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Full Length Research paper

Ameliorative effects of α-tocopherol on cypermethrin induced oxidative stress and lipid peroxidation in Wistar rats

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Environmental contamination of pyrethroids is responsible for increasing oxidative stress in man and animals. The present study was aimed to investigate the ameliorative effects of α-tocopherol on different stress parameters in rats. Significant ($p<0.05$) increased catalase activity in cypermethrin treated rats is maintained normal by the supplementation of α-tocopherol. Significantly reduced ($p<0.05$) SODs and GSH-Px activity by cypermethrin treated rats and activity of these enzymes were comparable to control animals by α-tocopherol supplementation. Significantly reduced GSH and increased ($p<0.05$) lipid peroxidation were observed in both groups, cypermethrin alone and along with the α-tocopherol treated groups. Observations from the present study suggest that α-tocopherol supplementation plays a protective role in cypermethrin induced oxidative stress in rats and linseed oil is not suitable as a vehicle for α-tocopherol.

Key words: Cypermethrin, oxidative stress, α-tocopherol, Wistar rats.

INTRODUCTION

Synthetic pyrethroid, cypermethrin is used extensively for the management of pests in crops and as an ectoparasiticide in man and animals (Taplin and Meinking, 1990). Environmental contamination and increased concentrations in different food products, therapeutic application and accidental/occupational exposure to pyrethroids are responsible for increasing oxidative stress in mammals (Daniel and Moser, 1993; Yousef et al., 2006). Pyrethroids delay the Na^+ channel closure which leads to spontaneous repetitive nerve firing, resulting in nervous disorders on chronic exposure (Zlotkin, 1999). Exposure to pyrethroids on *Paramecium tetraurelia* increases intracellular concentration of Ca^{++} ions (Symington et al., 1999) which may occur due to the direct effect of pyrethroids on the Ca^{++} channels (Kadous et al., 1994) or due to energy deficits resulting in the inability of cells to remove cytosolic Ca^{++} ion (Reddy et al., 1991). Cypermethrin and other pyrethroids are metabolized in the liver via hydrolytic ester cleavage and oxidative pathways by the CYP-450 enzymes yields reactive oxygen species (ROS), which may be responsible for oxidative stress in mammals (Floodstrom et al., 1988; Klimek, 1990). Increase in ROS/free radicals-mediated lipid peroxidation and increased cytosolic Ca^{++} concentration may lead to cytotoxicity and genotoxicity in higher vertebrates during exposure (Kadous et al., 1994; Gassner et al., 1997; Kale et al., 1999).

Nature produces an array of antioxidants to prevent free radical formation or to limit their damaging effects in the cell. Vitamins E and C, selenium, carotene, etc are the naturally occurring antioxidants of biological systems. α-Tocopherol (5,7,8-trimethyl tocol) is the predominant form of vitamin E and is considered to be the most important tocopherol since it constitutes about 90% of tocopherols in animal tissues (Azzi et al., 2002). Cypermethrin preferentially gets localized in the hydrophobic core of the membrane, where it increases lipid packing and consequently decreases membrane fluidity (Gabbianelli et al., 2002). α-Tocopherol is well known for its antioxidant properties in biological membranes, where it acts to prevent the peroxidation of lipid membranes by physiochemical interaction between its phytol side chain and the fatty acid chain of polyunsaturated phospholipids

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(Burton et al., 1986; Jiang et al., 2000). Vitamin E attenuates cycloxygenase (COX) activity by scavenging the oxidant hydro-peroxide, which is required for COX activation (Kulmacz et al., 1995) and inhibits the activetion of cytokine-induced nuclear factor-kß (NF-ß) as well (Suzuki and Packer, 1993). NF-ß is responsible for the activation of inducible nitric oxide synthase which plays an important role in inflammation (Kilbourn and Griffith, 1992). Considering that the involvement of ROS/oxidative stress has been implicated in the toxicity of cypermethrin, therefore the present study was designed to investigate the protective role of α-tocopherol on oxidative stress induced by dermal exposure to cypermethrin.

