cypermethrin and fenvalerate and its ester metabolites release cyanohydrins which are unstable under physiological conditions and decompose to cyanides and aldehydes (World Health Organisation, 1990). ROS such as superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($^{\bullet}OH$) and H_2O_2 enhance oxidative process and produce lipid peroxidative damage to cell membranes. The $^{\bullet}OH$ radical has been proposed as an initiator of LPO through an iron-catalysed Fenton reaction (Halliwell and Gutteridge, 1986, 1989). The erythrocytes may be susceptible to oxidative damage due to the presence of polyunsaturated fatty acids (PUFA), heme iron and oxygen which may produce oxidative changes in red cells. It is probable that pyrethroids transported through blood to the liver for metabolism may produce cellular damage to erythrocytes. LPO, a consequence of cellular injury (Spiteller, 1996) was studied in pyrethroid intoxicated rats as an index of oxidative stress. The antioxidant enzymes in erythrocytes such as SOD which dismutate $O_2^{\bullet -}$ and CAT which decompose H_2O_2, may counteract pyrethroid-induced oxidative stress. Other antioxidants such as glutathione (GSH) required to reduce H_2O_2 via glutathione peroxidase may also have an important function in mitigating the toxic effects of

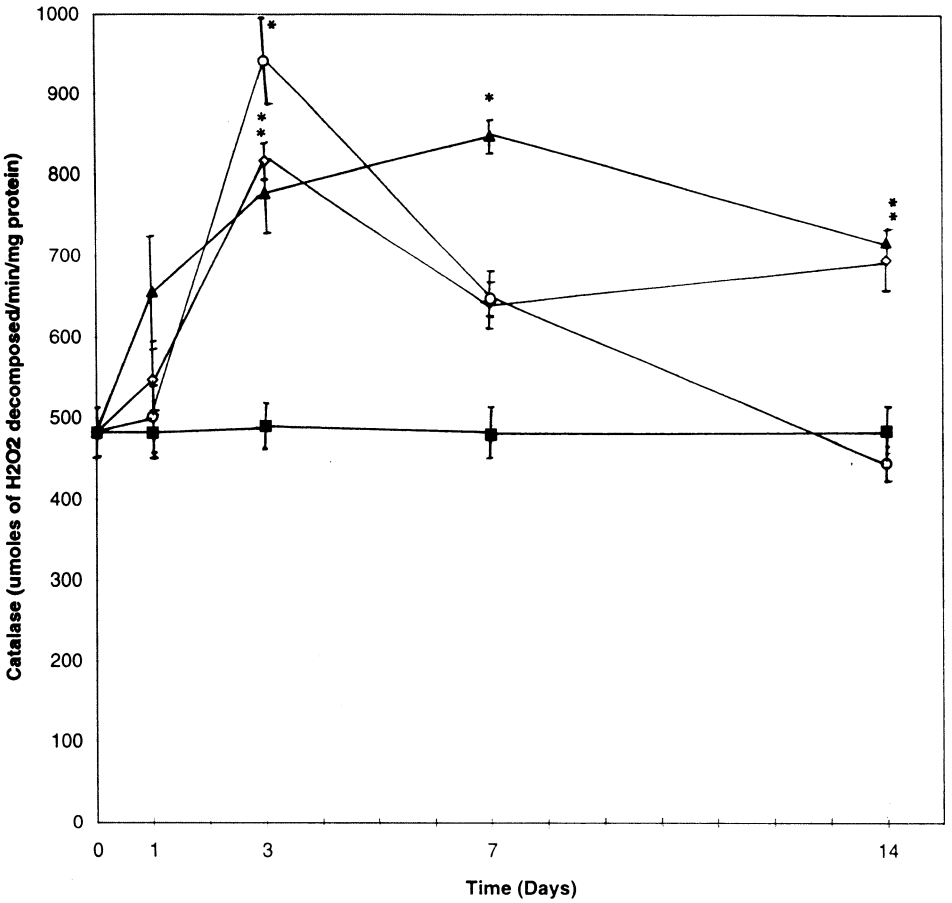

Fig. 3. Catalase activity in erythrocytes. Other details are as shown in legend to Fig. 1.

