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## The Effects of Vitamin E on Endosulfan - Induced Oxidative Stress in Rat Heart

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**Abstract:** Endosulfan, an organochlorine insecticide and acaricide, has been used in agriculture for several years. The aim of present study was to analyze the cardiotoxic effect of endosulfan which caused biochemical changes in heart of male rats and to evaluate the possible protective effect of vitamin E. Vitamin E (200mg/kg, twice a week), endosulfan (2mg/kg per day, once a day in corn oil) and Vitamin E (200mg/kg, twice a week) + endosulfan (2mg/kg per day, once a day in corn oil) combination were given to rats (n=6) orally via gavage for 4 weeks. GPx and CAT activities were measured in tissues samples. Gpx, CAT and SOD activities increased in the endosulfan - treated group heart tissues compared to control group ( $P<0.01$ ,  $P<0.05$  and  $P<0.01$  respectively). GPx and SOD activities decreased in the endosulfan+vitamin E - treated group compared to endosulfan - treated group ( $P<0.05$ ). Decrease of CAT activity was not significant in the endosulfan+vitamin E - treated group compared to endosulfan - treated group. We conclude that vitamin E significantly reduces endosulfan - induced oxidative stress in rat heart.

**Key words:** Vitamin E, Endosulfan, antioxidant enzymes, heart

### Introduction

Oxidative stress is defined as a disruption of the prooxidant - antioxidant balance in favour of the former, leading to potential damage (Sies, 1991). It is a result of one of three factors: An increase in reactive oxygen species (ROS), an impairment of antioxidant defense systems or an insufficient capacity to repair oxidative damage. Damage induced by ROS includes alterations of cellular macromolecules such as membrane lipids, DNA, and/or proteins. The damage may alter cell function through changes in intracellular calcium or intracellular PH, and eventually can lead to cell death (Kehrer *et al.*, 1990).

Under normal condition, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by enzymatic and non - enzymatic mechanisms including vitamin E and Glutathione. The antioxidant enzymes such as Gpx, SOD and CAT may also have an important function in mitigating the toxic effects of ROS (Adali *et al.*, 1999).

Endosulfan (6,7,8,9,10,10 - hexachloro - 1,5,5a,6,9,9a - methano - 2,4,3 - benzodioxathiepin - 3 - oxide) is a broad spectrum insecticide and acaricide first registered for use in the United States in 1954 to control agricultural insect and mite pests on a variety of field, fruit, and vegetable crops (US. EPA, 2002). It belongs to the group of cyclodienes, chemicals that are potent inhibitors of  $\text{Na}^+/\text{K}^+$  and  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ATP<sub>ase</sub>, essential for transport of ions across membranes, in mammals and fish. Actually, residual amounts of organochlorines (OC) are known to cause inhibition of acetylcholinesterase activity in the

target tissues and effect  $\text{Na}^+$ ,  $\text{K}^+$  ATP<sub>ase</sub>,  $\text{Mg}^{2+}$  ATP<sub>ase</sub> and  $\text{Ca}^{2+}$  activities of cells (Naqvi and Vaishnavi, 1993).

Recent studies indicate that pesticide intoxication produce oxidative stress by the generation of free radicals and induce tissue lipid peroxidation in mammals and other organisms (Çomelekoglu *et al.*, 2000). (Hincal *et al.*, 1995) reported the oxidant 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.

A major contributor to non - enzymatic protection against lipid peroxidation is vitamin E, a known free radical scavenger (Rikans *et al.*, 1991). 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).

This study is planned to evaluate the role of Vitamin E as a protective agent against endosulfan - induced cardiotoxicity by measuring the antioxidant enzymes Gpx, SOD and CAT.

### Materials and Methods

**Chemicals:** Endosulfan, technical purity 35%, Vitamin E ( $\alpha$ -tocopherol) was purchased from Sigma Chemical co.

**Animals and treatments:** Male Waster rats weighing 150-180 g were provided from a local animal research institute. Rats were housed in plastic cages under standard condition with free access to drinking water

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Table 1: Effect of endosulfan on Catalase (CAT), Glutathione peroxidase (GPx) and superoxide dismutase (SOD)activities in male rats heart tissues (mean  $\pm$  SD)