MATERIALS AND METHODS

Chemicals

Cypermethrin (10%) solution, commercially obtained from Meghmani Organic Limited, Ahemdabad was used. The reported LD_{50} value for acute dermal toxicity of cypermethrin in rats is 500 mg kg^{-1} b. wt (Luty et al., 1998). The selected dose was 50 mg kg^{-1} b. wt (1/10 of LD_{50}); it was applied dermally on the inter-scapular area daily for 10 days (Punareewattana et al., 2001). DL-α-tocopherol (High Media Laboratories Pvt. Ltd, Mumbai) at a dose of 100 mg kg^{-1} b. wt (Gabbianelli et al., 2004) was used orally for the study. α-Tocopherol was suspended in linseed oil for easy oral administration.

Animals and experimental protocol

Wister rats (200 – 250 gm b. wt.) of either sex procured from the Indian Institute of Integrated Medicine (CSIR, Lab) Jammu were maintained under standard environmental conditions. The animals were provided with free access to feed and water. The experiment was conducted strictly in accordance to the Institution's Animal Ethics Committee. The rats were divided randomly into 4 groups consisting of 8 animals each. Group A animals received distilled water, 0.5 ml; group B animals were exposed to linseed oil alone at 0.5 ml orally each; group C animals were exposed dermally with cypermethrin alone daily and group D animals were exposed dermally with cypermethrin along with α-tocopherol orally at 100 mg. kg^{-1} b. wt. All dosing were done in the morning continuously for ten days and body weights recorded at 3 days interval to adjust the dosage of application according to b. wt.

Blood enzyme assays

Whole blood samples were used for the estimation of blood glutathione (GSH) and hemoglobin. Erythrocyte lysate (1%) was used for catalase, superoxide dismutases (SODs) and glutathione peroxidase (GSH-Px), whereas 33% erythrocyte lysate was used for the determination of lipid peroxidation of the erythrocyte membrane. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive products (malondialdehyde) by the method of Ohkawa et al. (1979). The activity of SODs and catalase was measured according to the method described by Marklund and Marklund (1974) and Aebi et al. (1983), respectively. The GSH-Px and GSH activity in erythrocyte lysate was assayed by the methods of Hafeman et al. (1974) and Beutler et al. (1975), respectively.

Statistical analysis

The data were expressed as mean ± SE. Statistical analyses were done by one-way ANOVA followed by Dunnet's test with $P \leq 0.05$ as a limit of significance.

RESULTS AND DISCUSSION

In the present investigation, various oxidative stress parameters viz. catalase, SODs, GSH-Px, GSH and indicator of oxidative damage that is, lipid peroxidation were evaluated in control, linseed oil, cypermethrin alone and along with α-tocopherol treated animals. Values are presented in Table 1. Linseed oil treated animals show significantly reduced activity of ($p < 0.05$) SODs, GSH-Px, GSH level and significantly increased ($p < 0.05$) lipid peroxidation because of the high concentration of unsaturated fatty acids in it (Mary et al., 1991; Salobir et al., 2005). A significant increase ($p < 0.05$) in catalase activity was seen among animals that received cypermethrin. Although catalase activity did not change significantly in α-tocopherol treated animals as compared to the control group, it was significantly reduced ($p < 0.05$) in the cypermethrin treated animals. The catalase activity did not increase significantly in α-tocopherol treated rats. This may be due to the presence of linseed oil as a vehicle. Contrary to this, the significant increase in catalase activity was observed on subcutaneous injection of vitamin E in rats (Ahmet et al., 2005). The activity of erythrocyte SODs decreased significantly ($p < 0.05$) in all groups as compared to control, while animals treated with α-tocopherol had significantly ($p < 0.05$) greater (SODs) activity compared to cypermethrin treated group. The protective effect of α-tocopherol may be due to increased activity of pyrethroid-metabolizing enzymes that is, CYP-450 activity of these enzymes are dependent on the plasma α-tocopherol concentration (Floodstrom et al., 1988; Klimek, 1990; Azzi et al., 2002) or inhibition of protein kinase C activity (Zingg and Azzi, 2004), which is required for the production of the superoxide radicals (Islam et al., 1998; Cachia et al., 1998). Similarly, deltamethrin alone significantly decreased SODs activity while treatment with vitamin E increased the activities of SODs (Yousef et al., 2006). Cypermethrin and linseed oil groups had significantly ($p < 0.05$) reduced GSH-Px activity while in α-tocopherol treated rats, GSH-Px increased significantly ($p < 0.05$) as compared to cypermethrin treated group. The reduction in activity may be due to reduced level of GSH, which acts as a substrate for the enzyme. Similar results have also been observed in rats treated with permethrin (Gabbianelli et al., 2004). In α-tocopherol supplemented group, non-significant increase in GSH-Px activity as compared to control may be due to hindrance in the GSH-Px activity by linseed oil as a vehicle for the α-tocopherol and the study of Salobir et al. (2005) also reported that a high level of linseed oil in the diet of pigs reduces the activity of GSH-Px. This