ROS. Therefore, in the present study, the rats were administered orally, cypermethrin and/or fenvalerate at a low dose to determine its effect on LPO and antioxidant system in erythrocytes.

2. Materials and methods

Male Wistar rats weighing 150–180 g were housed in polypropylene cages under standard conditions with free access to drinking water and basal diet. The animals were orally administered 0.2 ml cypermethrin and/or fenvalerate (0.001% LD_{50}) as a single dose in 1 ml of groundnut oil. LD_{50}'s for cypermethrin and fenvalerate are 2500 and 450 mg/kg body wt, respectively. The animals were sacrificed at 0, 1, 3, 7 and 14 days after pesticide treatment. Blood was collected by cardiac puncture in vials containing citrate (2%). The blood was centrifuged and the erythrocytes were washed twice with 0.1 M phosphate buffered saline (PBS, 1:9), pH 7.4. Erythrocyte lysate was prepared according to the method of McCord and Fridovich (1969) for the assay of antioxidant enzymes.

2.1. Lipid peroxidation in erythrocytes

LPO in erythrocytes was estimated by the thiobarbituric acid (TBA) reaction with malonyldialdehyde (MDA), a product formed due to peroxidation of lipids by the method of Stocks and Dormandy (1971).

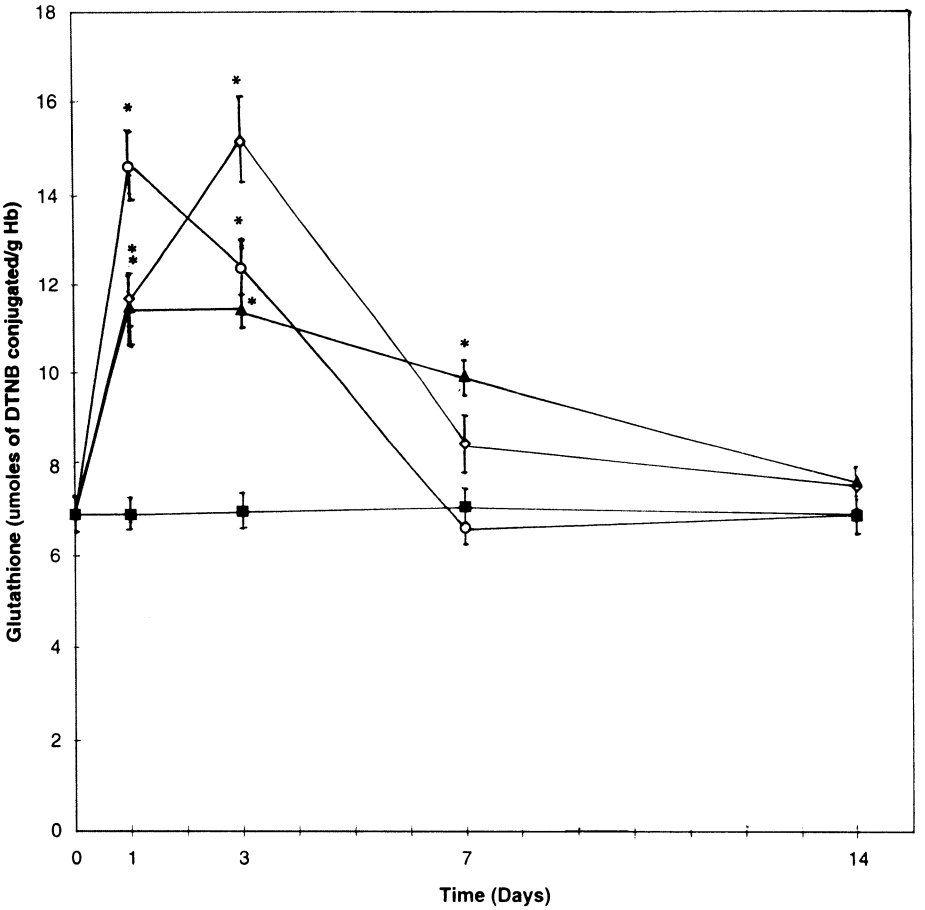

Fig. 4. Glutathione content in erythrocytes. Other details are as shown in legend to Fig. 1.