Groups (n=6)	CAT ( U/mg protein)	Gpx ( U/mg protein)	SOD (U/mg protein)
Control	30.4 $\pm$ 1.52	50.3 $\pm$ 2.39	25.5 $\pm$ 2.7
Vitamin E	32.6 $\pm$ 1.85	54.6 $\pm$ 3.21	29.4 $\pm$ 8.2
Endosulfan	55.4 $\pm$ 2.01	99.2 $\pm$ 2.47	67.9 $\pm$ 3.8
Vitamin E+ Endosulfan	45.4 $\pm$ 2.27	65.3 $\pm$ 2.12	32.9 $\pm$ 3.2
Control - Endosulfan	P<0.05	P<0.01	P<0.01
Control - vitamin E+Endosulfan	P<0.05	P<0.05	n.s
Vitamin E - Endosulfan	P<0.05	P<0.01	P<0.05
Endosulfan - Vitamin E+endosulfan	n.s.	P<0.05	P<0.05
Control - vitamin E	n.s	n.s	n.s

and basal diet. The animals were adapted to the laboratory condition for 7 days before use and were maintained in a room with controller temperature (20 - 22°C), relative humidity (50%) and 12h light/dark cycle.

The rats were divided into four groups, each including six animals;

1. control group: corn oil 0.2ml per animal per day was given through gavage to rats once a day
2. vitamin E-treated group: vitamin E ( 200mg/kg) twice a week
3. Endosulfan - treated group: endosulfan at a dose of 2mg/kg per day in corn oil (0.2ml per animal) once a day
4. vitamin E+ endosulfan- treated group vitamin E ( 200mg/kg, twice a week) 15 min before endosulfan (2mg/kg per day, once a day in corn oil).

At the end of the experiments animals were anaesthetized by diethylether. The hearts were removed and immediately rinsed in ice - saline. Then Homogenized in 10% w/v ice - cold 0.05 M potassium phosphate buffer (pH 7.4) and were used for the analysis.

**Biochemical analyses:** CAT activity were determined according to the method (Aebi, 1984). Supernatant (50  $\mu$ l) was added to a 3.0 ml cuvette that contained 1.95 ml of 50 mM phosphate buffer (pH 7.0). 1.0 ml of 30 mM hydrogen peroxide was added and changes absorbance were followed for 30 sec at 240 nm at 15 - sec intervals. Catalase activity was expressed as U/mg protein.

SOD activity was determined according to the method (Sun *et al.*, 1988). The principle of the method is based on the inhibition of nitroblue tetrazolium reduced by the xantine - xantine oxidase system as a superoxide generator. Activity was assessed in the ethanol/chloroform was added to the sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in NBT reduction rate. SOD activity was expressed as U/mg protein.

GPx activities were determined according to the method of (Lawrence and Burk, 1976). GPx activity in the supernatant was detected using GPx assay buffer (100 mM Tris/HCl pH 7.6, 5 mM EDTA, 1 mM sodium azide,

0.1% Triton X-100), supplemented with 3 mM glutathione, 600 mU/ml glutathione reductase and 0.1 mM NADPH. Reaction was started by addition of Cumene Hydro peroxide (CuOOH) to a final concentration of 50  $\mu$ M and detected by measurement of NADPH consumption at 340 nm. GPx activity was determined using the extinction coefficient of NADPH at 340 nm. GPx activity was expressed as U/mg protein. Protein concentration was determined according to (Lowry *et al.*, 1951).

**Statistical analysis:** The results were expressed as means $\pm$ SD. These results were analyzed by standard statistical analyses, One-way ANOVA with Duncan's test for multiple comparisons to determine significance between different groups. The value for p < 0.05 was considered statistically significant.

## Results

SOD activities significantly increased in the endosulfan - treated group compared to control group (P< 0.01). A significant increase of SOD activity was observed in the endosulfan - treated group compared to vitamin E - treated group (P<0.05). SOD activity showed no difference in vitamin E + endosulfan treated group compared to control group (Table 1, Fig. 1).

CAT activities significantly increased in the endosulfan and vitamin E + endosulfan - treated groups compared to control group (P< 0.05). A significant increase of CAT activity was observed in the endosulfan - treated group compared to vitamin E-treated group (P<0.05). CAT activity showed no difference in vitamin E + endosulfan treated group compared to endosulfan - treated group (Table 1, Fig. 2).

GPx activities significantly increased in the endosulfan - treated group compared to control and vitamin E -treated groups (P<0.01). GPx activities decreased in the vitamin E+Endosulfan-treated group compared to endosulfan - treated group (P< 0.05) (Table 1, Fig. 3).

