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Research Article

Vitamin E provides protection against *in vitro* oxidative stress due to pesticide (Chlorpyrifos and Endosulfan) in goat RBC

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Abstract

Over the centuries, humans have developed many ingenious methods in their attempts to control herbs, weeds, insects, invertebrates and microorganisms that constantly threatened the supply of food and fibre as well as posed a threat to public health. The aim of present study was to find out the protective nature of antioxidant property of vitamin E in against chlorpyrifos and endosulfan toxicity. Erythrocytes were collected from healthy goat and exposed to 10ppm chlorpyrifos and endosulfan pesticide individually and also along with vitamin E treatment. Results showed endosulfan was more toxic in comparison of chlorpyrifos. Activity of Superoxide dismutase (SOD) and Catalase significantly decreased and lipid peroxidation (LPO) and Glutathione S-transfarase (GST) was increased in comparison of control value. The result of the present study suggests that vitamin E is an effective antioxidant for chlorpyrifos and endosulfan pesticide toxicity in reducing oxidative stress burden. It can be concluded that organophosphate chlorpyrifos and endosufan insecticides induced oxidative stress and lipid peroxidation level in mammalian erythrocytes (RBCs) and conjugation and supplementation of vitamin E or in combination has amelionated these effects.

Keywords: Endosulfan, chlorpyrifos, erythrocytes, vitamin E, LPO, catalase, SOD.

Introduction

Due to increased civilization more goods are manufactured and used resulting into more waste thrown out, which causes various kind of pollution. Advent of agricultural and industrial revolution in India has added many pollutants in the environment which are potentially hazardous, out of which some may be toxic, inflammable, explosive or corrosive. Over the centuries, humans have developed many ingenious methods in their attempts to control herbs, weeds, insects, invertebrates and microorganisms that constantly threatened the supply of food and fibre as well as posed a threat to public health. Pesticide is a general term used for substance which is used as a poison for killing the weeds, insect, molds, rodents etc. However, not all the pesticides are actually toxic for humans or other non target species (Aspelin, 1994). Now a day synthetic pesticides have been popular with farmers because of their simplicity in application, efficacy and economic returns. In India, around 1979 active ingredients of different pesticides including botanicals and microbial products are registered for use.

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On the basis of using pattern markets share of insecticide in maximum (74%) followed by fungicides (14%), herbicides (11%) and others (1%). Pesticidal use is one of those methods that is practiced throughout the world with the aim to protect desirable crops and to maximize food production, reducing loss through infestation of pests. Notwithstanding their benefits, pesticides, which are intentionally introduced into the environment in substantial quantities, might produce a wide range of toxic or adverse effects in non-target species and jeopardize the ecological equilibrium of fragile ecosystems.

Endosulfan (6,7,8,9,10,10 - hexachloro - 1,5,5a,6,9,9a - methano - 2,4,3 – benzodioxathiepin - 3 - oxide) is an organochlorine insecticide and acaricide, and acts as a contact poison in a wide variety of insects and mites (US EPA, 2002). It is being easily absorbed by the stomach, lungs and through the skin, which meaning that all routes of exposure can pose a hazard. It enhances the effect of estrogens and act as an endocrine disruptor, causing reproductive and developmental damage in animals and humans as well as cause cancer. Recent studies indicate

that pesticide intoxication produces oxidative stress by the generation of free radicals and induced tissue lipid peroxidation in mammals and other organisms (Comelekoglu *et al*., 2000). Hincal *et al*. (1995) reported the oxidative stress inducing effects of endosulfan, with an increase of lipid peroxidation and a significant alteration in glutathione redox cycle in cerebral and hepatic tissues of rats.