2.2. Antioxidant enzymes in erythrocytes

SOD activity was determined by the ability of the enzyme to inhibit the autoxidation of pyrogallol by the method of Marklund and Marklund (1974). Catalase was assayed by the decomposition of hydrogen peroxide by the method of Aebi (1983).

2.3. Glutathione content in erythrocytes

Glutathione content was assayed using Ellman's reagent by the method of Beutler et al. (1963).

2.4. Acetylcholinesterase activity in erythrocytes and serum

Acetylcholinesterase (AChE) was assayed by the method of de la Huerga et al., as described by Varley (1969) using acetylcholine chloride as substrate.

Haemoglobin in blood was estimated using Drabkin's reagent by the method of Dacie and Lewis (1984). The protein content was determined by the method of Lowry et al. (1951).

2.5. Statistical analysis

The significance was calculated using one-way analysis of variance (ANOVA) and Student's

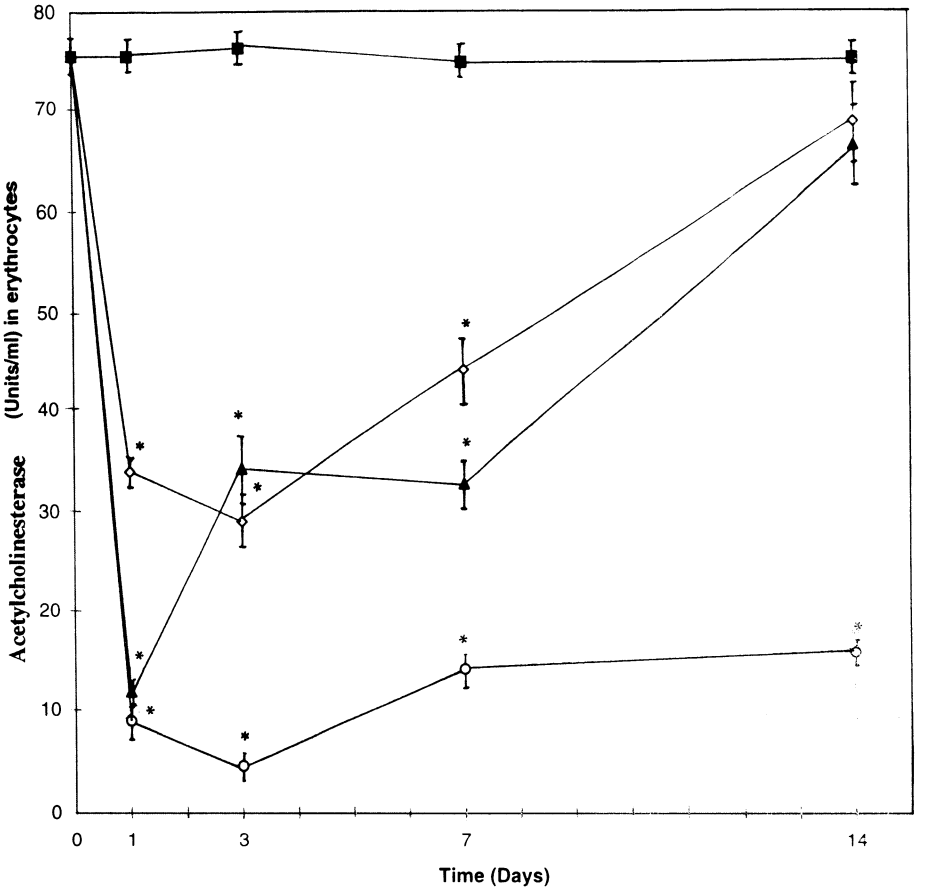

Fig. 5. Acetylcholinesterase activity in erythrocytes. Other details are as shown in legend to Fig. 1.

t-test. The values were ascribed significant at $P < 0.001$.