## Discussion

In the present study we demonstrated that Endosulfan, an organochlorine pesticide from the group of cyclodienes, influences antioxidative enzymes in heart

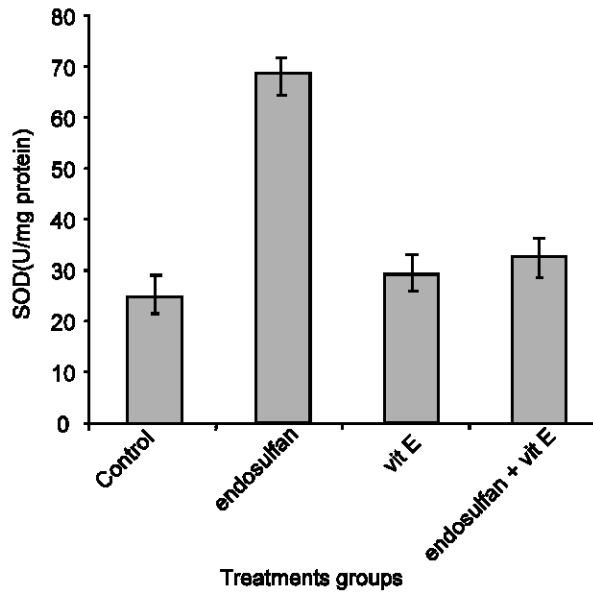


Fig. 1: Effects of interaction between endosulfan and vitamin E on superoxide dismutase (SOD) activity in male rats heart tissues (mean±SD).

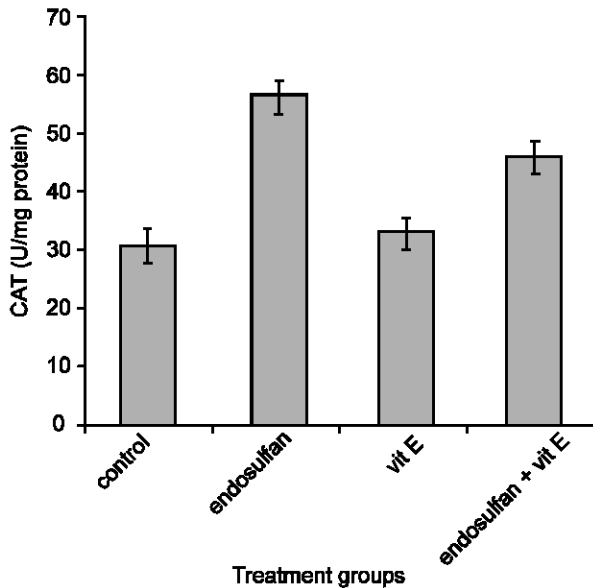


Fig. 2: Effects of interaction between endosulfan and vitamin E on catalase (CAT) activity in male rats heart tissues (mean±SD).

and, therefore heart injury associated with this insecticide may be due to oxidative tissue damage. While Vitamin E was inhibiting formation of free radical, also it decreased endosulfan cardiotoxicity. Oxidative stress, generated by xenobiotics, induces disturbances in antioxidant enzyme systems (Gabbianelli *et al.*, 2002). Free radicals play an important role in toxicity of pesticides and environmental

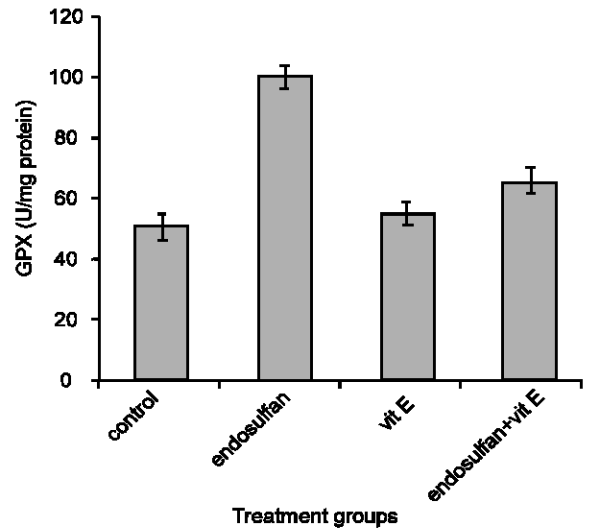


Fig. 3: Effects of interaction between endosulfan and vitamin E on Glutathione peroxidase (GPX) activity in male rats heart tissues (mean±SD).