On the other hand, chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl-phosphorothioate), is an organophosphorus (OP) insecticide and is widely used for a variety of agricultural and human health applications. OPs produces a wide range of toxicity in mammals by inhibiting acetylcholinesterase (AChE) and the consequent accumulation of the neurotransmitter acetylcholine (ACh) in synaptic junction leads to excessive stimulation of postsynaptic cells leading to cholinergic toxicity (Ecobichon, 1996). In fact, one of the molecular mechanisms of the toxicity of some pesticides seems to be lipid peroxidation, as a consequence these compounds can disturb the biochemical and physiological functions of the red blood cells (RBC) (Banerjee *et al*., 1999; Akhgari *et al*., 2003). The susceptibility of RBC to oxidative damage is due to the presence of polyunsaturated fatty acid, haem iron and oxygen, which may produce oxidative changes in RBC (Kale *et al*., 1999).

A major contributor to non-enzymatic protection against lipid peroxidation is vitamin E and Vitamin C, well known free radical scavengers (Rikans *et al*., 1991; Jain and More, 1998; Kumar *et al*., 2009). Vitamin E as a lipid soluble, chain-breaking antioxidant plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals in biological membranes (Kagan *et al*., 1992). Some investigators reported that administering Vitamin E may be useful in controlling the toxic effect of insecticides and chemicals (Atessahin *et al*., 2005). Keeping these points in mind, present study was planned to establish the antioxidant role of vitamin E on oxidative stress of rat RBC's induced due to pesticides.

Method and materials

Ten male healthy goats weighing about 18 – 22 kg were used in the study. About 3 ml peripheral blood was obtained from jugular vein puncture of goat by using EDTA-sodium salt as the anticoagulant for assays. Blood was centrifuged at 2000 rpm for 10 minutes. Plasma and buffy coat was removed. Subsequently the cells were washed three times with phosphate buffered saline (PBS), pH 7.2. The final red cell suspension was taken in test tubes for chlorpyrifos, endosulfan and vitamin E treatment, each in triplicate set. All pesticide and vitamin E (chlorpyrifos, endsulfan, vitamin E, chlorpyrifos and vitamin E, and endosulfan and vitamin E) was dissolved in di methyl sulphonic acid (DMSO), and made upto 100 ppm stock solution of each group, respectively. Above combinations were mixed and made to desired 10 ppm concentration in all groups (DMSO 5% of total volume and test was performed in triplicate set). As well as 5% DMSO was dissolved/mixed in control group. The tubes were incubated for 3 hours at 37^{0}C in shaking water bath. At the end of incubation, the tubes were removed and subjected for biochemical analysis.

Biochemical analysis

Chemicals for analysis

Alpha endosulfan (100%) and chlorpyrifos (100%) were purchased from ACCUSTANDARD, INC. USA. Tris cacodylic acid, diethylenetriamine-penta-acetic acid (DTPA) (99% pure), nitro blue tetrazolium (98%), pyrogallol (>98%), sodium-dodecyl-sulphate (>99%), bovine serum albumin (97%), triton X-100 and thiobarbituric acid (99%) were purchased from Sigma Chemicals, USA. Nitric acid (69%), NaH_2PO_4 (98%), KH_2PO_4 (99.5%), pyridine (99%) and 1-butanol (0.99%) extra pure grade were purchased from Qualigens Chemicals, India.

Lipid peroxidation in erythrocytes

The level of lipid peroxides (LPOs) in erythrocyte hemolysate was determined spectrophotometrically following the method of Placer *et al*. (1966). Lipid peroxidation was calculated using 1.56×10^5 as extinction coefficient (Utley *et al*., 1967) to express the value in nanomoles of malonyldialdehyde (MDA) per millilitre of hemolysate. Haemoglobin (Hb) in haemolysate was estimated by cyanomethaemoglobin method spectrophotometrically (Van-kampen and Ziglstra, 1961) and lipid peroxide level in the erythrocytes was expressed as nanomoles of MDA per milligram of hemoglobin.