Table 1. Protective effects of α-tocopherol on dermal exposure of cypermethrin on various oxidative stress parameters and lipid peroxidation in rats.

Parameter (unit)	Treatment given to rats			
	Group A	Group B	Group C	Group D
Catalase (μM of H_2O_2 decom.min^{-1} $mgHb^{-1}$)	49.68 ± 7.10[a]	30.76 ±2.70 [a]	128.34 ± 10.35 [b]	32.03 ± 4.32 [a]
SODs (Units $mgHb^{-1}$)	0.116 ± 0.015 [a]	0.016 ± 0.004 [b]	0.038 ± 0.002 [b]	0.057 ± 0.011 [c]
GSH-Px (Units $mgHb^{-1}$)	2.66 ± 0.09 [a]	0.58 ± 0.13 [b]	1.15 ± 0.23 [b]	3.02 ± 0.42 [a]
GSH (n mol ml^{-1})	110.85 ± 8.53 [a]	23.44 ± 2.30 [b]	23.68 ± 1.82 [b]	29.95 ± 2.71 [b]
Lipid peroxidation (n mol MDA produced g Hb^{-1} h^{-1})	4.19 ± 0.54 [a]	7.25 ± 0.32[b]	6.28 ± 0.42 [b]	6.55 ± 0.35 [b]

Values are expressed as mean ± SE of 8 animals.
[a, b, c] Mean with different superscript differ significantly at 5% (P ≤ 0.05) level of significance.
Group A, control animals without any treatment; Group B, animals supplemented with linseed oil orally (vehicle); Group C, animals exposed to dermal Cypermethrin at 50 mg kg^{-1} b. wt; Group D, cypermethrin exposure with same rate along with the oral feeding of α-tocopherol at 100 mg kg^{-1} b. wt.

This suggested that α-tocopherol has a protective effect on enzymatic activity.

GSH provides protection against free radicals/ ROS by scavenging from the biological system. In the present study, a significant ($p < 0.05$) reduction in GSH may be due to either decreased synthesis or increased utilization. The inability of α-tocopherol to maintain GSH level may be due to the presence of excess unsaturated fatty acids in linseed oil (Salobir et al., 2005). A significant ($p < 0.05$) increase in lipid peroxidation was observed in cypermethrin, linseed oil and α-tocopherol treated groups. Contrary to this, supplementation with vitamin E protected erythrocytes against plasma membrane lipids peroxidetion in rats (Belma et al., 2001; Gabbianelli et al., 2004; Ahmet et al., 2005) and vitamin E also protects deltamethrin induced lipid peroxidation (Yousef et al., 2006). In the present study, alpha tocopherol supplementation cannot reduce lipid peroxidation. This may be due to the presence of excess polyunsaturated fatty acids in the vehicle that is, linseed oil (Mary et al., 1991). The observations from the present study suggest that dermal cypermethrin exposure produces oxidative stress in rats and α-tocopherol supplementation has a protective effect on some stress parameters and linseed oil is not suitable as a vehicle for α-tocopherol.

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