chemicals. Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system (Banerjee *et al.*, 1999). Oxidative stress is known to be an important etiopathological factor in a variety of cardiac diseases, such as heart failure and ischemic heart disease. The effects of organophosphate insecticides on fish revealed that besides acetylcholinesterase inhibition, there were changes characteristic of oxidative stress (Malkovics, 1995). In humans, pesticides were shown to reduce the total cholesterol and phospholipids level of RBC membrane following phosphamidon and malathion, and increase lipid peroxides level following malathion. (John *et al.*, 2001). The basis of pesticide toxicity in the production of reactive oxygen species may be due to:

- 1) their "redox-cycling" activity - they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction.
- 2) generation of free radicals probably because of the alteration in the normal homeostasis of the body resulting in oxidative stress, if the requirement of continuous antioxidants is not maintained (Ryrfeldt *et al.*, 1992).

The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, resulting in oxidative cell damage (Kalra *et al.*, 1994).

The present study reports that oral administration of endosulfan caused oxidative stress in rat heart. As is reported by other authors, the activities of GPx are

linked in their capacity to prevent peroxidative tissue damage from oxidants. GPx converts toxic lipid hydroperoxides and H<sub>2</sub>O<sub>2</sub> using reducing equivalents generated by G6PDH (Frei *et al.*, 1989). However, in this study the increased activity of CAT, SOD and GPx was observed at the same time after intoxication with endosulfan.

A number of recent studies provided evidence for the capacity of organochlorine pesticides (endrin, HCH, lindane and others) to induce oxidative stress in different organs (Bagchi *et al.*, 1993a,b; Hincal *et al.*, 1995; Hassoun and Stohs, 1996; Ananya *et al.*, 2005).

Endosulfan was identified as a chemical inducing alteration in the activity of enzymes involved in oxidative stress and lipid peroxidation (Dorval *et al.*, 2003; Frederick and Panemangalore, 2003).

Several studies with liver, brain, and testes indicate that lindane (*an organochlorine insecticide*) causes oxidative stress (Sahoo *et al.*, 2000; Junqueira *et al.*, 1988; Abdollahi *et al.*, 2004). Though lindane is also distributed to the heart following oral absorption (Sauviat *et al.*, 2002).

A number of previous studies have reported that lindane causes oxidative stress in the liver (Khessiba *et al.*, 2005). Apart from the liver, oxidative stress has also been demonstrated in the brain (Sahoo *et al.*, 2000), testes (Chitra *et al.*, 2001) and myometrium (Krieger and Caruso, 2001).

The involvement of the two main cellular antioxidants, catalase and GPx in protection of cells against oxidative stress induced by endosulfan, was evaluated in the present study in enzymatically dispersed. The increased GPx, SOD and CAT levels in heart tissue indicate an elevated antioxidant status.

Epidemiological studies provide increasing evidence related to the importance of the human antioxidant defense system in assessing the risk of chronic and degenerative diseases. In recent years, several such investigations have provided strong circumstantial evidence for the beneficial effects of Vitamin E and have shown a highly significant correlation between lower risk to ischemic heart disease mortality and higher plasma Vitamin E levels. Beneficial effects of Vitamin E supplementation on human health are also noted in various chronic diseases and some acute clinical conditions (Packer, 1992).

Vitamin E allow free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions, the resulting antioxidant radicals being a relatively unreactive species (Pascoe *et al.*, 1987). In many studies vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect (John 2001; Aldana *et al.*, 2001).

Therefore Vitamin E supplementation sufficient to protect

the organism from toxic agents and free radical damage is a time consuming process. It is concluded that Vitamin E is an essential component of the kidney for the protection of this tissue against peroxidative damage (Champe and Harvey, 1987).

Vitamin E has also been used to prevent oxidative damage by interrupting the propagation of the oxidation of polyunsaturated fatty acids. Some investigators reported that administering Vitamin E may be useful in controlling the hepatotoxic effects of insecticides and chemicals (Adali *et al.*, 1999; Aldana *et al.*, 2001). Our findings showed protective role of vitamin E. When vitamin E was used alone, GPx and CAT activities were not found significant compared to control group. But when vitamin E was given together with endosulfan, we observed that it decreased toxic effect of endosulfan.

We concluded that Vitamin E administration might be useful in detoxification of heart tissue against the toxic effects of endosulfan.

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