Antioxidant enzymes

The erythrocyte homogenate was used for analysis of antioxidant enzymes after suitable dilution. Activity of superoxide dismutase (SOD), an important antioxidant defense enzyme, was measured in the 10% RBC haemolysate following the method of Marklund and the

values were expressed in units per milligram of haemoglobin for erythrocytes. Glutathione-S-transferase (GST) activity in RBC hemolysate was estimated spectrophotometrically at wave length of 340 nm as per method of Habig *et al*. (1974) and the values were expressed in units per milligram of haemoglobin for erythrocytes.

Statistical analysis

The data obtained were statistically analyzed using two-way analysis of variance to find out significance of difference within a group at different periods of observation, and students t-test to find out the significance between the groups at particular period of observation (Snedecor and Cocharan, 1989).

Results

Data pertaining to Hb concentration and enzyme activity are presented in Table-1. There was significantly higher ($P<0.05$) Hb levels in chlorpyrifos exposed group and lowest in vitamin E RBC lysate (12.30 ± 0.03). The level of LPO in cell lysate was significantly ($P<0.05$) greater in the group exposed endosulfan and chlorpyrifos, and lowest in control group. The LPO level in cell lysate reduced significantly ($P<0.05$) in vitamin E treated groups as compared to their respective non-treated group pesticides. The SOD activity was significantly lower in chloropyrifos and endosulfan exposed groups 14.30±0.08 and 27.73±012 , respectively. Goat erythrocytes exposed to chlorpyrifos and endosulfan at 10 ppm and on treatment with vitamin E showed greater SOD activity in erythrocytes compared with non-treated pesticide exposed group. It was observed that vitamin E treatment improved SOD activity with duration treatment in both pesticide groups. However, vitamin E treatment with in both pesticide exposures increased catalase activities in erythrocytes. The catalase activity in erythrocytes decreased gradually following both pesticide exposures.

Discussion

Our studies comprised as a part of comparative toxicology aimed to identify the biochemical and physiological alteration in red blood cells exposed to two different pesticides. The approach is known to help in understanding the mechanisms of toxic action due to xenobiotics (Siebert *et al*., 1994).

There are several pathways by which pesticide is thought to induce oxidative stress. It inhibits the mitochondrial electron-transfer chain reaction, leading to accumulation of semi ubiquitous, which enables it to transfer one electron (e^-) to molecular oxygen to form superoxide radicals (Wang *et al*., 2004). Further, it may also interference with cellular antioxidant defense system via alteration in activities of antioxidant enzymes *viz.* SOD and catalase and status of glutathione (Panda *et al*., 1997; Sandrini *et al.*, 2006). LPO level in rat erythrocytes treated with vitamin E was comparable to control, suggesting that the pesticides (chlorpyrifos and endosulfan) acts as a catalyst in the oxidative deterioration of biological macromolecules and this effect could be minimized by treatment with antioxidants. These indirectly suggest an increased production of oxygen free radicals in erythrocytes. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product.

	Hb (g/dl)	**LPO** (nmol MDA/ mg of Hb)	**SOD** (unit/mg of Hb)	**CAT** (unit/mg of Hb)	**GST** (unit/mg of Hb)
(N=6)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Control	12.35 ± 0.03 ^{b}	3.53 ± 0.00 ^{a}	41.39 ± 0.00 ^{f}	69.83 ± 0.31 ^{f}	1.64 ± 0.01 ^{b}
Chlorpyrifos	12.80 ± 0.03 ^{d}	5.55 ± 0.07 ^{f}	14.30 ± 0.07 ^{a}	50.07 ± 0.89 ^{a}	1.31 ± 0.04 ^{f}
Endosulfan	12.51 ± 0.03 ^{bc}	4.86 ± 0.04 ^{e}	27.73 ± 0.11 ^{c}	58.00 ± 0.37 ^{b}	1.47 ± 0.03 ^{e}
Vitamin E	12.30 ± 0.03 ^{a}	3.78 ± 0.03 ^{b}	39.87 ± 0.06 ^{e}	66.17 ± 0.48 ^{e}	1.72 ± 0.02 ^{a}
Chloro + Vit E	12.61 ± 0.04 ^{c}	4.32 ± 0.04 ^{d}	25.99 ± 0.01 ^{b}	60.08 ± 0.49 ^{c}	1.49 ± 0.08 ^{d}
Endosulfan + Vit E	12.40 ± 0.09 ^{ab}	3.99 ± 0.07 ^{c}	39.01 ± 0.14 ^{d}	63.10 ±0.52 ^{d}	1.56 ± 0.02 ^{c}