3. Results

3.1. Lipid peroxidation in erythrocytes

Treatment with cypermethrin as well as fenvalerate increased LPO in erythrocytes by day 3 of treatment (Fig. 1). However, LPO in erythrocytes decreased towards control by day 14 of pesticide treatment. A combination of pesticides, when administered together, showed higher LPO in erythrocytes during 1–14 days of treatment.

3.2. Superoxide dismutase activity in erythrocytes

Treatment with a single dose of cypermethrin showed increase in erythrocyte SOD activity up to day 14 of pesticide treatment (Fig. 2). Treatment with fenvalerate also showed an increase in erythrocyte SOD activity up to day 7 of pesticide treatment with the gradual decrease in erythrocyte SOD activity by the day 14 of treatment. Treatment with both the pesticides together increased SOD activity within 3 days of treatment followed by a decrease towards control by day 14 of pesticide treatment.

3.3. Catalase activity in erythrocytes

CAT activity in erythrocytes increased by day 3 of cypermethrin and/or fenvalerate treatment, fol-

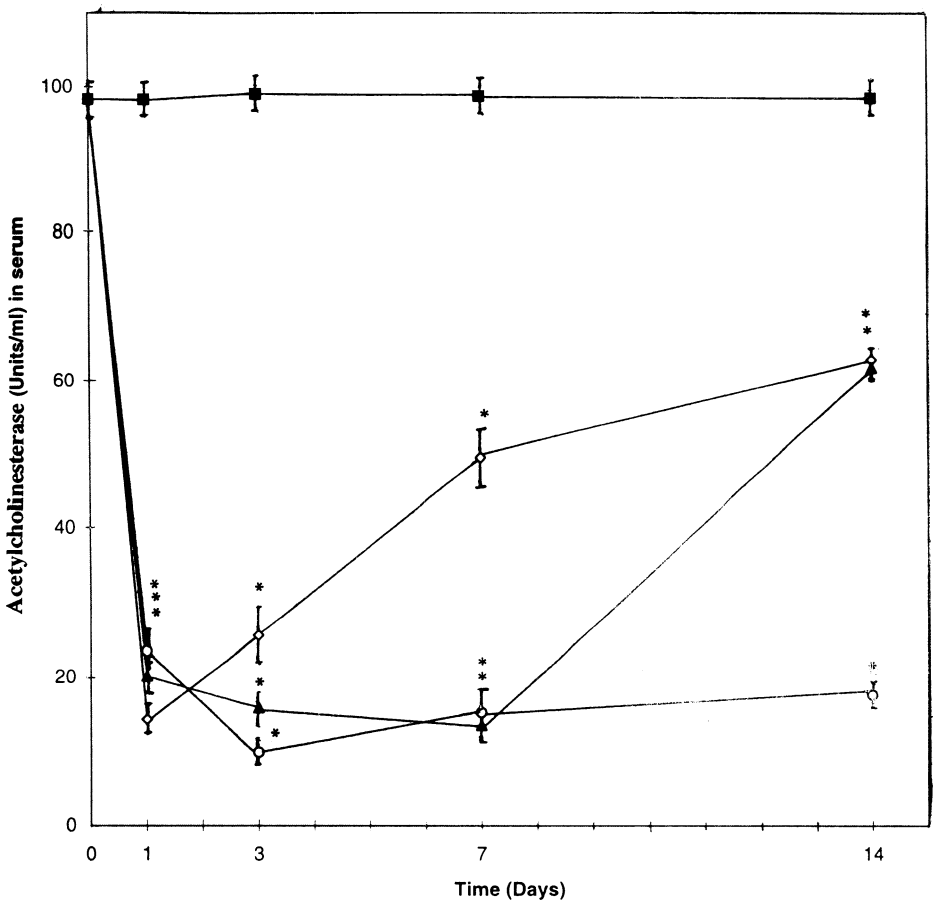

Fig. 6. Acetylcholinesterase activity in serum. Other details are as shown in legend to Fig. 1.

lowed by a decrease in erythrocyte CAT activity by day 14 of treatment (Fig. 3).

3.4. Glutathione content in erythrocytes

The treatment with cypermethrin and/or fenvalerate showed an increased GSH content in erythrocytes within day 3, followed by a decrease towards control by day 14 of treatment (Fig. 4).

3.5. Acetylcholinesterase activity in erythrocytes and serum

The erythrocytes and serum AChE activities were markedly inhibited by day 1 of pesticide treatment followed by a recovery in AChE activity towards control by day 14 of treatment (Figs. 5 and 6). However, treatment with both the pesticides showed inhibited AChE activity in erythrocytes and serum with very low recovery in AChE activity over the period of treatment. A significant correlation between erythrocyte LPO and erythrocyte AChE activity was observed ($r = -0.97$, -0.57, -0.80 with cypermethrin, fenvalerate or treatment with both the pesticides. r value > -0.70 show significance at $P < 0.05$).

4. Discussion

The treatment with cypermethrin and/or fenvalerate showed increased LPO in erythrocytes. The effect on LPO was more pronounced when both the pesticides were administered. The result

show that cypermethrin and/or fenvalerate induce oxidative stress in erythrocytes. The generation of $O_2^{\bullet -}$, $^{\bullet}OH$ radicals and H_2O_2 in pyrethroid toxicity result in increased LPO. Fenvalerate and cypermethrin as well as their esters, form cyanohydrins, which decompose to cyanides and aldehydes. Cyanide ions are mainly converted to thiocyanate and CO_2. The major metabolic reactions are ester cleavage and hydroxylation at the 4-positon and formation of a lipophilic conjugate, 2[R]-2-(4-chlorophenyl) isovalerate. This conjugate have been detected in adrenals, liver and mesentric lymph nodes in rats, mice and some other species (World Health Organisation, 1990). The aldehydes and other lipophilic conjugates may produce oxidative stress in pyrethroid toxicity.

The increase in SOD and CAT activities in erythrocytes after pyrethroid intoxication appears to be due to increased generation of ROS. Both, SOD and CAT activities decreased oxidative stress by dismutation of $O_2^{\bullet -}$ and decomposition of H_2O_2. The gradual decrease in erythrocyte SOD and CAT activities over a period of time show a decrease in ROS generation due to metabolism and excretion of pyrethroids. However, the increase in GSH content in erythrocytes may be an adaptive response to neutralise pyrethroid induced oxidative stress. GSH provide the –SH group for conjugation by glutathione-*S*-transferase (GST), which detoxifies a variety of electrophilic compounds to less toxic forms. It is probable that the initial increase in erythrocyte GSH provide GSH for GST activity to decrease pyrethroid toxicity.

In the present study, the erythrocyte and serum AChE activity was markedly inhibited after day 1 of cypermethrin and/or fenvalerate treatment. The AChE activity in erythrocytes and serum recovered by day 14 of cypermethrin or fenvalerate treatment. However, when both the pesticides were administered together the recovery in erythrocyte and serum AChE activity was less than 20% at day 14 of treatment and the AChE activity remain depressed for a longer period. In diisopropylphosphorofluoridate (DFP) intoxicated rats the erythrocyte AChE activity has been shown to remain low for the duration of red cell life probably due to irreversible nature of AChE inhibitor and the AChE activity fully regenerates when there has been a full turnover and replacement of red cells (Yang and Dettbarn, 1996). However, serum AChE activity may return to normal within few weeks because it is rapidly replaced by new enzymes synthesized in the liver (Murphy, 1986). The inhibition in AChE activity in target tissues is often followed as a measure of pesticide intoxication (Jayaratnam and Maroni, 1994). In many of the earlier studies, correlation between blood AChE inhibition and inhibition in target tissues has been shown (Yang and Dettbarn, 1996; Su et al., 1971; Koshakji et al., 1973). The present result also show a significant correlation between increase in erythrocyte LPO and inhibition in AChE activity in erythrocytes. The data indicate that the toxic effect of pyrethroids on AChE activity in erythrocytes and serum may exist upto day 14 of intoxication, however, the oxidative stress in erythrocytes may considerably reduce during this period. The enhancement of erythrocyte LPO and alteration in antioxidant enzymes suggest of involvement of free radical intermediates in pyrethroid toxicity.

Acknowledgements

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