$^{a, b, c, d, e, f}$ Values bearing different superscripts in a row differ significantly ($P<0.05$).

Table-1: Antioxidant enzymes activities in erythrocytes of the treatment groups.

Chlorpyrifos have been reported to induce oxidative stress, as shown by enhanced MDA production (Banerjee *et al*., 1999; Gultekin *et al*., 2001; Goel *et al*., 2005). The use of vitamin E in conjunction with chlorpyrifos affected such elevation in the level of MDA; bringing it within the normal limits ($p<0.05$). The normalization of LPO following vitamin E treatment is very likely due to its antioxidant properties, as has been shown previously (Chvapil *et al*., 1972; Cabre *et al*., 1999).

However, the antioxidant enzymes SOD, GST and CAT limit the effects of oxidant molecules on tissues and are active in the defense against oxidative cell injury by means of their being free radical scavengers (Kyle *et al*., 1987; Jalaili *et al*., 2007). These enzymes work together to eliminate active oxygen species and small deviations in physiological concentrations may have a dramatic effect on the resistance of cellular lipids, proteins and DNA to oxidative damage.

Our results revealed that chlorpyrifos and endosulfan caused a statistically significant decrease ($p<0.05$) in SOD activity in goat erythrocytes compared to the control value. Supplementation of vitamin E to chlorpyrifos and endosulfan treated groups of gots erythrocyte normalized the levels of SOD. Treatment with vitamine E alone did not result in significant alteration in SOD activity compared to control treatment. Our results agree with previous studies (Gultekin *et al*., 2001; Gultekin *et al*., 2000) regarding the effect of chlorpyrifos *in vivo* on rat erythrocytes or *in vitro* on human erythrocytes. The decrease in the activity of superoxide dismutase in chlorpyrifos- intoxicated animals may be owed to the consumption of this enzyme in converting the O^{2-} to H_2O. The dismutation of O^{2-} to H_2O is catalyzed by SOD which contains both copper and zinc.

In comparison to the control group, the activities of GST was significantly ($p<0.05$) higher by chlorpyrifos treatment in goats erthrocytes. Considering that glutathione- S-transferases are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (Hayes and Pulford, 1995), the significant increase of GST activity in the rats erythrocytes after exposure of chlorpyrifos and endosulfan may indicate sufficient detoxification of pesticide in goat erythrocytes while, the use of vitamin E with pesticide goes near to the control sample.

Catalase is ubiquitously present in a wide range of aerobic cell types, with the highest activities in mammals being found in liver, kidney and red blood cells (Deisseroth and Dounce, 1970) It is found as a soluble protein in erythrocytes, where it may protect haemoglobin from peroxidation. Table shows that chlorpyrifos and endosulfan caused significant decrease in CAT activity in erythrocytes of goats erythrocytes In compare, vitamin E with chlorpyrifos and endosulfan treated erythrocytes maintained the levels of CAT at the normal values ($p<0.05$).

It can be concluded that organophosphate chlorpyrifos and cndosufan insecticides induced oxidative stress and lipid peroxidation level in mammalian erythrocytes (RBCs) and conjugation and supplementation of vitamin E or in combination has amelionated these effects. Lipid peroxidation is one of the molecular mechanisms involved in pesticide induced toxicity.

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